Clinical value of immune-inflammatory parameters to assess the severity of coronavirus disease 2019

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ABSTRACT

Objective: To explore the clinical value of immune-inflammatory markers to assess the severity of coronavirus disease 2019 (COVID-19).

Methods: 127 consecutive hospitalized patients with confirmed COVID-19 were enrolled in this study, and classified into non-severe and severe groups. Demographics, symptoms, underlying diseases and laboratory data were collected and assessed for predictive value.

Results: Of 127 COVID-19 patients, 16 cases (12.60%) were classified into the severe group. High level of interleukin-6 (IL-6), C-reaction protein (CRP) and hypertension were independent risk factors for the severity of COVID-19. The risk model based on IL-6, CRP and hypertension had the highest area under the receiver operator characteristic curve (AUROC). Additionally, the baseline IL-6 was positively correlated with other immune-inflammatory parameters and the dynamic change of IL-6 in the severe cases were parallel to the amelioration of the disease.

Conclusion: Our study showed that high level of IL-6, CRP and hypertension were independent risk factors for assessing the severity of COVID-19. The risk model established upon IL-6, CRP and hypertension had the highest predictability in this study. Besides, IL-6 played a pivotal role in the severity of COVID-19 and had a potential value for monitoring the process of severe cases.

1. Introduction

Since December 2019, an unexperienced pneumonia emerged in Wuhan, China, and soon spread globally (Guo et al., 2020, Wu et al., 2020). This pneumonia was verified to be caused by a novel coronavirus and named as coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO). Based on phylogeny, taxonomy and established practice, this novel coronavirus was designated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the Coronavirus Study Group (CSG) (Gorbalenya et al., 2020). Similar to the former two pathogenic coronaviruses, severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) at the beginning of the 21st century (Cui et al., 2019), SARS-CoV-2 is likely originated from bat (Zhou et al., 2020a), have the ability to spread from person to person (Chan et al., 2020), cause pneumonia and severe respiratory syndrome (Chen et al., 2020b) with a typic ground glass on imaging (Xu et al., 2020). COVID-19 is becoming a threat to people's life and health. Up to 27 March, 2020, 509299 people were infected by SARS-CoV-2 worldwide, with 23338 cases dead (http://2019ncov.chinacdc.cn/2019-nCoV/global.html).

Regardless of most COVID-19 patients are mild, patients with severe type may quickly progress to acute respiratory distress syndrome (ARDS), multiple organ failure (MOF) and even death (Wang et al., 2020c). Therefore, exploring potential risk factors for the severity of COVID-19 is crucial for delay or halt the progression of

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the disease. Previous studies have revealed that patients with old age and underlying diseases are more likely to be aggravated (Wang et al., 2020b, Wang et al., 2020d), and aberrant immune-inflammatory response and cytokine storm may played an important role in the disease progression (Zhou et al., 2020b). Therefore, a retrospective study was performed to compare the clinical features, immune-inflammatory parameters and cytokines between the severe and non-severe groups, and tried to establish a predict model for assessing the severe cases in Hwa Mei Hospital, University of Chinese Academy of Sciences, Ningbo, Zhejiang province, China.

2. Methods

2.1. Data collection

127 consecutive hospitalized patients with confirmed COVID-19 at Hwa Mei Hospital, University of Chinese Academy of Sciences during January 23 to February 20, 2020 were enrolled into this retrospective study. All patients were diagnosed according to the guidelines for diagnosis and treatment for COVID-19 (Trail Version 6), and classified into mild, moderate, severe and critical type based on the severity of symptoms. Severe patients should meet at least one of the following criterions: First, shortness of breath with respiration rate (RR) $\geq$30 times/min. Second, oxygen saturation $\leq$93% in resting state. Third, partial pressure of arterial oxygen (PaO_2)-to-fraction of inspired oxygen (FiO_2) ratio $\leq$300 mm Hg. Obvious lesion progression >50% within 24-48 hours on pulmonary imaging were also recognized as severe cases. Critical cases were defined when one of the following conditions met: First, respiratory failure and require mechanical ventilation. Second, shock occurred. Third, combined with other organ failure and treated in intensive care unit. Mild and moderate cases were defined as non-severe group, while severe and critical patients were categorized as severe group in this study. All medical record information including demographics, symptoms, underlying diseases and laboratory data were obtained.

This study was approved by the Ethics Committee of Hwa Mei Hospital, University of Chinese Academy of Sciences (Certificate no. PJ-NBEY-KY-2020-061-01), and a written informed consent was obtained from all enrolled patients.

Table 1
Demographic and clinical characteristics in patients with COVID-19

Variables	All patients (n = 127)	Non-severe group (n = 111)	Severe group (n = 16)	P value
Gender				0.457
Female	82 (64.57)	38 (34.23)	7 (43.75)	
Male	45 (35.43)	73 (65.77)	9 (56.25)	
Age (years)	50.90 ± 15.26	49.95 ± 15.52	57.50 ± 11.70	0.03
Body mass index (kg/m^2)	24.01 ± 3.53	23.71 ± 3.04	26.04 ± 5.63	0.013
Time interval (days)*	5.35 ± 3.72	5.13 ± 3.79	6.88 ± 2.83	0.078
Underlying disease	52 (40.94)	40 (36.04)	12 (75.00)	0.003
Diabetes	10 (7.87)	10 (9.01)	0 (0.00)	0.451
Hypertension	31 (24.41)	23 (20.72)	8 (50.00)	0.025
Cardiovascular disease	6 (4.72)	4 (3.60)	2 (12.50)	0.348
Hepatic disease	7 (5.51)	5 (4.50)	2 (12.50)	0.469
Chronic lung disease	6 (4.72)	4 (3.60)	2 (12.50)	0.348
Cancer	5 (3.94)	4 (3.60)	1 (6.25)	>0.999
Symptoms				
Fever	108 (85.04)	92 (82.88)	16 (100)	0.156
Highest temperature (°C)				
<37.5	40 (31.50)	40 (36.04)	0 (0.00)	0.004
37.5-38	43 (33.86)	36 (32.43)	7 (43.75)	0.371
38.1-39	34 (26.77)	31 (27.93)	3 (18.75)	0.636
>39	10 (7.87)	4 (3.60)	6 (37.50)	<0.001
Rhinorrhoea	7 (5.51)	6 (5.41)	1 (6.25)	>0.999
Nasal congestion	6 (4.72)	6 (5.41)	0 (0.00)	0.747
Sore throat	14 (11.02)	13 (11.71)	1 (6.25)	0.822
Headache	23 (18.11)	22 (19.82)	1 (6.25)	0.332
Dizziness	23 (18.11)	21 (18.92)	2 (12.50)	0.782
Chill	18 (14.17)	16 (14.41)	2 (12.50)	>0.999
Dry mouth	14 (11.02)	13 (11.71)	1 (6.25)	0.822
Bitter taste	9 (7.09)	9 (8.11)	0 (0.00)	0.509
Fatigue	80 (62.99)	67 (60.36)	13 (81.25)	0.106
Anorexia	59 (46.46)	49 (44.14)	10 (62.50)	0.169
Night sweat	7 (5.51)	7 (6.31)	0 (0.00)	0.655
Myalgia	12 (9.45)	11 (9.91)	1 (6.25)	0.991
Chest pain	4 (3.15)	3 (2.70)	1 (6.25)	>0.999
Chest distress	30 (23.62)	22 (19.82)	8 (50.00)	0.019
Shortness of breath	10 (7.87)	7 (6.31)	3 (18.75)	0.218
Dyspnea	4 (3.15)	1 (0.9)	3 (18.75)	0.002
Cough	105 (82.68)	90 (81.08)	15 (93.75)	0.369
Expectoration	75 (59.06)	65 (58.56)	10 (62.50)	0.764
Nausea	57 (44.88)	54 (48.65)	3 (18.75)	0.025
Diarrhea	43 (33.86)	38 (34.23)	5 (31.25)	0.814
Abdominal pain	4 (3.15)	3 (2.70)	1 (6.25)	>0.999
Anxiety	1 (0.79)	0 (0.00)	1 (6.25)	0.258
Delirium	1 (0.79)	0 (0.00)	1 (6.25)	0.258
Rash	4 (3.15)	4 (3.60)	0 (0.00)	0.995

P values indicated the comparison between non-severe group and severe groups.
Data are presented as mean ± standard deviation or n (%).
COVID-19: coronavirus disease 2019.
* Refer to the time from illness onset to hospitalization.

2.2. Laboratory examination

Throat-swab, nasopharynx-swab and sputum specimens from all suspected SARS-CoV-2 infection patients were collected, and SARS-CoV-2 RNA was detected at Hwa Mei Hospital, University of Chinese Academy of Sciences by real-time reverse transcription polymerase chain reaction (RT-PCR) method. The specific primers, probes and the determination of result for detection SARS-CoV-2 were following the recommendation by the National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention.

Routine blood tests were detected by the XS-1000i hematology analyzer (Sysemx, Japan). Biochemical indictors were tested by the ADVIA2400 Chemistry System (Siemens, Germany). Coagulation tests were analyzed by the ACLTOP750 automatic coagulation analyzer (Instrumentation Laboratory, USA). Plasma cytokines were measured by the flow cytometry with human Th1/2 cytokine kit (CellGene, China). Cardiac troponin I (cTnI) and N-terminal prohormone of brain natriuretic peptide (NT-proBNP) were tested by fluorescence immunochromatography assay (Wondof, China). Arterial blood was analyzed by cobasb221 blood gas analyzer (Roche, Switzerland) immediately after sampling. The baseline laboratory examinations were performed within 5 days of admission.

2.3. Statistical analyses

Normally distributed continuous data were expressed as mean ± standard deviation, and the significance was detected by the Student's t-test. Abnormally distributed variables were reported as medians and inter-quartile ranges (IQR), and the significance was analyzed by the Mann-Whitney U test. Classification variables were described as percentages, and the significance was tested by the chi square or Fisher's exact test. A multivariate logistic regression analysis was performed by taking the severity of COVID-19 (yes or no) as dependent variable and variables which found significant during univariate analysis were selected as independent variables. The receiver operator characteristic (ROC) curves were built to assess predictive values. The correlations between interleukin-6 (IL-6) and other variables were analyzed by the Spearman correlation analysis. Abnormal distribution data from repeated measures were compared by the generalized linear mixed model. $P < 0.05$ was considered statistically significant. All data were analyzed by SPSS statistical 16.0 software (IBM, Armonk, NY, USA) and GraphPad PRISM 5.0 software (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Demographic and clinical characteristics

A total of 127 consecutive hospitalized patients with SARS-CoV-2 infection were included in this study, among them 82 (64.57%) were woman and 45 (35.43%) were man, the mean age was 50.90 years. Fever (108 [85.04%]), cough (105 [82.86%]), fatigue (80 [62.99%]), expectoration (75 [59.06%]), anorexia (59 [46.46%]) and nausea (57 [44.88%]) were the most common symptoms. Hypertension (31 [24.42%]) was the most common underlying disease.

According to severity of the disease, 111 cases (87.40%) were classified as non-severe group (11 as mind and 100 as moderate)

Table 2
Laboratory parameters of patients with COVID-19.

Variables	All patients (n = 127)	Non-severe group (n = 111)	Severe group (n = 16)	P value
Immune-inflammatory parameters				
WBC count ($\times 10^9$)	5.00 (4.20-6.80)	5.00 (4.30-6.50)	5.35 (4.13-7.53)	0.568
Neutrophil% (%)	67.20 (60.00-75.10)	66.50 (59.60-73.90)	75.70 (64.53-88.98)	0.018
Lymphocyte% (%)	23.60 (17.80-30.30)	24.30 (18.70-31.40)	17.45 (8.23-22.25)	0.012
Neutrophil count ($\times 10^9$)	3.37 (2.54-4.55)	3.29 (2.54-4.40)	3.89 (2.25-6.57)	0.309
Lymphocyte count ($\times 10^9$)	1.21 (0.87-1.48)	1.23 (0.90-1.53)	0.74 (0.47-1.18)	0.004
Platelet count ($\times 10^9$)	198.00 (158.00-243.00)	205.00 (165.00-246.00)	155.00 (125.75-206.00)	0.01
NLR	2.91 (1.95-4.15)	2.75 (1.90-3.95)	4.24 (3.00-10.87)	0.015
PLR	161.37 (127.04-223.75)	160.00 (129.64-215.00)	210.46 (116.33-300.88)	0.299
D-dimer (ng/ml)*	100.00 (82.00-163.45)	100.00 (82.00-158.00)	160.95 (73.75-282.75)	0.195
Fibrinogen (mg/dl)	432.00 (372.40-574.30)	423.00 (363.00-550.00)	574.30 (405.30-668.00)	0.018
Erythrocyte sedimentation rate (mm/h)[a]	68.00 (41.50-96.00)	67.00 (40.50-93.50)	89.00 (60.50-105.75)	0.083
Sialic acid (U/L)	76.20 (68.60-88.40)	75.10 (67.90-86.60)	85.75 (82.68-97.38)	0.01
C-reactive protein (mg/L)	8.47 (3.18-25.55)	8.47 (2.79-16.19)	36.64 (15.33-69.94)	<0.001
Interleukin-2 (pg/ml)	0.91 (0.54-1.70)	0.93 (0.55-1.73)	0.90 (0.47-1.60)	0.49
interleukin-4 (pg/ml)	1.88 (1.43-2.55)	1.87 (1.43-2.55)	1.99 (1.26-2.73)	0.777
Interleukin-6 (pg/ml)	4.26 (2.41-11.78)	3.82 (2.19-9.87)	24.11 (1.14-54.37)	<0.001
Interleukin-10 (pg/ml)	3.13 (2.22-4.84)	3.13 (2.15-4.57)	6.41 (3.24-11.02)	0.001
Interferon-γ (pg/ml)	1.27 (0.98-1.69)	1.24 (0.93-1.57)	1.93 (1.25-2.29)	0.003
Tumor Necrosis Factor-α (pg/ml)	1.39 (1.12-1.73)	1.35 (1.12-1.73)	1.48 (1.39-1.74)	0.495
Cardiac dysfunction biomarkers				
cTnI (ng/ml)[b]	<0.03 (<0.03-0.04)	<0.03 (<0.03-0.04)	<0.03 (<0.03- <0.03)	0.082
NT-proBNP (pg/ml)[b]	119.30 (78.38-219.98)	118.00 (78.38-218.75)	196.45 (75.85-405.10)	0.25
Arterial blood gas analysis				
pH[c]	7.45 (7.42-7.48)	7.45 (7.40-7.48)	7.47 (7.42-7.49)	0.234
pO2 (mmHg)[c]	84.00 (65.90-113.00)	102.00 (77.00-123.00)	63.00 (57.75-74.50)	<0.001
pCO2 (mmHg)[c]	37.00 (35.00-42.00)	40.00 (35.00-43.00)	35.90 (33.33-37.45)	0.015
Lactate (mmol/L)[c]	1.90 (1.20-2.70)	1.50 (1.00-2.70)	2.30 (1.23-2.70)	0.215

Data are presented as medians and inter-quartile ranges.
P values indicated the comparison between non-severe group and severe group.
COVID-19: coronavirus disease 2019; WBC: white blood cell; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; cTnI: cardiac troponin I; NT-proBNP: N-terminal prohormone of brain natriuretic peptide.
* The number of COVID-19 patients who tested D-dimer was 95 and 10 in the non-severe group and severe group, respectively.
[a] The number of COVID-19 patients who tested erythrocyte sedimentation rate was 97 and 12 in the non-severe group and severe group, respectively.
[b] The number of COVID-19 patients who tested cTnI and NT-proBNP was 104 and 16 in the non-severe group and severe group, respectively.
[c] The number of COVID-19 patients who tested pH, pO2, pCO2 and Lactate was 35 and 16 in the non-severe group and severe group, respectively.

and 16 cases (12.60%) were categorized as severe group (13 as severe and 3 as critical). Compared with patients in the non-severe group, patients in the severe group were older (57.50 ± 11.70 years vs 49.95 ± 15.52 years, $P = 0.03$), had higher body mass index (BMI) (26.04 ± 5.63 kg/m^2 vs 23.71 ± 3.04 kg/m^2, $P = 0.013$) and were more likely to have hypertension (8 [50%] vs 23 [20.72%], $P = 0.025$), highest temperature > 39 °C (6 [37.50%] vs 4 [3.60%], $P < 0.001$), chest distress (8 [50.00%] vs 22 [19.82%], $P = 0.019$) and dyspnea (3 [18.75%] vs 1 [0.90%], $P = 0.002$), while nausea was more often in the non-severe group (3 [18.75%] vs 54 [48.65%], $P = 0.025$) (Table 1).

3.2. Laboratory findings

Table 2 showed the baseline laboratory parameters of included patients. Neutrophil%, neutrophil-to-lymphocyte ratio (NLR), fibrinogen, sialic acid (SA), C-reaction protein (CRP), IL-6, interleukin-10 (IL-10) and interferon-γ (IFN-γ) in the severe group were significantly higher than those in the non-severe group ($P < 0.05$). While lymphocyte%, lymphocyte count and platelet count were significantly lower in the severe group when compared with the non-severe group ($P < 0.05$). There were no significant differences in cTnI and NT-proBNP between the severe and non-severe groups. As for the arterial blood gas parameters, higher levels of pO_2 and pCO_2 were found in the non-severe group ($P < 0.05$), while pH and lactate showed no differences between the two groups.

Table 3.

3.3. Risk factors the severity of COVID-19

The result of multivariate logistic regression analysis demonstrated that the high level of peripheral blood cytokine IL-6, CRP and hypertension were independent risk factors for assessing the severity of COVID-19, with odd ratios (ORs) of 1.090 (95% confidence interval [CI]: 1.040-1.147), 1.030 (95%CI: 1.005-1.055) and 4.380 (95%CI: 1.012-18.993), respectively. A risk model was established based on IL-6, CRP and hypertension, and ROC curves were performed to assess the value of risk model and other single parameters. The area under ROC curve (AUROC) was 0.835 for IL-6, superior to other single immune-inflammatory parameters (ranging from 0.682 to 0.802), and the sensitivity was 87.50% (95% CI: 61.60-98.10), specificity was 74.77% (95% CI:65.60-82.50). Moreover, the risk model combined with IL-6, CRP and hypertension enlarged the predictability, with AUROC of 0.900 (95% CI: 0.831-0.968), sensitivity of 100% (95% CI: 79.20-100.00) and specificity of 65.77% (95% CI:56.20-74.50) (Table 4, Fig. 1).

3.4. Correlations between IL-6 and other variables

The baseline IL-6 was positively correlated with neutrophil% ($r = 0.398$, $P < 0.001$), NLR ($r = 0.428$, $P < 0.001$), fibrinogen ($r = 0.370$, $P < 0.001$), SA ($r = 0.420$, $P < 0.001$), CRP ($r = 0.468$, $P < 0.001$), IL-10 ($r = 0.638$, $P < 0.001$) and IFN-γ ($r = 0.434$, $P < 0.001$). Meanwhile, it was negatively correlated with lymphocyte% ($r = -0.438$, $P < 0.001$), lymphocyte count ($r = -0.446$, $P < 0.001$) and platelet count ($r = -0.375$, $P < 0.001$). Additional, IL-6 was higher in patients with hypertension than without hypertension ($P = 0.001$)) (Fig. 2).

3.5. Dynamic changes of IL-6

The dynamic changes of IL-6 were analyzed in 45 non-severe cases and 12 severe cases. The level of IL-6 in the severe group was significantly higher than non-severe group at baseline and 5-10 days after disease onset, but dropped gradually day-by-day, and reached a level equal to non-severe group at ≥ 10 days after treatment. Furthermore, we took a generalized linear mixed model to find that the severity of disease ($F = 12.624$, $P = 0.001$) and curing time ($F = 10.926$, $P = 0.002$) were two factors related to the level of IL-6 (Fig. 3).

4. Discussion

This study was performed at Hwa Mei Hospital, University of Chinese Academy of Sciences, Ningbo, a largest local designated

Table 3
Logistic regression analysis of variables associated with the severity of COVID-19.

Variables	Univariate analysis			Multivariate analysis		
	OR	95%CI	P value	OR	95%CI	P value
Age(years)	1.037	0.997-1.079	0.068			
BMI (kg/m2)	1.186	1.029-1.367	0.019			
Hypertension	5.201	1.745-15.508	0.003	4.380	1.012-18.993	0.048
WBC count (×10^9)	1.009	0.845-1.206	0.917			
Neutrophil% (%)	1.062	1.012-1.116	0.015			
Lymphocyte% (%)	0.932	0.877-0.989	0.021			
Neutrophil count (×10^9/L)	1.054	0.906-1.226	0.497			
Lymphocyte count (×10^9/L)	0.160	0.041-0.632	0.009			
Platelet count (×10^9/L)	0.990	0.980-1.000	0.041			
NLR	1.090	1.003-1.185	0.043			
PLR	1.004	1.000-1.008	0.06			
D-dimer (ng/ml)[*]	0.899	0.998-1.002	0.899			
Fibrinogen (mg/dl)	1.004	1.001-1.008	0.007			
Erythrocyte sedimentation rate (mm/h)[a]	1.015	0.996-1.034	0.118			
Sialic acid (U/L)	1.049	1.011-1.088	0.011			
C-reactive protein (mg/L)	1.033	1.015-1.052	<0.001	1.030	1.005-1.055	0.017
Interleukin-2 (pg/ml)	0.790	0.389-1.604	0.515			
interleukin-4 (pg/ml)	1.020	0.529-1.967	0.952			
Interleukin-6 (pg/ml)	1.086	1.041-1.133	<0.001	1.090	1.040-1.147	<0.001
Interleukin-10(pg/ml)	1.117	1.017-1.228	0.021			
Interferon-γ (pg/ml)	0.994	0.947-1.043	0.803			
Tumor Necrosis Factor-α (pg/ml)	0.917	0.386-2.182	0.845			

COVID-19: coronavirus disease 2019; WBC: white blood cell; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; OR: odd ratio; CI: confidence interval.
[*] The number of COVID-19 patients who tested D-dimer was 95 and 10 in the non-severe group and severe group, respectively.
[a] The number of COVID-19 patients who tested erythrocyte sedimentation rate was 97 and 12 in the non-severe group and severe group, respectively.

Table 4
AUROCs of risk model and other single immune-inflammatory parameters for the severity of COVID-19.

Variables	AUROC	Std. Error	P value	Asymptotic 95% CI Lower Bound	Upper Bound
Neutrophil%	0.684	0.083	0.018	0.521	0.846
Lymphocyte%	0.695	0.076	0.012	0.546	0.844
Lymphocyte count	0.722	0.076	0.004	0.574	0.87
NLR	0.689	0.078	0.015	0.537	0.842
Platelet count	0.7	0.078	0.01	0.548	0.853
Fibrinogen	0.682	0.073	0.019	0.539	0.824
Sialic acid	0.698	0.073	0.01	0.555	0.842
C-reactive protein	0.802	0.058	<0.001	0.688	0.915
Interleukin-6	0.835	0.065	<0.001	0.708	0.962
Interleukin-10	0.756	0.074	0.001	0.611	0.901
Interferon-γ	0.733	0.061	0.003	0.613	0.852
Risk model	0.900	0.035	<0.001	0.831	0.968

COVID-19: coronavirus disease 2019; AUROC: area under the receiver operator characteristic curve; CI: confidence interval; NLR: neutrophil-to-lymphocyte ratio.
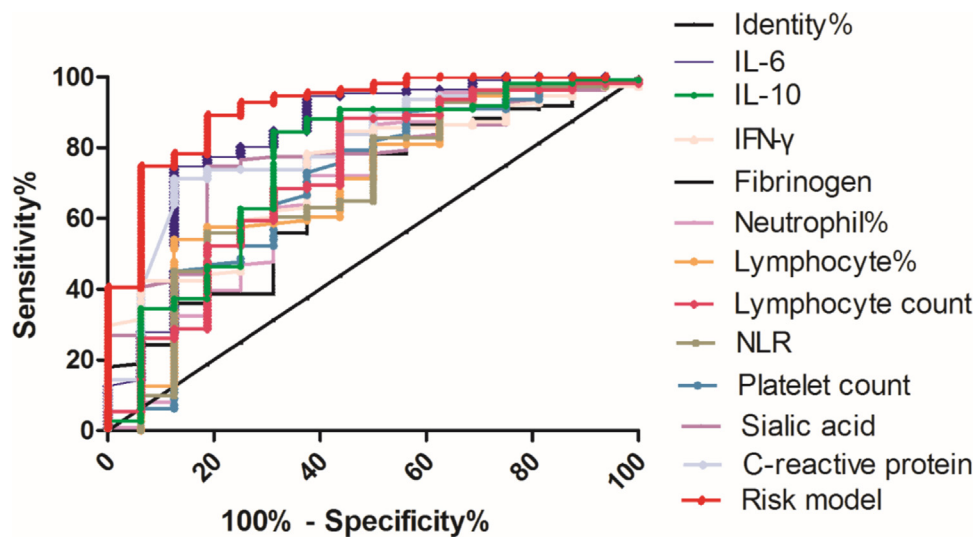
Fig. 1. ROC curves of risk model and other single immune-inflammatory parameters for the severity of COVID-19. COVID-19: coronavirus disease 2019; ROC: Receiver operator characteristic; NLR: neutrophil-to-lymphocyte ratio.

hospital treating COVID-19. Different from Wuhan, all patients treated immediately once relative symptoms appeared. The time interval from illness onset to hospitalization was 5.35 ± 3.72 days, most of them (87.40%) were non-severe cases. Therefore, it may sever as a representative of the general situation, except for the severely affected area. We found higher age, BMI index and proportion of hypertension, highest temperature > 39°C, chest distress and dyspnea in the severe group, but nausea was more often in non-severe group. We also observed significant increases of neutrophil%, NLR, fibrinogen, SA, CRP, IL-6, IL-10, IFN-γ, pO$_2$ and pCO$_2$ and decreases of lymphocyte%, lymphocyte count and platelet count in the severe group.

Previous studies have indicated that the severe and aberrant host immune response are responsible for the severity of COVID-19 (Zhou et al., 2020b). Meanwhile, it was companied by a sharp change in the peripheral blood immune-inflammation parameters. Therefore, emerging studies were focus on these accessible laboratory data for assessing the severity of disease. The increased of neutrophil counts indicate the intensity of inflammatory response, while the decreased of lymphocyte counts suggest the damage of immune system, therefore, higher NLR maybe a potential maker for risk factor (Liu et al., 2020a). A study by Liu et al built a nomogram based on NLR to found a high c-index of 0.807 for predicting the severe illness, and established a cut-off of 3.13 (Liu et al., 2020b). Liu et al took a further step to analyze the

kinetic change of lymphocyte subsets and observed neutrophil-to-CD8$^+$ T cell ratio (N8R) to be the most powerful prognostic factor (Liu et al., 2020a). Besides, a recent study indicated the AUROCs of three inflammation markers white blood cell (WBC), CRP and serum amyloid A (SAA) in single and combine forms were 0.814, 0.742, 0.673, and 0.924 respectively for predicting severe and critically severe COVID-19 cases (Shi et al., 2020). Our study confirmed again for the predictive value of immune-inflammation parameters, with AUROCs ranging from 0.682 to 0.835 in this study, among them, IL-6 had the largest AUROC. However, only IL-6, CRP and hypertension entered the final risk model revealed by multivariate logistic regression analysis and the AUROC of the risk model was as high as 0.900.

CRP is an acute phase reactive protein, and parallels to the severity of inflammatory. A previous study and a recent study have revealed its role in predicting the severity of patients with SARS and SARS-COV-2 (Shi et al., 2020, Wang et al., 2004). It was confirmed again in this study.

Cytokines are small protein molecules aimed for cell-to-cell communications, and play an immunomodulating function (Chousterman et al., 2017). However, in some infection diseases, excessive inflammation activate cytokine storm, lead to serious pathological changes, even is responsible for multiorgan dysfunction (Liu et al., 2016). Cytokine storm have been implicated in severe influenza (Liu et al., 2016), SARS and MERS
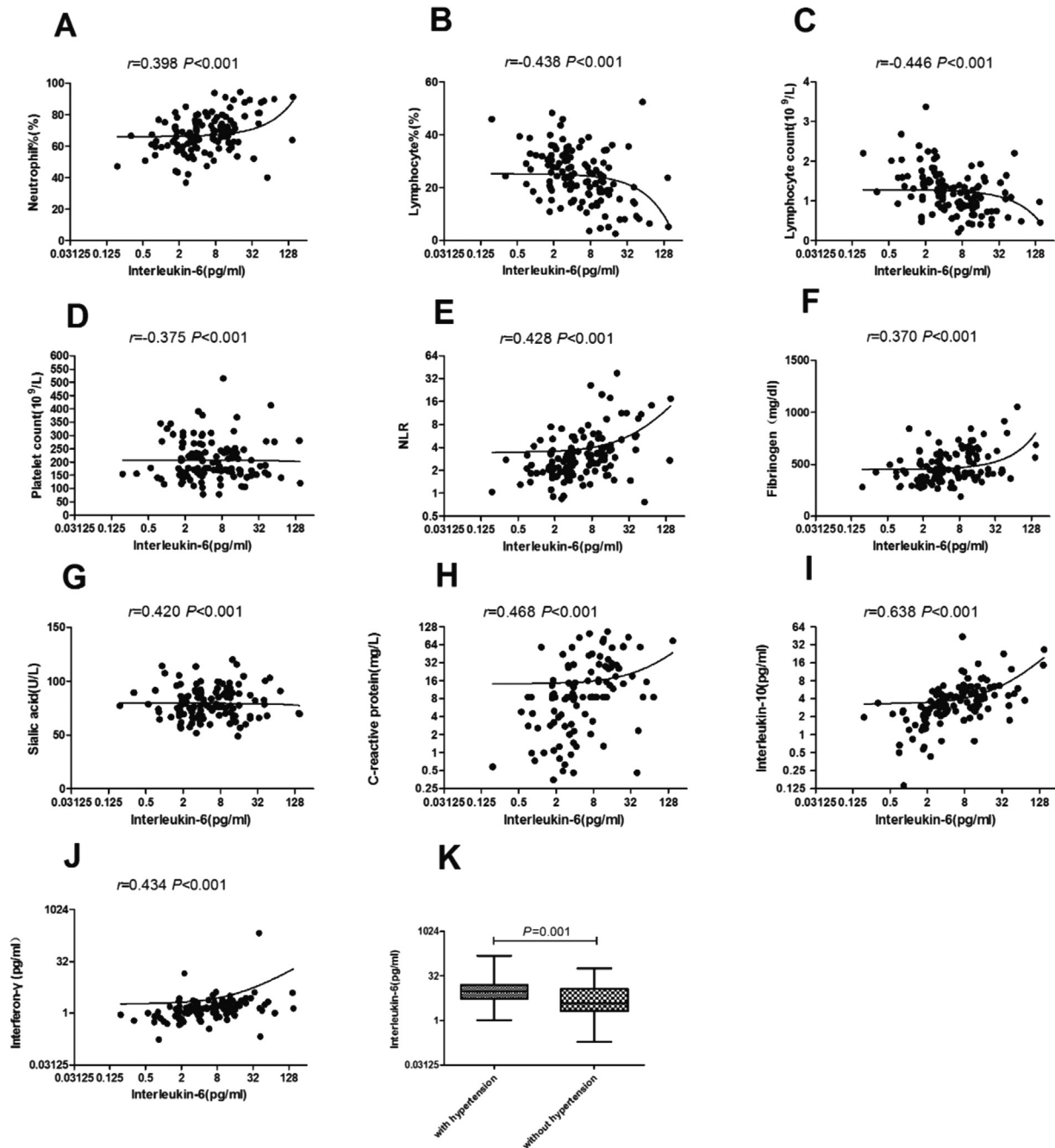
Fig. 2. Correlations between interleukin-6 and neutrophil% (A), lymphocyte%(B), lymphocyte count (C), platelet count (D), NLR (E), fibrinogen (F), sialic acid (G), C-reaction protein (H), interleukin-10 (I), interferon-γ (J) in patients with COVID-19. The levels of interleukin-6 in COVID-19 patients with and without hypertension (K). COVID-19: coronavirus disease 2019; NLR: neutrophil-to-lymphocyte ratio.

(Channappanavar and Perlman, 2017), and evidence has revealed that cytokine storm may as a cause for deleterious consequence during SARS-COV-2 infection (Zhou et al., 2020b). In consist with the previous studies (Liu et al., 2020a), cytokine IL-6, IL-10 and IFN-γ in the severe group were significantly higher than those in the non-severe group, especially for IL-6, and it was recognized as an independent risk factor by using multivariate logistic regression analysis, with OR of 1.090 (95% CI: 1.040-1.147).

As mentioned above, IL-6 had the highest AUROC among the inflammation parameters in this study, so we further analyzed their correlations. It was found that IL-6 was significantly correlated with other inflammation parameters, and a most close relationship was observed between IL-6 and IL-10 ($r = 0.638$, $P < 0.001$). IL-10 is an anti-inflammatory cytokine, the increased of IL-10 maybe reflect a self-protection during cytokine storm, the level of IL-10 was also directly related to the degree of inflammation (Chousterman et al., 2017), and high IL-10 was associated with immunosuppression in sepsis (Hotchkiss et al., 2013).

IL-6 plays a key role in cytokine storm owing to its pleiotropic property (Gupta et al., 2020). Apart from its robust proinflammatory function, it induces a variety of acute-phase proteins, such as CRP, SAA, antitrypsin, hepcidin, fibrinogen and complement components to deteriorate inflammatory reaction and activate coagulation cascade to enlarge disseminated intravascular coagulation (DIC) (Tanaka et al., 2016). A recent study confirmed the role of IL-6 by using the peripheral blood of severe COVID-19 cases for
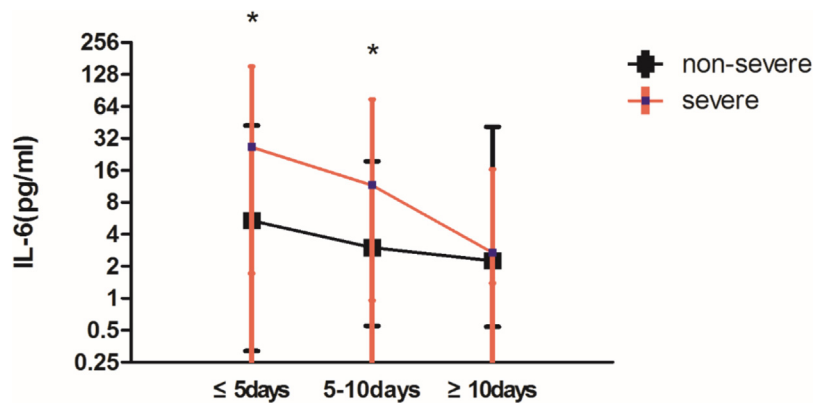
Fig. 3. The dynamic changes of interleukin-6 in patients with COVID-19. COVID-19: coronavirus disease 2019. *$P< 0.05$ between non-severe and severe groups.

immune analysis, indicated that GM-CSF+ Th1 cells and CD14+CD16+ monocytes with high expression of IL-6 accelerated the excessive immune response and deteriorated the disease (Zhou et al., 2020b). In addition, two prior studies reported the dynamic change of IL-6 in severe COVID-19 cases, from a high level at baseline to a low level when cured (Liu et al., 2020a, Liu et al., 2020c). Our study was in consist with them, and considered it has a potential value for monitoring the condition of severe COVID-19 cases.

A very recent study reported that massive mucus was aggregated in the distal respiratory tract by autopsying two body donors of fatal cases with COVID-19 (Wang et al., 2020a). IL-6 may linked to the increased mucus production in COVID-19 patients for its ability of stimulating the predominant mucin genes, MUC5AC and MUC5B expression in tracheobronchial epithelial cells (Chen et al., 2003). In view of the pivotal role of IL-6 in cytokine storm and mucus production, and tocilizumab, a humanized anti-IL-6 receptor monoclonal antibody, have achieved an effective treatment for severe cytokine release syndrome (CRS) (Gupta et al., 2020, Tanaka et al., 2016), IL-6 blockade treatment is worthy of expectation for treating the severe COVID-19 patients.

In line with prior studies, our study also demonstrated that severe cases were older and more likely to have underlying diseases (Wang et al., 2020b, Wang et al., 2020d). However, apart from hypertension, the number of patients with and without diabetes, cardiovascular disease, hepatic disease, chronic lung disease and cancer showed no differences in non-severe and severe groups. Low number of patients in these underlying diseases may cause certain bias, while a relatively large number in hypertension is sufficient to make a certain comparison. Additionally, the level of IL-6 in patients with hypertension was higher than those without hypertension in this study, demonstrated a severe inflammation lesion in the hypertension patients. Hypertension patients are always associated with immune activation, resulted in immune system impaired (Norlander et al., 2018). Therefore, we speculated that the abnormal immune function of hypertension patients may played a role in the COVID-19 progression. Intriguingly, angiotensin-converting enzyme 2 (ACE-2) played a protect role in hypertension (Povlsen et al., 2020), while SARS-COV-2 use ACE-2 to enter target cells, and may reduce ACE-2 expression just like SARS-COV do (Kuba et al., 2005), which was likely to carry a burden to those weakness patients and aggravate the disease eventually. In this study, the existing of hypertension severed as an independent risk factor for the severity of COVID-19, it reminded us to take specially care of these high-risk patients to prevent the development of disease.

cTnI and NT-proBNP are two biomarkers for cardiac dysfunction. The baseline level of cTnI in the most patients with COVID-19 was below 0.03 ng/ml, no significant difference was found between the severe and non-severe groups, so did the level of NT-proBNP. It was consistent with the study by Peng et al., in which tests were performed on admission (Peng et al., 2020). However, Chen et al. found the elevated cTnI and NT-proBNP during hospitalization were significantly correlated with the critical disease status (Chen Chen et al., 2020a). Unfortunately, we did not test these two markers dynamically, the value of them deserve further investigation.

A blood gas analysis can measures the level of oxygen, carbon dioxide, lactate and pH in the blood, which is helpful to show how well the lungs are working. A previous study has indicated that higher level of lactate and lower levels of pO_2 and pCO_2 were found in the death patients with COVID-19 (Peng et al., 2020). The present study also showed lower levels of pO_2 and pCO_2 in the severe group, but pH and lactate showed no differences between the two groups. However, only few patients in the non-severe group performed blood gas analyses and partial patients received oxygen therapy before blood gas analysis, thus making it difficult to explore their role in the present study.

However, this study has some limitations. First, IL-6 is not a routine laboratory test, which would limit its usage. Second, the sample size included in this study is relatively small, especially of those in severe group. Third, it is a retrospective single-center study, the effectiveness of this model has not been validated by us and others. Forth, some laboratory analyses (e.g. blood gas analysis) were performed on partial patients. Therefore, a prospective multi-center study with a larger sample size is warranted.

5. Conclusion

Our study demonstrated that high level of peripheral blood cytokine IL-6, CRP and the existence of hypertension were independent risk factors for assessing the severity of COVID-19. The risk model established upon IL-6, CRP and hypertension had the highest predictability value in this study. Application of this model might be beneficial to delay or halt the progression of the disease. Besides, IL-6 played a pivotal role in the severity of COVID-19 and had a potential value for monitoring the process of severe cases. It reminds us to emphasize the cytokine storm in the progression of COVID-19, and IL-6 blockade treatment maybe a novel therapeutic strategy for treating the severe patients.

Authors' Contribution

Ethics approval

Conflicts of interest

Funding

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