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Association of interleukin-6 and CD4+ T cells and two-week prognosis of patients with COVID-19: a predictive role

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Abstract. – OBJECTIVE: The aim of this study was to determine the association of inflammation and immune responses with the outcomes of patients at various stages, and to develop risk stratification for improving clinical practice and reducing mortality.

PATIENTS AND METHODS: We included 77 patients with primary outcomes of either death or survival. Demographics, clinical features, comorbidities, and laboratory tests were compared. Linear, logistic, and Cox regression analyses were performed to determine prognostic factors.

RESULTS: The average age was 59 years (35-87 years). There were 12 moderate cases (16.2%), 42 severe cases (54.5%), and 23 critical cases (29.9%); and 41 were male (53.2%). Until March 20, 68 cases were discharged (88.3%), and nine critically ill males (11.7%) died. Interleukin-6 (IL-6) levels on the 1st day were compared with IL-6 values on the 14th day in the severe and the critically ill surviving patients (F=4.90, *p*=0.034, β=0.35, 95% CI: 0.00-0.10), and predicted death in the critically ill patients (*p*=0.028, β=0.05, OR: 1.05, 95% CI: 1.01-1.10). CD4+ T-cell counts at admission decreased the hazard ratio of death (*p*=0.039, β=-0.01, hazard ratio=0.99, 95% CI: 0.98-1.00, and median survival time 13.5 days).

CONCLUSIONS: The present study demonstrated that IL-6 levels and CD4+ T-cell count at admission played key roles of predictors in the prognosis, especially for critically ill patients. High levels of IL-6 and impaired CD4+ T-cells are seen in severe and critically ill patients with COVID-19.

Key Words:
COVID-19, SARS-CoV-2, Interleukin-6, CD4+ T, Prognosis.

Introduction

Coronavirus Disease 2019 (COVID-19) is an acute respiratory pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1]. This highly contagious disease has spread throughout the whole world quickly. As of May 7, 2020, the World Health Organization (WHO) reported that COVID-19 has caused over 3.6 million cases with more than 250 thousand deaths globally[2].

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Evidence[2] has suggested that excessive inflammation and exaggerated immune responses contribute to COVID-19 pathology. It includes high levels of proinflammatory cytokines and inflammatory markers, including interleukin-6 (IL-6)[3] and C-reactive protein (CRP)[4], as well as severe lymphopenia[4]. In SARS-CoV-infected animals, apparent inflammatory and immune responses activate a "cytokine storm", vascular leakage, and abnormal T cell responses. Subsequently, it induces severe acute respiratory distress syndrome (ARDS), or even death[5]. Similarly, cytokine storm syndrome occurred in patients with severe COVID-19, including ARDS, and even deteriorated within a short period. It induces death from multiple organ failure[6].

The primary clinical characteristic of COVID-19 infection is severe pneumonia[3], with dyspnea and high respiration rates reflecting the severity of lung lesions caused by infection. Common complications during hospitalization include ARDS and bacterial infections[7]. Increased levels of procalcitonin (PCT) in patients with viral infections typically mirror bacterial infections[8]. It may then contribute to driving the clinical course toward unfavorable progression[9]. Among patients who develop dyspnea and hypoxemia, the median time from the onset of symptoms was 5-8 days, with ARDS developing in a smaller subset at 7-10 days[10-12]. Abnormal inflammatory and immune responses occur during the COVID-19 infections. Therefore, in the present study, we investigated the association of abnormal inflammatory and immune responses concerning outcomes in patients with COVID-19 infection. We further explored the hazard ratios of survival in critically ill patients.

Patients and Methods

Participants

This retrospective, single-center study involved 77 patients with COVID-19 starting from January 31, 2020, at the East Hospital of People's Hospital of Wuhan University. This is a designated hospital capable to receive patients with COVID-19. Real-time reverse transcription PCR tests of SARS-CoV-2 for all patients' samples were positive. All patients were diagnosed and admitted by the Diagnosis and Treatment of Novel Coronavirus Pneumonia (7th trial version) released by the National Health Commission of the People's Republic of China.

The primary outcomes were survival and death. The final date of observation was March 20, 2020. Patients who did not meet the discharge standard continued hospitalization for treatment. The patients discharged and remaining in the hospital until the final follow-up date were considered survivors. The clinical classifications of COVID-19 are as follows: (1) Moderately ill patients showed fever and respiratory tract symptoms, with pneumonia revealed on imaging. (2) Severely ill patients were those with one of the following symptoms, including respiratory rate ≥30 breaths/min, finger oxygen saturation ≤93% at rest, and arterial partial pressure of oxygen/fraction of inspired oxygen ≤300 mmHg, as well as pulmonary imaging revealing significant progression within 24-48 h of >50%. (3) Critically ill patients met any of the following conditions, including respiratory failure, the requirement for mechanical ventilation, shock, and complications of other organ failures that require monitoring and treatment in the ICU. (4) Critically ill patients were further subdivided into a critically ill survivor and critically ill mortality groups. Bacterial infection was diagnosed if the patient produced purulent sputum when admitted to the hospital and it was combined with a positive culture of respiratory secretions or laboratory examination of CRP and PCT. Patients were excluded if they had abnormal thyroid function, systemic lupus erythematosus, rheumatism, human immunodeficiency, neoplastic disease, and other immune diseases in the present study.

The present study was approved by the Medical Ethics Committee of the People's Hospital of Wuhan University with the exception of the requirement of informed consent (WDRY2020-K120).

Data Collection

From medical records, we obtained demographic information and clinical characteristics, including age, gender, comorbidities, clinical presentation, and survival time after admission to the hospital.

Laboratory tests included counts of $CD4^+$ T cells, $CD8^+$ T cells, lymphocytes (LYMPH), CD4+/CD8+ lymphocyte ratio (LYMPHR), and levels of PCT, IL-6, and CRP on the 1st day of admission, and on the 7th and 14th days.

Statistical Analysis

Categorical variables were expressed as numbers (%). They were compared using the Chi-square test or Fisher's exact test among the four groups. Continuous variables were expressed as mean ± standard deviation (SD). In order to compare the laboratory results of repeated measurements, the analysis of variance (ANOVA) of repeated measurements was performed using time as an in-subject variable (on the 1st day admission, on the 7th day, and the 14th day). Groups were used as inter-subject variables for analysis of variance (moderate, severe, critical survival, and critical death groups). Laboratory findings were taken as dependent variables. To determine whether group differences changed over time, the interaction (Group × Time) was tested first. When the interaction terms for all laboratory findings were not significant, the interaction term was dropped and the main effect of group status (or time) was tested. When the interaction effects and main effects were found, the post-hoc and simple effect tests were performed using the Bonferroni correction. Partial Eta-squared was calculated for effect size. For the evaluation of effect size calculation, 0.01 is considered small, 0.09 is medium, and 0.25 is large. Stepwise linear regression and binary logistic regression were performed to identify the risk factors for "cytokine storm" and poor prognosis, respectively. A multivariate analysis of these variables was subsequently performed using the Cox regression model for survival analysis of critical cases. Statistical analysis was performed using SPSS Statistics 23 (IBM Corp., Armonk, NY, USA), as well as GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA, USA) for generating figures. A two-sided $\alpha<0.05$ was considered statistically significant.

Results

Demographics and 1st-day Clinical Characteristics

Among the 77 patients, there were 12 moderate cases (16.2%), 42 severe cases (54.5%), and 23 critical cases (29.9%). The mean age was 59 years, and 41 were males (53.2%); 37.7% of the patients had comorbidities, and 10.4% had two or more comorbidities. Common comorbidities included hypertension (23, 29.9%), diabetes (9, 11.7%), and cardiovascular disease (6, 7.8%). The most common symptom was fever, occurring in 58 (75.3%) patients. Dry cough (25, 32.5%), expectoration (16, 20.8%), dyspnea or chest tightness (8, 10.04%), and fatigue (9, 11.7%) were also common. Until March 20, 2020, 68 patients were discharged (88.3%), and nine critically ill patients (11.7%) died. The patients who died were all males. There were no significant differences between the groups in terms of hypertension, cardiovascular disease, diabetes, or chronic obstructive pulmonary disease (all $p>0.05$). Notably, more patients were complaining of dyspnea/chest tightness and expectoration in the mortality group (6/9 *vs.* 2/68, 7/9 *vs.* 11/68; $p<0.001$). There were significant differences in heart rate, respiration rate, and systolic blood pressure between the groups (all $p<0.05$) (Table I). The critically ill patients suffered from bilateral pneumonia. Five patients died of ARDS within 2 weeks due to progressive aggravation of pulmonary infection. Three patients contracted severe bacterial infections resulting in an inflammatory storm and died of respiratory failure in about one month. Another patient died of multiple organ dysfunction syndrome due to respiratory failure.

Laboratory Findings

Repeated measures ANOVAs revealed a significant Group × Time effect concerning lymphocyte ratio (LYMPHR) and IL-6 levels ($F_{(6,\ 124)}=2.34$, $p=0.035$, $\eta_p^2=0.10$; $F_{(3,\ 60)}=5.99$, $p=0.001$, $\eta_p^2=23$) (Table II). Significant main effects of both time and group status for $CD4^+$ T, $CD8^+$ T, LYMPH, and CRP were found ($CD4^+$ T: $F_{(2,\ 96)}=6.15$, $p=0.003$, $\eta_p^2=0.11$ for time and $F_{(3,48)}=4.60$, $p=0.007$, $\eta_p^2=0.22$ for group; $CD4^+8$ T: $F_{(2,96)}=8.84$, $p=0.001$, $\eta_p^2=0.16$ for time and $F_{(3,\ 48)}=3.55$, $p=0.021$, $\eta_p^2=0.18$ for group; LYMPH: $F_{(2,\ 106)}=6.42$, $p=0.004$, $\eta_p^2=0.09$ for time and $F_{(3,\ 62)}=4.43$, $p=0.007$, $\eta_p^2=0.18$ for group; CRP: $F_{(2,\ 124)}=7.72$, $p=0.001$, $\eta_p^2=0.11$ for time and $F_{(3,62)}=6.29$, $p=0.001$, $\eta_p^2=10.23$ for group) (Table II).

Simple effect testing showed that LYMPHR in the moderately ill group was higher than that of the other three groups on the 1st day (F=15.94, $p<0.001$). At day 7, survival and mortality were significantly higher in the moderate and severe disease groups than in the critical disease group (F=11.43, $p<0.001$). The moderate and severe disease groups were significantly higher than the death group on day 14. (F=5.39, $p=0.002$). IL-6 levels of deaths were significantly higher than that of the other three groups on the 1st day and the 14th day (F=13.97 and F=10.53, all $p<0.001$) (Figure 1).

Post-hoc testing of time revealed that the counts of $CD4^+$ T cells, $CD8^+$ T cells, and LYMPH on the 14th day were significantly higher than those on the 1st day ($p<0.05$). $CD8^+$ T cell counts rose significantly from the 7th day to the 14th day ($p<0.05$). CRP levels on both the 7th and 14th days were significantly higher than those on the 1st day (both $p<0.05$, Table III). For group status, post hoc test-

Table I. Distribution of demographic data and clinical characteristics at admission.

Characteristics	Total (n=77)	Moderate (n=12)	Severe (n=42)	Critical Living (n=14)	Critical Death (n=9)	χ^2/F	*p*-value (dead *vs.* Survival)
Age, year, median, (IQR)	59 (35-87)	56 (44-68)	63.5 (36-87)	56 (36-70)	52 (39-70)	3.002	0.036
Male, No. (%)	41 (53.2)	6/12 (50)	22/42 (52.3)	10/14 (71.4)	9/9 (100)	11.444	0.010
Smoking, No. (%)	9/77 (11.7)	1/12 (8.3)	3/42 (7.14)	3/14 (21.4)	2/9 (22.2)	2.961	0.398
Infection at admission, No. (%)	35/77 (45.5)	3/12 (25.0)	17/42 (40.5)	8/14 (57.1)	7/9 (77.8)	7.264	0.064
Comorbidities, No. (%)							
Hypertension	23/77 (29.9)	3/12 (25.0)	13/42 (31.0)	4/14 (28.6)	3/9 (30.0)	1.19	0.757
Diabetes	9/77 (11.7)	1/12 (8.33)	6/42 (14.3)	1/14 (7.14)	1/9 (11.1)	0.73	0.867
Cardiovascular disease	6/77 (7.80)	0/12 (0)	5/42 (11.9)	1/14 (7.14)	0/9 (0)	4.28	0.233
Chronic obstructive pulmonary disease	6/77 (7.80)	1/12 (8.33)	3/42 (7.14)	1/14 (7.14)	1/9 (11.1)	0.16	0.984
Symptoms							
Fever	58/77 (75.3)	8/12 (66.7)	33/42 (78.6)	10/14 (71.4)	7/9 (77.8)	0.84	0.840
Dry cough	25/77 (32.5)	4/12 (33.3)	13/42 (31.0)	7/14 (50)	1/9 (11.1)	4.14	0.247
Expectoration	16/77 (20.8)	0/12 (0)	5/42 (11.9)	4/14 (28.6)	7/9 (77.8)	21.75	0.000
Fatigue	9/77 (11.7)	2/12 (16.7)	0/42 (0)	3/14 (21.4)	4/9 (44.4)	17.82	0.000
Dyspnea/Chest tightness	8/77 (10.4)	0/12 (0)	1/42 (2.38)	1/14 (7.14)	6/9 (66.7)	23.26	0.000
Diarrhea	3/77 (3.87)	0/12 (0)	3/42 (7.14)	0/14 (0)	0/9 (0)	3.74	0.291
Anorexia/Nausea	2/77 (2.69)	0/12 (0)	1/42 (2.38)	1/14 (7.14)	0/9 (0)	1.89	0.595
Dizziness/Headache	3/77 (3.87)	0/12 (0)	1/42 (2.38)	1/14 (7.14)	1/9 (11.1)	2.42	0.490
Vital signs							
Heart rate, median (IQR), (bpm)	80 (66-102)	73 (68-85)	80 (66-96)	80 (75-92)	85 (76-102)	14.76	0.002
SBP, median (IQR), (mmHg)	20 (98-156)	115 (98-140)	120 (100-150)	110 (104-145)	138 (118-156)	5.02	0.003
DBP, median (IQR), (mmHg)	120 (64-92)	71 (64-85)	75.5 (66-92)	70 (68-92)	76 (70-88)	0.71	0.551
Respiration rate, median (IQR), (times per min)	72 (16-25)	19 (17-21)	20 (16-24)	20 (19-23)	21 (19-25)	13.11	0.004

IQR, interquartile range; SBP, systolic blood pressure; DBP, diastolic blood pressure.

ing demonstrated that $CD4^+$ T cell counts in the moderately ill group were significantly higher than those in the other three groups. $CD8^+$ T cell counts in the moderately ill group were higher than those in the critically ill survivors. LYMPH in the moderately ill group was higher than that of the severely ill and critically ill survivor groups. However, CRP levels among the mortality group were significantly higher than those of the moderately ill and severe-ill groups. Among the critically ill survivors, CRP levels were higher than those in the moderately ill group (all $p<0.05$, Table IV) (Figure 2).

Regression Analysis

Considering the effect of pro-inflammatory on the prognosis of severely ill and critically ill survivors, stepwise linear regression was performed for IL-6 levels on the 14th day as a dependent variable with all laboratory findings on the 1st day as independent variables. IL-6 level was a significant contributor (F=4.90, p=0.034, β=0.35, 95% CI: 0.00-0.10, adjustment R^2=0.10). Binary logistic regression revealed that IL-6 level on the 1st day was significantly associated with death (p=0.028, β=0.05, OR: 1.05, 95% CI: 1.01-1.10). The Cox regression model for each laboratory finding was performed in the critically ill group. It revealed that $CD4^+$ T cell counts on the 1st day decreased the hazard of dying (p=0.039, β=-0.01, hazard ratio=0.99, 95% CI: 0.98-1.00, and median survival time, 13.5 days).

Discussion

This study was conducted to determine the associations of abnormal inflammatory and immune responses with outcomes of patients with COVID-19 infection. The primary finding was that abnormal inflammatory and immune responses in severely and critically ill patients de-

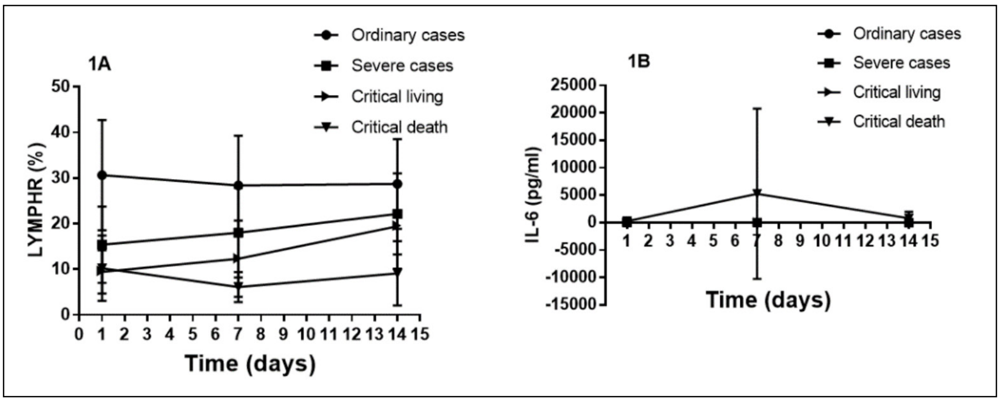

Figure 1. Simple effect testing shows differences and changes in lymphocyte ratio (LYMPHR) (**A**) and interleukin 6 (IL-6) (**B**) levels among four groups at three-time points.

creased the risk of death. High IL-6 levels on day 1 were associated with adverse outcomes and CD4+ T count on day 1. Previous studies[13,14] showed that IL-6 level plays a key role in the inflammatory cascade as an early responder. IL-6 was a reflection of the degree of systemic inflam-

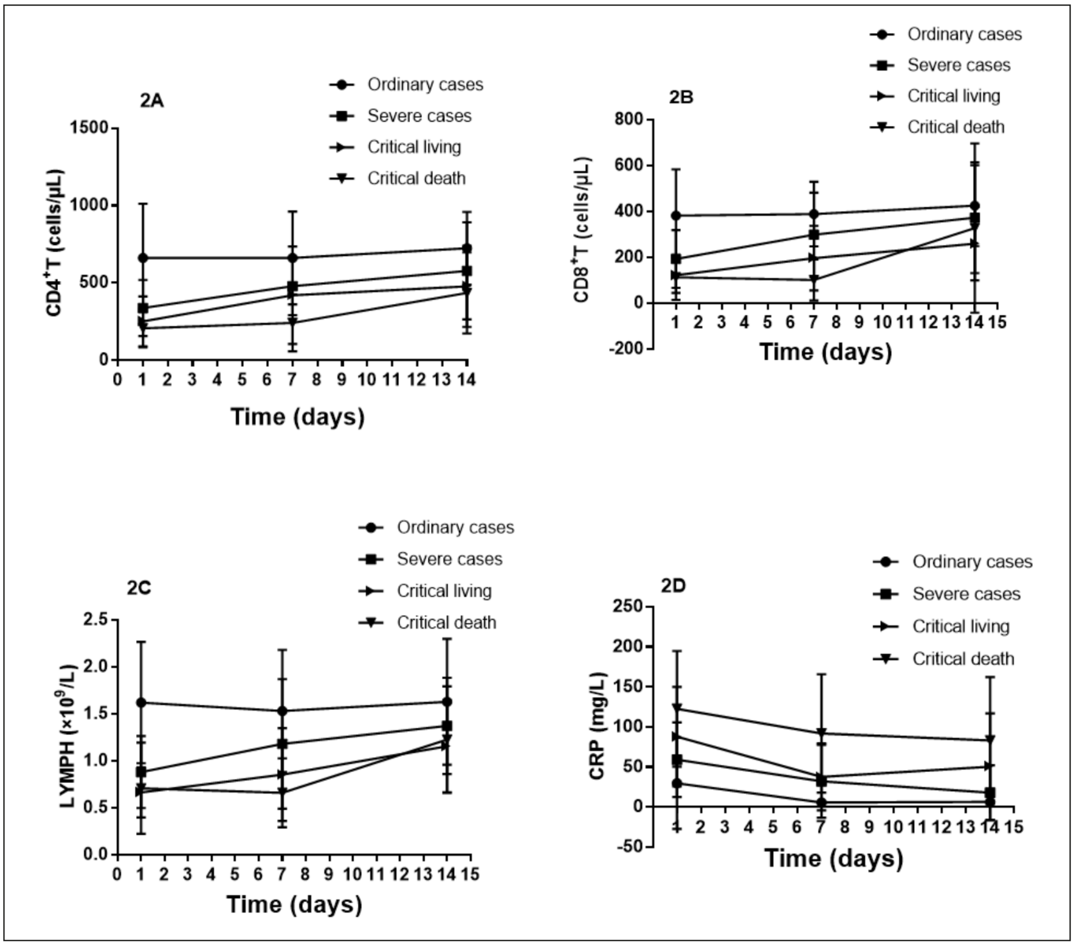

Figure 2. Post-hoc testing shows differences and changes in CD4+ (**A**) and CD8+ (**B**) T cell counts, lymphocyte counts (LYMPH) (**C**), and C-reactive protein (CRP) (**D**) levels among four groups at three-time points.

Table II. Changes in laboratory findings of four groups at 3 time-point using repeated measures ANOVA.

						Time × group	Time	Group
	Normal range		On the 1st day	On the 7th day	On the 14th day	F_1(df), p_1, $\eta^{2a}_{p\,1}$	F_2(df), p_2, $\eta^{2a}_{p\,2}$	F3(df),p_3, $\eta^{2a}_{p\,3}$
LYMPHR	20-50	Moderate group	30.63±12.09	28.38±10.89	28.74±9.86	2.34 (6, 124)	2.63 (2, 124)	10.32 (3, 62)
		Severe group	15.40±8.39	18.03±9.83	22.13±8.89	0.035	0.080	0.000
		Critical survival group	10.05±1.46	13.14±2.60	18.81±2.86	0.10		0.33
		Critical death group	14.15±4.65	7.58±1.54	10.28±3.78			
		Moderate group	6.40±8.50	4.25±2.99	4.06±5.08	5.99 (3, 60)	10.42 (1, 60)	6.83 (3, 60)
IL-6 (pg/ml)	<10	Severe group	23.16±46.22	16.893±30.979	7.99±7.54	0.001	0.002	0.000
		Critical survival group	27.12±9.48	17.56±4.51	23.23±10.12	0.23	0.15	0.26
		Critical death group	325.66±192.10	11,692.82±11,642.86	790.65±600.55			
		Moderate group	661.83±350.06	661.90±300.33	724.43±234.74	0.73 (6, 96)	6.15 (2, 96),	4.60 (3, 48)
CD4+T (cells/uL)	404-1,612	Severe group	338.57±181.29	479.03±188.21	577.53±314.57	0.63	0.003	0.007
		Critical survival group	291.82±48.09	462.27±98.35	490.55±81.76		0.11	0.22
		Critical death group	251.00±51.36	248.00±59.42	444.00±151.57			
CD8+T (cells/uL)	220-1,219	Moderate group	382.50±201.80	389.90±141.22	425.86±176.80	1.88 (6, 96)	8.84 (2, 96)	3.55 (3, 48)
		Severe group	193.83±126.19	299.81±183.76	373.73±241.31	0.09	0.001	0.021
		Critical survival group	136.55±24.69	217.82±43.58	271.73±49.32		0.16	0.18
		Critical death group	139.25±61.99	97.75±40.18	336.25±212.87			
LYMPH (×10⁹/L)	1.1-3.2	Moderate group	1.62±0.65	1.53±0.65	1.63±0.67	1.58 (5, 106)	6.42 (2, 106)	4.43 (3, 62)
		Severe group	0.88±0.38	1.18 ±0.69	1.37 ±0.51	0.17	0.004	0.007
		Critical survival group	0.71±0.08	0.96±0.14	1.16±0.16		0.09	0.18
		Critical death group	0.94±0.32	0.68±0.12	1.10±0.28			
CRP (mg/L)	0-10	Moderate group	29.51±57.16	5.64±1.96	6.47±4.29	0.58 (6, 124)	7.72 (2, 124)	6.29 (3, 62)
		Severe group	59.24±46.69	32.12 ±45.43	17.94±34.42		0.001	0.001
		Critical survival group	92.98±20.12	33.53±13.39	54.38±20.61		0.11	0.23
		Critical death group	83.78±40.03	66.30±45.46	91.18±44.57			
PCT(ng/ml)	<0.1	Moderate group	0.09±0.16	0.04±0.03	0.05±0.03	1.80 (4, 81)	1.83 (1, 81)	0.84 (3, 63)
		Severe group	0.20±0.39	0.43±2.31	0.05±0.03	0.139	0.178	0.477
		Critical survival group	0.16±0.04	0.52±0.20	0.31±0.14			
		Critical death group	0.10±0.04	0.14±0.07	2.17±1.98			
CD4+T /CD8+T	0.9-2.0	Moderate group	1.90±0.76	3.23±4.49	6.50±12.25	0.52 (4, 64)	1.18 (1, 640)	0.33 (3, 48)
		Severe group	2.26±1.74	3.30±5.46	4.87±8.84	0.72	0.298	0.802
		Critical survival group	2.59±0.61	2.52±0.44	2.09±0.25			
		Critical death group	2.58±0.86	3.37±0.98	3.92±2.44			

[a]Partial Eta Squared η^2_p: effect size (0.01-0.08 is small, 0.09-0.24 is medium, and 0.25+ is large). LYMPH, Lymphocyte count; LYMPHR, Lymphocyte ratio; PCT, procalcitonin; Interleukin-6, IL6; C-reactive protein, CRP.

Table III. Result of post-hoc comparisons for time status.

Items	(i)Time	(j)Time	Mean difference(i-j)	Stand error	p	95% CI for the difference	
						Lower bond	Upper bond
CD4+T (cells/uL)	1st day	7th day	-93.82	37.82	0.050	-187.64	0.001
		14th day	-161.23*	47.99	0.005	-280.27	-42.19
	7th day	14th day	-67.41	51.63	0.594	-195.49	60.68
CD8+T (cells/uL)	1st day	7th day	-25.91	22.36	0.757	-81.36	29.55
		14th day	-108.18*	30.35	0.003	-183.46	-32.89
	7th day	14th day	-82.27*	27.28	0.012	-149.95	-14.59
LYMPH ($\times 10^9$/L)	1st day	7th day	-0.07	0.10	1	-0.31	0.16
		14th day	-.310*	0.07	0.000	-0.48	-0.14
	7th day	14th day	-0.24	0.10	0.07	-0.49	0.01
CRP (mg/L)	1st day	7th day	37.93*	10.73	0.002	11.52	64.34
		14th day	32.71*	11.37	0.016	4.74	60.68
	7th day	14th day	-5.22	9.17	1	-27.78	17.34

LYMPH, Lymphocyte count; C-reactive protein, CRP; confidence interval, CI. *means $p < 0.05$.

Table IV. Result of post-hoc comparisons for group status.

Items	(i) Group	(j) Group	Mean difference (i-j)	Stand error	p	95% CI for the difference	
						Lower bond	Upper bond
CD4+T (cells/uL)	Moderate group	Severe group	248.69*	81.71	0.023	23.81	473.57
		Critical survival group	301.91*	91.97	0.012	48.81	554.99
		Critical death group	354.37*	113.23	0.018	42.77	665.97
	Severe group	Critical survival group	53.21	66.72	1.000	-130.40	236.83
		Critical death group	105.68	93.88	1.000	-152.69	364.05
	Critical survival group	Critical death group	52.47	102.93	1.000	-230.80	335.73
CD8+T (cells/uL)	Moderate group	Severe group	143.17	66.40	0.217	-39.56	325.89
		Critical survival group	230.27*	74.73	0.020	24.62	435.92
		Critical death group	223.38	92.00	0.114	-29.81	476.57
	Severe group	Critical survival group	87.10	54.21	0.688	-62.09	236.30
		Critical death group	80.21	76.28	1.000	-129.72	290.15
	Critical survival group	Critical death group	-6.89	83.63	1.000	-237.05	223.27
LYMPH ($\times 10^9$/L)	Moderate group	Severe group	0.47*	0.16	0.034	0.02	0.91
		Critical survival group	0.68*	0.19	0.005	0.15	1.21
		Critical death group	0.61	0.25	0.099	-0.06	1.28
	Severe group	Critical survival group	0.21	0.15	0.883	-0.18	0.61
		Critical death group	0.14	0.21	1.000	-0.43	0.71
	Critical survival group	Critical death group	-0.07	0.23	1.000	-0.71	0.57
CRP (mg/L)	Moderate group	Severe group	-20.23	11.45	0.493	-51.44	10.9701
		Critical survival group	-42.08*	13.68	0.019	-79.38	-4.79
		Critical death group	-65.35*	17.31	0.002	-112.52	-18.17
	Severe group	Critical survival group	-21.85	10.21	0.218	-49.69	5.99
		Critical death group	-45.11*	14.72	0.019	-85.23	-4.99
	Critical survival group	Critical death group	-23.26	16.52	0.98	-21.76	68.28

LYMPH, Lymphocyte count; C-reactive protein, CRP; confidence interval, CI. *means $p < 0.05$.

matory response. The overflow of inflammatory cytokines in the circulatory system may lead to a systemic cytokine storm, resulting in damage to multiple organ functions. In line with the current study, several studies[15] demonstrated that IL-6 is an independent predictor of outcome and in-hospital mortality[16-18] with the highest diagnostic value for infection[19]. The optimal cut-off point for predicting death is serum IL-6>229 pg/mL on admission[14].

In our study, follow-up IL-6 levels were measured at three-time points. Significant decreases were seen in the survivor group, but sharp increases were seen among those who died.

IL-6 promotes the differentiation of naïve $CD4^+$ T lymphocytes to perform an important function in acquired immune responses, and $CD4^+$ T cell is the central cell of the immune system and an effector cell that inhibits viral replication[20]. Lower levels of $CD4^+$ T cells and lack of early antiviral therapy have been considered independent risk factors for severe disease, according to a report[21]. Similar to human immunodeficiency virus infection, SARS-CoV-2 infection has a strong association of survival with initial $CD4^+$ T cell counts. Lower CD4 counts correlated with shorter survival times[22,23].

We also found that $CD8^+$ T cells, LYMPH, and LYMPHR dropped out of the normal range in severely and critically ill patients on admission and progressively increased to the normal range by the end of two weeks, except in patients who died. $CD8^+$ T cell responses are critical for controlling viral infection[24], and their responses aid viral clearance by direct killing infected cells[25]. The differentiation of $CD8^+$ T cells is induced by cytotoxic T cells to kill infected cells[26]. An early study[27] showed that high $CD8^+$ T cell counts correlated significantly with survival from infectious diseases. The depletion of $CD8^+$ T cells often suggests[28] enhanced viral infection as manifested by increased viral replication and lethality. Severe lymphopenia has been observed[4] in COVID-19 pneumonia[4]. Lymphopenia is typically associated with various infections directly as a result of immune suppression because of the underlying disease[29]. Persistent lymphopenia after admission is associated with a 1.7-fold increased risk of mortality[30]. Both $CD4^+$ and $CD8^+$ T cells were affected severely, suggesting that a severe drop in the number of circulating lymphocytes, especially constantly declining in the death group, might be the reason for the decrease of LYMPHR.

Other findings of the current study included markedly high CRP levels in severely and critically ill patients at admission that progressively decreased with improvement except for the patients in the critically ill mortality group. Their subsequent increase of CRP levels returned to the initial levels. A previous study[31] suggested that CRP levels are positively correlated with the degree of inflammation, and the concentration is not affected by age, sex, or physical condition. Elevated CRP concentrations were found in viral respiratory infection[32], and in patients with severe pneumonia[33]. The present study likewise showed that CRP levels continued to elevate in the critically ill mortality group and decreased among the survivors, even in the critically ill survivor group.

Limitations

There were some limitations to the present study. First, it is difficult to evaluate risk factors for disease severity and mortality with multivariable-adjusted methods because of the limited number of cases. It is necessary to explore a larger cohort to further define the clinical presentation and risk factors. Second, even though the causative pathogen has been identified, laboratory testing was not available. This could have provided more information regarding the characteristics of COVID-19. Finally, although we found a low fever ratio, similar to another study[34] (32169119), this was different from the findings of other studies[10,35]. Furthermore, no female deaths were reported in our cohort, and this should be considered a potential exposure bias in this study. More effort should be made to answer these questions in future studies.

Conclusions

The relationship between SARS-CoV-2 infection and abnormal inflammatory and immune responses is complex. The current study revealed that IL-6 levels and $CD4^+$ T cell counts at admission help predict the outcome of COVID-19 infection, especially in critically ill patients. Our findings suggest that the measurement of IL-6 levels and $CD4^+$ T cell counts should be monitored after admission, especially in severely ill and critically ill patients with COVID-19 infection.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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None.

Informed Consent

Patients and/or their families signed informed consent forms.

Authors' Contributions

FW, QM, and YL designed this study. FW, QM, and YL provided funding. FW revised the manuscript. QM and HY finished the manuscript and analyzed the data. QM, HY, KW, and KL collected the clinical data. JB contributed to the literature search.

Data Availability

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics Approval

The present study was approved by the Medical Ethics Committee of the People's Hospital of Wuhan University with the exception of the requirement of informed consent (WDRY2020-K120).

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