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JCI Insight. 2020;5(13):e139024. <https://doi.org/10.1172/jci.insight.139024>.

Clinical Medicine

Infectious disease

Pulmonology

Fatal cases of COVID-19 are increasing globally. We retrospectively investigated the potential of immunologic parameters as early predictors of COVID-19.

A total of 1018 patients with confirmed COVID-19 were enrolled in our 2-center retrospective study. Clinical feature, laboratory test, immunological test, radiological findings, and outcomes data were collected. Univariate and multivariable logistic regression analyses were performed to evaluate factors associated with in-hospital mortality. Receiver operator characteristic (ROC) curves and survival curves were plotted to evaluate their clinical utility.

The counts of all T lymphocyte subsets were markedly lower in nonsurvivors than in survivors, especially CD8⁺ T cells. Among all tested cytokines, IL-6 was elevated most significantly, with an upward trend of more than 10-fold. Using multivariate logistic regression analysis, IL-6 levels of more than 20 pg/mL and CD8⁺ T cell counts of less than 165 cells/ μ L were found to be associated with in-hospital mortality after adjusting for confounding factors. Groups with IL-6 levels of more than 20 pg/mL and CD8⁺ T cell counts of less than 165 cells/ μ L had a higher percentage of older and male patients as well as a higher proportion of patients with comorbidities, ventilation, intensive [...]

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IL-6 and CD8⁺ T cell counts combined are an early predictor of in-hospital mortality of patients with COVID-19

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BACKGROUND. Fatal cases of COVID-19 are increasing globally. We retrospectively investigated the potential of immunologic parameters as early predictors of COVID-19.

METHODS. A total of 1018 patients with confirmed COVID-19 were enrolled in our 2-center retrospective study. Clinical feature, laboratory test, immunological test, radiological findings, and outcomes data were collected. Univariate and multivariable logistic regression analyses were performed to evaluate factors associated with in-hospital mortality. Receiver operator characteristic (ROC) curves and survival curves were plotted to evaluate their clinical utility.

RESULTS. The counts of all T lymphocyte subsets were markedly lower in nonsurvivors than in survivors, especially CD8⁺ T cells. Among all tested cytokines, IL-6 was elevated most significantly, with an upward trend of more than 10-fold. Using multivariate logistic regression analysis, IL-6 levels of more than 20 pg/mL and CD8⁺ T cell counts of less than 165 cells/ μ L were found to be associated with in-hospital mortality after adjusting for confounding factors. Groups with IL-6 levels of more than 20 pg/mL and CD8⁺ T cell counts of less than 165 cells/ μ L had a higher percentage of older and male patients as well as a higher proportion of patients with comorbidities, ventilation, intensive care unit admission, shock, and death. Furthermore, the receiver operating curve of the model combining IL-6 (>20 pg/mL) and CD8⁺ T cell counts (<165 cells/ μ L) displayed a more favorable discrimination than that of the CURB-65 score. The Hosmer-Lemeshow test showed a good fit of the model, with no statistical significance.

CONCLUSION. IL-6 (>20 pg/mL) and CD8⁺ T cell counts (<165 cells/ μ L) are 2 reliable prognostic indicators that accurately stratify patients into risk categories and predict COVID-19 mortality.

FUNDING. This work was supported by funding from the National Natural Science Foundation of China (no. 81772477 and 81201848).

Introduction

Over the past 3 months, coronavirus disease 2019 (COVID-19), caused by severe acute respiratory coronavirus 2 (SARS-CoV-2), has quickly spread around the world. As of April 9, 2020, there have been 1,436,198 confirmed cases of COVID-19 and 85,522 deaths globally. With the global epidemic worsening, additional fatalities will occur. An initial study of 41 confirmed COVID-19 cases indicated that 63% of patients had decreased lymphocytes, and various cytokines could be associated with disease severity (1). Chen et al. described the immunologic features of severe and moderate COVID-19 and found that SARS-CoV-2 infection might affect CD4⁺ T and CD8⁺ T cell numbers (2). Our previous studies as well as others have shown that severe COVID-19 cases had high levels of IL-2R, IL-6, IL-10, and TNF- α compared with moderate cases (2, 3). A recent multicenter retrospective study of 150 confirmed COVID-19 cases in Wuhan, China, confirmed that IL-6 is significantly increased in nonsurvivors (4). Wan et al. discovered a positive relationship between CD4⁺ T cells and CD8⁺ T cells, IL-6, and IL-10 in a mild group, but not in a group of patients with severe COVID-19 (5). We recruited a larger cohort to further explore the association between immunologic features (including cytokines and lymphocyte subsets) and in-hospital mortality of patients with COVID-19. In this study, we aimed to conduct a 2-center retrospective study to systematically investigate the potential prognostic roles of immunological parameters in patients with COVID-19.

Authorship note: SW and HL contributed equally to this work.

Conflict of interest: The authors have declared that no conflict of interest exists.

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Submitted: April 13, 2020

Accepted: June 3, 2020

Published: June 16, 2020.

Reference information: *JCI Insight*. 2020;5(13):e139024.
<https://doi.org/10.1172/jci.insight.139024>.

Table 1. Sociodemographic and clinical characteristics of patients with COVID-19 in this cohort

	All patients (n = 1018)	Survivors (n = 817)	Nonsurvivors (n = 201)
Sociodemographic			
Age in years, median (IQR)	61.00 (49.00–69.00)	57.00 (46.00–66.00)	69.00 (62.00–78.00)
Male/female, n (%)	521 (51.2)/497 (48.8)	388 (47.5)/429 (52.5)	133 (66.2)/68 (33.8)
Comorbidities			
Hypertension, n (%)	365 (35.9)	256 (31.3)	109 (54.2)
Coronary heart disease, n (%)	83 (8.2)	53 (6.5)	30 (14.9)
Diabetes mellitus, n (%)	164 (16.1)	115 (14.1)	49 (24.4)
Underlying pulmonary diseases ^A , n (%)	56 (5.5)	38 (4.7)	18 (9.0)
Vital signs and scores			
Temperature in °C, median (IQR)	36.70 (36.50–37.30)	36.70 (36.40–37.20)	36.80 (36.50–37.50)
Heart rate in bpm, median (IQR)	88.00 (80.00–102.00)	88.00 (80.00–100.00)	94.00 (81.50–108.00)
RR in breaths/min, median (IQR)	20.00 (20.00–24.00)	20.00 (20.00–22.00)	25.00 (20.00–32.00)
MAP in mmHg, median (IQR)	96.33 (88.33–104.22)	96.00 (88.33–103.66)	98.00 (88.50–105.00)
SOFA score, median (IQR)	0.00 (0.00–3.00)	0.00 (0.00–1.00)	4.00 (3.00–4.00)
Types of COVID-19			
Mild/normal, n (%)	645 (63.4)	645 (78.9)	0
Severe, n (%)	164 (16.1)	152 (18.6)	12 (6.0)
Critical, n (%)	209 (20.5)	20 (2.5)	189 (94.0)
Outcomes			
NPPV, n (%)	228 (22.4)	80 (9.8)	148 (73.6)
IMV, n (%)	130 (12.8)	5 (0.6)	125 (62.2)
ICU, n (%)	173 (17.0)	18 (2.2)	155 (77.1)
Shock, n (%)	163 (16.0)	2 (0.2)	161 (80.1)
Hospital stay time in days, median (IQR)	14.00 (8.00–22.00)	14.00 (9.00–22.00)	11.00 (5.50–19.00)
Time of onset to discharge or death in days, median (IQR)	29.00 (21.00–36.00)	30.00 (22.00–37.00)	25.00 (17.00–33.00)

Data are presented as medians (interquartile range [IQR]) or n (%). ^AUnderlying pulmonary disease includes chronic obstructive pulmonary disease, asthma, bronchiectasis, and tuberculosis, etc. COVID-19, coronavirus disease 2019; MAP, mean arterial pressure; SOFA, Sequential Organ Failure Assessment; NPPV, noninvasive positive pressure ventilation; IMV, invasive mechanical ventilation; ICU, intensive care unit.

Results

T lymphocyte subset counts and serum levels of cytokines in surviving and deceased patients with COVID-19. Sociodemographic and clinical characteristics of patients with COVID-19 in this cohort are shown in Table 1. We detected T lymphocyte subsets and plasma cytokine levels in enrolled patients. The counts of all T lymphocyte subgroups were markedly lower in the nonsurvivor group than in the survivor group ($P < 0.001$), especially in CD8⁺ T cells with less than half the count (96.89 vs. 203.98 cells/ μ L, $P < 0.001$) (Table 2). The serum levels of all tested cytokines on admission, including IL-2R, IL-6, IL-8, IL-10, and TNF- α , were significantly higher in the nonsurvivor group ($P < 0.001$). Among them, IL-6 was elevated most significantly, with an upward trend of more than 10 times (56.16 vs. 5.36 pg/mL, $P < 0.001$). As shown in Figure 1A, the area under curve (AUC) derived from CD8⁺ T cells was much larger than that derived from CD3⁺ cells or CD4⁺ cells (AUC_{CD8⁺} = 0.832 [0.804–0.861] vs. AUC_{CD3⁺} = 0.758 [0.726–0.791] or AUC_{CD4⁺} = 0.721 [0.686–0.755], $P < 0.001$). As shown in Figure 1B, the AUC of IL-6 (0.899 [0.878–0.920]) was larger than that of other cytokines tested ($P < 0.001$), such as IL-2R (0.848 [0.816–0.879]), IL-8 (0.820 [0.787–0.854]), IL-10 (0.748 [0.704–0.791]), and TNF- α (0.763 [0.723–0.804]). Therefore, we assumed that CD8⁺ T cell counts and IL-6 are the 2 most important indicators associated with in-hospital mortality among all the tested immunologic parameters, including T cell subsets and cytokines. Additionally, we also investigated the correlation between CD8⁺ T cell counts and inflammatory status. Our results showed that plasma IL-6 levels in patients with COVID-19 were positively correlated with plasma C-reactive protein (CRP) ($R^2 = 0.424$, $P < 0.001$) (Figure 2A). A significant negative correlation was found between CD8⁺ T cell counts and IL-6 levels ($R^2 = 0.255$, $P < 0.001$); (Figure 2B). The plasma CRP levels in patients with COVID-19 were negatively correlated with CD8⁺ T cell counts ($R^2 = 0.294$, $P < 0.001$); (Figure 2C). These findings showed that CD8⁺ T cell counts were negatively correlated with the inflammatory indicators of CRP and IL-6.

Table 2. Comparisons of T cell subset count and cytokines levels between patients with COVID-19 in the survival and nonsurvival groups

Variables	Normal range	Survivors (n = 817), median (IQR)	Nonsurvivors (n = 201), median (IQR)	P value
T cell subset counts				
CD3 ⁺ , cells/ μ l	690–2540	611.01 (420.12–858.10)	39,120 (262.95–504.46)	<0.001
CD4 ⁺ , cells/ μ l	410–1590	367.99 (242.39–543.00)	245,00 (161.64–317.64)	<0.001
CD8 ⁺ , cells/ μ l	190–1140	203.98 (142.54–313.05)	96.89 (60.65–140.08)	<0.001
CD4 ⁺ /CD8 ⁺ ratio	0.71–2.78	1.70 (1.30–2.21)	2.37 (1.77–3.36)	<0.001
Cytokines				
IL-2R, U/mL	223–710	464.60 (294.90–673.04)	1063.00 (726.62–1483.00)	<0.001
IL6, pg/mL	<7.0	5.36 (2.32–15.85)	56.16 (25.53–136.75)	<0.001
IL8, pg/mL	<62	12.31 (8.10–18.80)	29.90 (17.90–57.53)	<0.001
IL10, pg/mL	<9.1	5.25 (5.00–6.80)	9.10 (5.79–16.00)	<0.001
TNF- α , pg/mL	<8.1	6.90 (5.68–8.43)	10.30 (7.34–17.35)	<0.001

P values were derived from Mann-Whitney U test. COVID-19, coronavirus disease 2019; IQR, interquartile range.

IL-6 elevation and CD8⁺ T cell count reduction were correlated with in-hospital mortality in COVID-19. Univariate logistic regression analysis was performed to investigate the risk factors associated with in-hospital mortality in patients with COVID-19. On the basis of the receiver operator characteristic (ROC) curve for CD8⁺ T cells, the cutoff value was defined from Youden's index as 165 cells/ μ L. The count of CD8⁺ T cells below 165 cells/ μ L was regarded as low. We defined a high concentration of IL-6 to be more than 20 pg/mL, according to a previous study (6). Then, by multivariable logistic regression analysis, 2 indicators were identified to be independent risk factors associated with in-hospital mortality, including IL-6 levels of more than 20 pg/mL (OR = 9.781; 95% CI, 6.304–15.174; $P < 0.001$) and CD8⁺ T cell counts of less than 165 cells/ μ L (OR = 5.930; 95% CI, 3.677–9.562; $P < 0.001$), after adjusting for confounding factors including age, sex, and underlying diseases (hypertension, coronary heart disease, diabetes mellitus, and underlying pulmonary diseases) (Table 3).

Clinical features and outcomes of patients with COVID-19 with IL-6 levels of more than 20 pg/mL and CD8⁺ T cell counts of less than 165 cells/ μ L. We divided the enrolled patients into 4 groups: group I (IL-6 \leq 20 pg/mL and CD8⁺ T cells \geq 165 cells/ μ L, $n = 487$), group II (IL-6 > 20 pg/mL and CD8⁺ T cells \geq 165 cells/ μ L, $n = 98$), group III (CD8⁺ T cells < 165 cells/ μ L and IL-6 \leq 20 pg/mL, $n = 203$), and group IV (CD8⁺ T cells < 165 cells/ μ L and IL-6 > 20 pg/mL, $n = 230$). Table 4 shows sociodemographic data, comorbidities, clinical features, and outcomes of patients among the 4 groups. The median age (interquartile range [IQR]) of patients from group I to group IV was 56.00 (43.00–65.00), 61.00 (50.75–70.00), 62.00 (53.00–69.00), and 68.00 (62.00–77.00), respectively. The proportion of older patients (age \geq 60 years) in group IV exceeded 79.6%. In each group, 216 (44.4%), 55 (56.1%), 96 (47.3%), and 154 (67.0%) participants were male. Group IV had more comorbidities, including hypertension, coronary heart disease, diabetes, and underlying pulmonary diseases, with 122 (53.0%), 28 (12.2%), 49 (21.3%), and 18 (7.8%) patients, respectively. More importantly, more patients from group IV required ventilation (122 [53.0%]), including noninvasive positive pressure ventilation (NPPV) and 94 (40.9%) with invasive mechanical ventilation (IMV). In group IV, 121 patients (52.6%) were admitted to the intensive care unit (ICU) for more intensive monitoring and treatment. In groups I–IV, the number of patients with shock was 6 (1.2%), 20 (20.4%), 21 (10.3%), and 116 (50.4%), respectively. The number of patients who died among the 4 groups was 6 (1.2%), 22 (22.4%), 29 (14.3%), and 144 (62.6%), respectively. However, we observed that the hospitalization time of group IV was not extended more significantly than that of group II or III, as the majority of individuals in group IV were nonsurvivors with shorter survival times.

In addition, in group IV, the white blood cell and neutrophil counts as well as the levels of d-dimer, blood urea nitrogen (BUN), lactate dehydrogenase (LDH), procalcitonin (PCT), and CRP were markedly elevated, while the counts of lymphocytes and platelets were lower (Table 5). There was no significant difference in the concentration of hemoglobin among the 4 groups ($P = 0.335$). In the 4 groups, the vast majority of patients had bilateral lung lesions, with proportions of 88.1% (429–487 patients), 96.9% (95–98 patients), 98.0% (199–203 patients), and 99.1% (228–230 patients), respectively.

Early in-hospital mortality prediction of IL-6 combined with CD8⁺ T cell count in patients with COVID-19. Kaplan-Meier survival curves indicated that groups I–IV had different survival times (Figure 3).

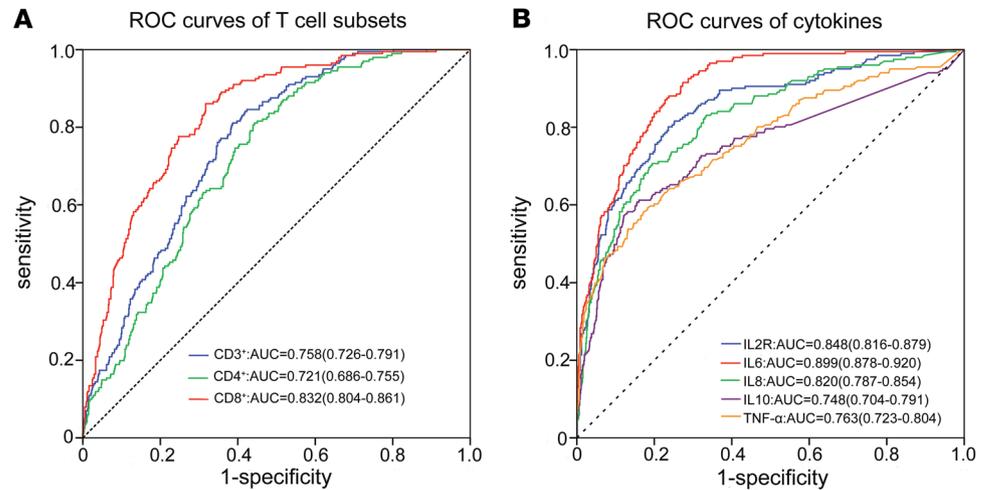


Figure 1. ROC curves of T lymphocyte subsets and cytokines. (A) ROC curves of each category of T lymphocyte subgroup. (B) ROC curves for each category of serum cytokines. AUC, area under the ROC curve; ROC, receiver operating characteristic.

Compared with group I, the patients from the other 3 groups had worse survival times ($P < 0.001$). More importantly, the patients from group IV had a much shorter survival time than those from groups II or III ($P < 0.001$). There was no statistical difference between groups II and III ($P = 0.205$) (Figure 3). Moreover, the ROC curve of the model combining IL-6 elevation and CD8⁺ T cell count reduction had a larger AUC (0.907 [0.886–0.928] vs. 0.843 [0.810–0.877], $P < 0.001$) (Figure 4). The Hosmer-Lemeshow test showed a good fit of the model with no statistical significance ($P = 0.581$), indicating that no statistically significant difference existed between the observed and expected values. Therefore, reduced CD8⁺ T cell counts combined with elevated IL-6 had a good ability to predict in-hospital mortality in patients with COVID-19.

Discussion

A series of previous studies have summarized the clinical features of patients with COVID-19 (1, 7–9). However, studies of the association between immunologic indexes and outcomes of COVID-19 are lacking. This study is the first investigation to our knowledge to discover the role of these 2 indicators, IL-6 elevation and CD8⁺ T cell count reduction, in contributing to the outcome of COVID-19. We also attempted to stratify patients into more accurate prognostic groups. It is very important for clinicians to better understand immunologic dysregulations in fatal cases and provide potential target interventions.

Flow cytometry analysis indicated that CD3⁺, CD4⁺, and CD8⁺ T cell counts were significantly lower in nonsurvivors than in survivors. The absolute count of CD8⁺ T cells was below 100 cells/ μ L in nonsurvivors,

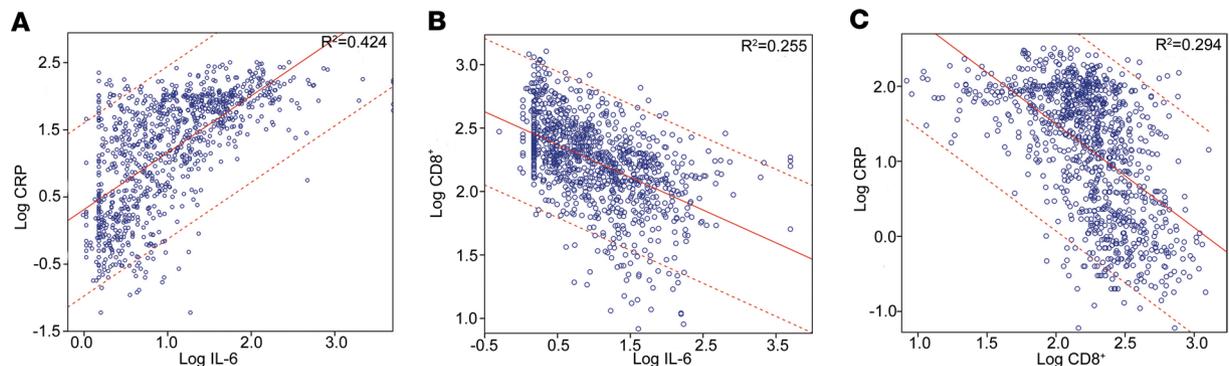


Figure 2. Correlation analysis of indicators of patients with COVID-19. (A) Correlation analysis between plasma IL-6 and CRP levels ($R^2 = 0.424$, $P < 0.001$). (B) Correlation analysis between plasma IL-6 levels and CD8⁺ T cell counts ($R^2 = 0.255$, $P < 0.001$). (C) Correlation analysis between plasma CRP levels and CD8⁺ T cell counts ($R^2 = 0.294$, $P < 0.001$). IL-6, C-reactive protein (CRP), and CD8⁺ T cell counts were collected on admission.

Table 3. Univariable and multivariable logistic regression analysis of mortality-related risks in patients with COVID-19

Variables	Univariate				Multivariate ^A			
	β	OR	CI	<i>P</i> value	β	OR	CI	<i>P</i> value
IL-6 >20 pg/mL	2.954	19.176	12.815–28.696	<0.001	2.280	9.781	6.304–15.174	<0.001
CD8 ⁺ <165 cells/ μ l	2.583	13.326	8.649–20.257	<0.001	1.780	5.930	3.677–9.562	<0.001

^AAdjusting for age, sex, and underlying diseases, including hypertension, coronary heart disease, diabetes mellitus, and underlying pulmonary diseases

which is less than half of the total number of surviving cells. Furthermore, an interesting finding in our study is that the AUC of the CD8⁺ T cell subset was larger than that of the CD3⁺ or CD4⁺ T cell subset. These findings indicated a more obvious change in CD8⁺ T cells in nonsurvivors with COVID-19. Multivariable analysis indicated that lower CD8⁺ T cell counts were an independent mortality-related risk factor in patients with COVID-19 after adjusting for confounding factors, including age, sex, and underlying diseases (hypertension, coronary heart disease, diabetes mellitus, and underlying pulmonary diseases). Therefore, it is reasonable to consider that CD8⁺ T cell count is a more important risk factor for predicting mortality than CD4⁺ T cells or total CD3⁺ T cells in patients with COVID-19. These results also support the need for lymphocyte classification tests and suggest that CD8⁺ T cells are more vulnerable to the effects of infectious patients with SARS-CoV-2. Similarly, the reduction of CD8⁺ T cell counts in our cohort was in agreement with that in other studies that have examined these markers. Chen et al. described the immunologic features in severe and moderate COVID-19 and found that SARS-CoV-2 infection might affect CD4⁺ T and CD8⁺ T cell numbers as well as IFN- γ production (2). A study also indicated that both Th cells and suppressor T cell numbers were significantly decreased in patients with severe COVID-19, while the percentage of naive Th cells was increased (10). Previous studies have found MERS-CoV-specific CD8⁺ T cell responses in most infectious individuals, and the level of T cell response in patients with MERS was related to disease severity (11, 12). In addition, another team discovered a similar phenomenon where depletion of CD8⁺ T cells facilitated hosts at risk of MERS-CoV-induced infection (13). Our results may provide a potential strategy for later therapy targeting CD8⁺ T cell activation in patients with COVID-19.

Studies have shown that the main cause of death of critical viral pneumonia is the excessive inflammatory response triggered by the virus infection (cytokine release syndrome), leading to disease progression, multiple organ dysfunction, and finally death (14–16). Zhou et al. clarified the pathogenic mechanism of the inflammatory storm in patients with severe COVID-19 (17). Pathogenic Th1 cells were found to form and produce human GM-CSF after SARS-CoV-2 infection to construct a cytokine microenvironment, which leads to IL-6 overexpression by inflammatory monocytes. Through a positive feedback mechanism, huge abnormal pathogenic Th1 cells and inflammatory monocytes may enter the pulmonary circulation and play an important role in immune injury, resulting in loss of lung function (17). Consistent with the results of a previous study (3), the levels of IL-2R and IL-6 in the nonsurvivors were statistically higher than those in survivors. The same trend also occurred in TNF- α , IL-8, and IL-10 in our study. This result was documented in another study, which showed that severe COVID-19 cases had higher levels of IL-2R, IL-6, IL-10, and TNF- α than moderate cases (2, 3). Although both IL-8 and IL-10 showed a significant increase, the values did not exceed the upper limit, suggesting their limited value. Our results showed that the AUC of IL-6 was larger than that of IL-2R, IL-8, IL-10, or TNF- α . Furthermore, multivariate analysis indicated that increased levels of IL-6 were an independent risk factor that contributed to mortality in patients. Therefore, IL-6 was another outstanding indicator for predicting mortality in patients with COVID-19. Recently, Zhang et al. documented a case in order to prove that humanized anti-IL-6 receptor antibody (tocilizumab) was effective in the treatment of COVID-19 in multiple myeloma with obvious clinical recovery (18). Another intervention study found that tocilizumab can effectively improve the condition of severe patients with COVID-19. Within 5 days of tocilizumab treatment, 75.0% (15 of 20) of patients had reduced oxygen intake, and CT results of 90.5% (19 of 20) of patients showed significant absorption of lung lesions. The percentage of peripheral blood lymphocytes was decreased in 85.0% (17 of 20) of patients before treatment and returned to normal in 52.6% (10 of 19) of patients on the fifth day. A total of 90.5% (19 of 20) of patients were discharged at an average of 13.5 days after treatment with tocilizumab, and the remainder are recovering well (19). Jacobs et al. retrospectively analyzed 32 patients with COVID-19 treated by

Table 4. Demographic data, clinical features, and outcomes among 4 groups of patients with COVID-19

Variables	Group I (n = 487)	Group II (n = 98)	Group III (n = 203)	Group IV (n = 230)	P value ^A
Increased IL-6	-	+	-	+	
Reduced CD8 ⁺ T cells	-	-	+	+	
Sociodemographic data					
Age in years, median (IQR)	56.00 (43.00–65.00)	61.00 (50.75–70.00)	62.00 (53.00–69.00)	68.00 (62.00–77.00)	<0.001 ^B
18–30, n (%)	37 (7.6)	3 (3.1)	3 (1.5)	1 (0.4)	
30–60, n (%)	281 (57.7)	44 (44.9)	91 (44.8)	46 (20.0)	
60–75, n (%)	143 (29.4)	39 (39.8)	86 (42.4)	121 (52.6)	
75+, n (%)	26 (5.3)	12 (12.2)	23 (11.3)	62 (27.0)	
Male/female, n (%)	216 (44.4)/ 271 (55.6)	55 (56.1)/ 43 (43.9)	96 (47.3)/ 107 (52.7)	154 (67.0)/ 76 (33.0)	<0.001
Comorbidities					
Hypertension, n (%)	128 (26.3)	32 (32.7)	83 (40.9)	122 (53.0)	< 0.001
Coronary heart disease, n (%)	30 (6.2)	9 (9.2)	16 (7.9)	28 (12.2)	0.052
Diabetes, n (%)	56 (11.5)	23 (23.5)	36 (17.7)	49 (21.3)	0.001
Underlying PD ^C , n (%)	20 (4.1)	6 (6.1)	12 (5.9)	18 (7.8)	0.226
Malignancy, n (%)	15 (3.1)	4 (4.1)	7 (3.4)	9 (3.9)	0.876 ^D
Vital signs and score					
Temperature in °C, median (IQR)	36.60 (36.40–37.00)	37.41 (36.70–38.23)	36.60 (36.40–37.00)	36.90 (36.50–37.66)	<0.001 ^B
Heart rate in bpm, median (IQR)	87.00 (79.00–100.00)	94.00 (82.00–107.25)	86.00 (79.00–94.00)	94.00 (82.00–106.00)	<0.001 ^B
RR in breaths/min, median (IQR)	20.00 (20.00–22.00)	20.00 (20.00–22.00)	20.00 (20.00–24.00)	23.00 (20.00–30.00)	<0.001 ^B
MAP in mmHg, median (IQR)	96.67 (89.33–105.00)	96.50 (88.58–103.42)	94.67 (86.67–102.00)	96.17 (88.58–104.33)	0.116 ^B
SOFA score, median (IQR)	0.00 (0.00–1.00)	1.00 (0.00–3.00)	0.00 (0.00–2.00)	3.00 (1.00–4.00)	< 0.001 ^B
Outcomes					
NPPV, n(%)	32 (6.6)	31 (31.6)	43 (21.2)	122 (53.0)	<0.001
IMV, n (%)	3 (0.6)	14 (14.3)	19 (9.4)	94 (40.9)	<0.001
ICU, n (%)	8 (1.6)	19 (19.4)	25 (12.3)	121 (52.6)	<0.001
Shock, n (%)	6 (1.2)	20 (20.4)	21 (10.3)	116 (50.4)	<0.001
Death, n (%)	6 (1.2)	22 (22.4)	29 (14.3)	144 (62.6)	<0.001
Hospital stay time in days, median (IQR)	12.00 (7.00–20.00)	20.00 (12.00–29.00)	15.00 (10.00–22.00)	15.00 (8.75–22.00)	<0.001 ^B
Time of onset to discharge or death in days, median (IQR)	31.00 (21.00–38.00)	30.00 (22.00–36.40)	27.00 (21.00–36.00)	26.00 (18.00–34.00)	<0.001 ^E

Data are presented as medians (interquartile range [IQR]) or n (%). ^AP values derived from χ^2 test, unless otherwise specified. ^BP values derived from Kruskal-Wallis test. ^CUnderlying pulmonary disease includes chronic obstructive pulmonary disease, asthma, bronchiectasis, and tuberculosis, etc. ^DP values derived from Fisher's exact test. ^EP values derived from log-rank test. RR, respiratory rate; MAP, mean arterial pressure; NPPV, noninvasive positive pressure ventilation; IMV, invasive mechanical ventilation; ICU, intensive care unit.

extracorporeal membrane oxygenation and found that 2 of the 5 survivors had received anti-IL-6 receptor monoclonal antibodies (tocilizumab or sarilumab) (20). In the future, targeting IL-6 or IL-6 receptors may be a promising therapy option for critically ill patients with COVID-19.

In this cohort, individuals were divided into 4 groups according to the level of IL-6 and CD8⁺ T cell counts. Median age in different sections, comorbidities (hypertension and diabetes mellitus, and vital signs (temperature, heart rate, and respiratory rate) were significantly different among the 4 groups. The patients from group IV had higher white blood cell, neutrophil, d-dimer, LDH, and SOFA scores as well as lower lymphocytes and platelets. These indicators were confirmed to be associated with the death of patients with COVID-19 in recent studies (9, 21). The proportion of patients who had ventilation (NPPV and IMV), ICU admission, shock, and death in group IV was significantly higher than those in any other group. The time of hospital stay or time of onset to discharge or death in group IV was not shown to be longer than that of any other group. More importantly, the patients from group IV had much worse survival than those in the

Table 5. Laboratory results and radiological findings among 4 groups of patients with COVID-19

Variables	Normal range	Group I (n = 487)	Group II (n = 98)	Group III (n = 203)	Group IV (n = 230)	P value ^A
Increased IL-6		-	+	-	+	
Reduced CD8 ⁺ T cells		-	-	+	+	
Laboratory results						
White blood cell count in $\times 10^9/L$, median (IQR)	3.50–9.50	5.50 (4.29–6.96)	6.73 (4.96–9.69)	5.66 (4.20–7.78)	8.24 (5.07–11.74)	<0.001
Neutrophil count in $\times 10^9/L$, median (IQR)	1.80–6.30	3.37 (2.47–4.53)	5.11 (3.80–7.94)	4.20 (2.29–6.30)	7.25 (3.92–10.74)	<0.001
Lymphocyte count in $\times 10^9/L$, median (IQR)	1.10–3.20	1.38 (1.00–1.87)	0.91 (0.61–1.21)	0.80 (0.57–1.14)	0.64 (0.44–0.83)	<0.001
Red blood cell count in $\times 10^9/L$, median (IQR)	4.30–5.80	4.25 (3.89–4.63)	4.24 (3.81–4.57)	4.17 (3.80–4.55)	4.10 (3.65–4.50)	0.010
Hemoglobin in g/L, median (IQR)	130.00–175.00	128.00 (118.00–142.00)	132.00 (118.50–140.00)	126.40 (115.00–138.00)	127.50 (115.75–139.25)	0.355
Platelet count in $\times 10^9/L$, median (IQR)	125.00–350.00	216.00 (168.00–272.00)	196.00 (142.75–266.25)	205.00 (152.00–262.00)	161.00 (120.00–208.00)	<0.001
APTT in seconds, median (IQR)	29.00–42.00	37.00 (34.26–40.46)	41.70 (37.77–44.90)	36.60 (33.00–40.32)	40.05 (35.95–45.63)	<0.001
PT in seconds, median (IQR)	14.00–19.00	13.30 (12.60–14.00)	13.90 (13.20–15.03)	13.50 (12.80–14.30)	14.80 (13.70–16.63)	<0.001
D-dimer in mg/L, median (IQR)	<0.50	0.36 (0.22–0.72)	0.82 (0.43–2.01)	0.66 (0.31–1.73)	1.79 (0.68–12.28)	<0.001
ALT in U/L, median (IQR)	≤ 40.00	20.00 (13.00–34.00)	27.00 (18.75–49.25)	24.00 (15.00–39.00)	28.00 (17.00–44.00)	<0.001
AST in U/L, median (IQR)	≤ 40.00	21.00 (17.00–29.00)	37.00 (23.75–58.25)	27.00 (19.00–42.00)	36.50 (25.75–58.00)	<0.001
TP in g/L, median (IQR)	64.00–83.00	66.40 (61.70–71.30)	67.35 (62.08–73.05)	64.30 (58.90–69.90)	63.75 (58.05–69.00)	<0.001
ALB in g/L, median (IQR)	35.00–52.00	39.30 (36.50–42.20)	33.70 (31.45–36.63)	35.20 (32.50–38.30)	31.60 (28.58–34.70)	<0.001
TBil in $\mu\text{mol/L}$, median (IQR)	≤ 26.00	8.39 (6.20–11.54)	10.35 (6.59–15.15)	9.05 (6.60–11.80)	11.71 (8.60–17.83)	<0.001
BUN in mmol/L, median (IQR)	3.60–9.50	4.31 (3.31–5.30)	4.87 (3.50–6.31)	5.23 (3.80–6.97)	7.25 (4.80–10.85)	<0.001
SCr in $\mu\text{mol/L}$, median (IQR)	59.00–104.00	64.00 (53.00–77.00)	72.00 (56.00–89.00)	68.00 (55.00–86.00)	81.50 (64.00–104.25)	<0.001
LDH in U/L, median (IQR)	135.00–225.00	201.00 (170.00–262.00)	320.50 (262.25–456.75)	291.00 (216.00–387.00)	415.50 (302.00–591.05)	<0.001
PCT in ng/mL, median (IQR)	0.02–0.05	0.04 (0.03–0.04)	0.08 (0.04–0.18)	0.04 (0.04–0.10)	0.16 (0.07–0.68)	<0.001
CRP in mg/L, median (IQR)	<1	3.40 (0.90–18.79)	67.30 (36.35–104.98)	32.30 (10.40–71.10)	85.63 (54.75–124.93)	<0.001
K ⁺ in mmol/L, median (IQR)	3.50–5.10	4.02 (3.76–4.34)	4.29 (3.75–4.57)	3.97 (3.62–4.42)	4.05 (3.69–4.56)	0.023
CT scans, n (%)						<0.001 ^B
Unilateral lesions, n (%)		58 (11.9)	3 (3.1)	4 (2.0)	2 (0.9)	
Bilateral lesions, n (%)		429 (88.1)	95 (96.9)	199 (98.0)	228 (99.1)	

Data are presented as medians (interquartile range [IQR]) or n (%). ^AP values derived from Kruskal-Wallis test, unless otherwise specified. ^BP values derived from χ^2 test. COVID-19, coronavirus disease 2019; APTT, activated partial thromboplastin time; PT, prothrombin time; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TP, total protein; ALB, albumin; TBil, total bilirubin; BUN, blood urea nitrogen; SCr, serum creatinine; LDH, lactate dehydrogenase; K⁺, serum potassium; PCT, procalcitonin; CRP, C-reactive protein.

other groups. Based on the evidence above, IL-6 elevation combined with CD8⁺ T cell count reduction was found to be associated with poor outcomes in patients with COVID-19. Through univariate and multivariate logistic regression analysis of mortality-related risks in patients with COVID-19, we confirmed that IL-6 (>20 pg/mL) and CD8⁺ T cell counts (<165 cells/ μL) were 2 vital mortality-related risk factors. Therefore, we further incorporated them to build a model in order to predict death risk. By ROC analysis, we found that our model had a better ability to predict in-hospital mortality earlier in comparison with the commonly used CURB-65 score (age 65 years, respiratory rate, confusion, urea, blood pressure) (22). Therefore, we emphasize the necessity of observing lymphocyte T subsets and cytokines in clinical laboratory examinations. This facilitates an early discrimination of fatal cases and provides opportunities for effective interventions.

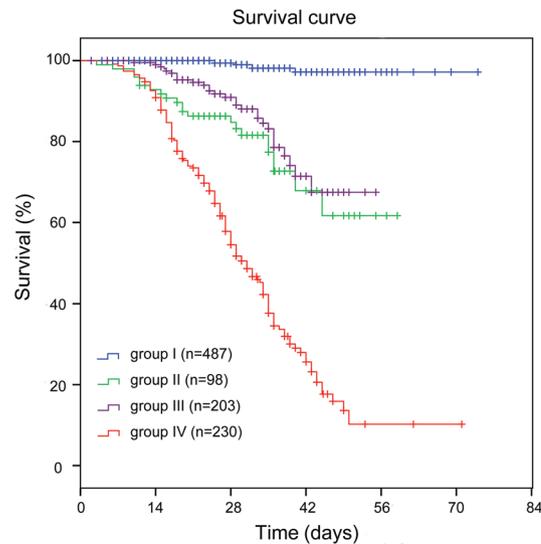


Figure 3. Kaplan-Meier survival curves in 4 groups of patients with COVID-19. The survival rates of groups I–IV were 98.8% (481 of 487), 77.6% (76 of 98), 85.7% (174 of 203), and 37.4% (86 of 230) at the observed endpoint during hospitalization, respectively. Compared with group I, the patients from the other 3 groups had worse survival rates ($P < 0.001$). The patients from group IV had a much shorter survival time than those from groups II or III ($P < 0.001$). However, there was no statistical difference between groups II and III ($P = 0.205$). Group I, IL-6 ≤ 20 pg/mL and CD8⁺ T cells ≥ 165 cells/ μ L, $n = 487$; group II, IL-6 > 20 pg/mL and CD8⁺ T cells ≥ 165 cells/ μ L, $n = 98$; group III, IL-6 ≤ 20 pg/mL and CD8⁺ T cells < 165 cells/ μ L, $n = 203$; and group IV, IL-6 > 20 pg/mL and CD8⁺ T cells < 165 cells/ μ L, $n = 230$.

This study has several limitations. First, the retrospective design may cause an unavoidable and inherent selection bias in enrolling diagnosed participants. Second, this research has limited generalizability as all the patients enrolled in our study were Chinese. The clinical features of patients might be different in other countries or areas. Additionally, there may be some inherent biases by using this study format. Our results should be further validated by a multiple-center, prospective study.

In conclusion, via this 2-center retrospective study of patients diagnosed with COVID-19 in Wuhan, we first identified 2 reliable prognostic indicators, IL-6 (> 20 pg/mL) and CD8⁺ T cell counts (< 165 cells/ μ L), which can accurately stratify patients into risk categories and effectively predict mortality of patients with COVID-19. These 2 indicators may serve as a guide to clinicians to evaluate patient prognoses, make appropriate decisions, and optimize medical resources.

Methods

Study design and participants. We retrospectively analyzed 1018 patients who died or were discharged between January 9, 2020, and March 31, 2020, from Wuhan Pulmonary Hospital and Tongji Hospital, Huazhong University of Science and Technology. These are designated hospitals to treat patients with SARS-CoV-2 pneumonia. All patients were diagnosed with SARS-CoV-2 pneumonia according to the WHO interim guidelines (23). The study was conducted with strict and reasonable inclusion and exclusion criteria. The inclusion criteria were as follows: (a) adults (aged over 18 years), who understood and agreed to participate in this experiment and (b) PCR test was positive for virus nucleic acid of SARS-CoV-2. The exclusion criteria were as follows: (a) patients with a cause of death that could not be explained by COVID-19; (b) patients with blood system diseases, such as leukemia, which has a great influence on hematological examinations; (c) patients who were critically ill and died before routine hematologic examinations could be conducted; (d) children and adolescents younger than 18 years of age and pregnant and lactating women; and (e) patients who were transferred out of the hospital, and we were unable to follow up on the outcome. According to clinical guidelines, the diagnosed patients can be divided into the following 4 types. These types include mild, in which mild clinical symptoms and no pneumonia manifestations on imaging are seen; normal, cases with fever and respiratory tract symptoms, etc., and pneumonia manifestation that could be seen on imaging; severe, in which, in addition to the above symptoms, cases meet any of the following conditions: (a) shortness

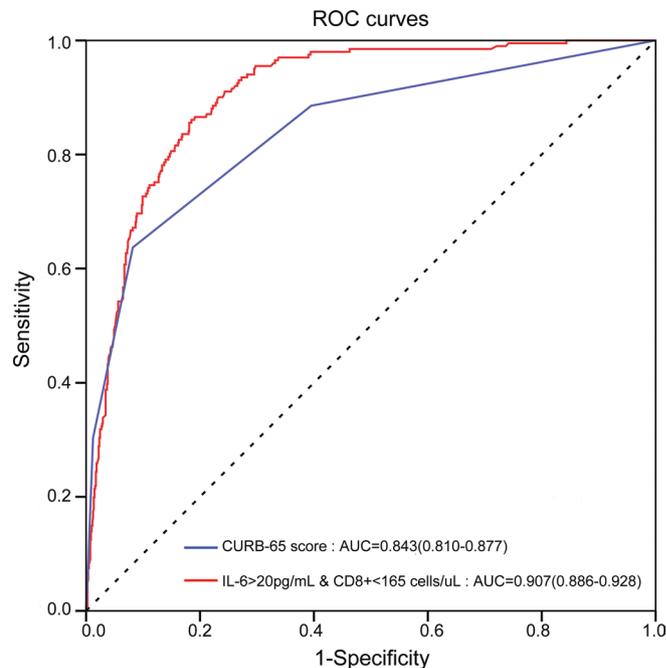


Figure 4. ROC curve analysis to predict in-hospital mortality in patients with COVID-19. ROC curves derived from the model of combined elevated IL-6 and reduced CD8⁺ T cell counts and CURB-65 scores in our cohort. The ROC curves of this predictive model showed a better performance than that of the CURB-65 score ($P < 0.001$).

of breath (≥ 30 breaths/min), (b) oxygen saturation $\leq 93\%$ at rest, (c) arterial partial pressure of oxygen (PaO₂)/fraction of inspired oxygen (FiO₂) ≤ 300 mmHg (1 mmHg = 0.133 kPa), and (d) chest imaging that showed obvious lesion progression within 24–48 hours $>50\%$; and critical, in which, in addition to the above symptoms, cases meet 1 of the following conditions: (a) cases with respiratory failure and the requirement for mechanical ventilation; (b) cases with shock; and (c) cases with other organ failure and the requirement for ICU monitoring treatment.

Data collection. The data were collected from the electronic medical record systems of Tongji Hospital and Wuhan Pulmonary Hospital, which included sociodemographic information, comorbidities, clinical symptoms, routine laboratory tests, immunological tests, CT results, clinical interventions, and outcomes. For early prediction of mortality, the results of laboratory examinations and CT conducted within the first 3 days of hospitalization were collected for analysis. The CURB-65 predictive model is available to evaluate the prognosis of pneumonia and includes the following clinical indicators: (a) disturbance of consciousness, (b) BUN >7 mmol/L, (c) respiratory rate ≥ 30 times/min, (d) systolic blood pressure <90 mmHg or diastolic blood pressure ≤ 60 mmHg, and/or (e) age ≥ 65 years old (24). The CURB-65 score of each patient was calculated. The entry and calculation of all relevant data were verified by 2 experienced clinical researchers. The clinical outcomes of patients included in this clinical study were observed until April 4, 2020.

Cytokines measurement. According to the operating instructions given by the manufacturer, the principle of chemiluminescence immunoassay was used to detect the concentrations of relevant cytokines in the blood samples of patients, including IL-2R, IL-6, IL-8, IL-10, and TNF- α . The entire process was completed using a fully automated analyzer (Immulite 1000, DiaSorin Liaison, or Cobas e602, Roche Diagnostics). The IL-2R kit (LKIP1), IL-8 kit (LK8P1), IL-10 kit (LKXP1), and TNF- α kit (LKNF1) were purchased from DiaSorin. The IL-6 kit (05109442 190) was purchased from Roche Diagnostics.

Measurement of T lymphocyte subsets. Various immune cells have different labels on their surfaces, which can be combined with their specific fluorescence staining or labeled antibodies to achieve cell separation using a BD FACS Canto II Flow Cytometry System. The following antibodies were used: anti-CD8 (RPA-T8, PE-Cy7, 557746), anti-CD3 (SK7, APC-Cy7, 557832), and anti-CD4 (RPA-T4, V450, 560345). All reagents were purchased from BD. The results of flow sorting were further analyzed with the BD FACS Diva software.

Statistics. The percentages of missing values of variables in our cohort were <50%. The multiple imputation method was used to impute missing data in our cohort with guidance from a previous study (25). Continuous variables were expressed as median (IQR). Comparisons between 2 groups were made with the Mann-Whitney *U* test and comparisons among 4 groups were made with the Kruskal-Wallis *H* test. Categorical variables were expressed as a number (%) and compared using the χ^2 test or Fisher's exact test. All tests were considered statistically significant when the 2-sided *P* value was less than 0.05. For mortality-predictive model establishment, continuous variables were categorized by a cutoff point or clinical reference threshold. The cutoff value was confirmed from Youden's index of the ROC curve. Survival analysis among the 4 groups was also performed using Kaplan-Meier analysis. Statistical analysis was conducted using the Statistical Package for Social Sciences 24.0 and R software 3.5.0.

Study approval. The ethics commissions of Tongji Hospital and Wuhan Pulmonary Hospital approved this study. Informed consent was obtained from patients before enrollment, and data were collected retrospectively.

Author contributions

SW and HL had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. SW, HL, and ML conceived and designed the study. ML and JL acquired, analyzed, or interpreted data. ML and SW drafted the manuscript. ML, SW, and HL provided critical revision of the manuscript for important intellectual content. ML provided statistical analysis. JL, WJ, and SY provided administrative, technical, or material support.

Acknowledgments

This work was supported by funding from the National Natural Science Foundation of China (no. 81772477 and 81201848) awarded to SW. We thank all the patients who enrolled in this study as well as their families.

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