Characteristics of Lymphocyte Subsets and Cytokine Profiles of Patients with Coronavirus Disease 2019

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Research

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Abstract

Background: To explore the changes in lymphocyte subsets and cytokine profiles in patients with coronavirus disease 2019 (COVID-19) and their relationship with disease severity.

Methods: This study included 228 patients with COVID-19 who were treated at Chongqing University Three Gorges Hospital from January 1, 2020 to February 20, 2020. The characteristics of lymphocyte subsets and cytokine profiles of severe and mild COVID-19 patients were compared. Of the 228 patients enrolled, 48 were severe patients and 180 were mild patients.

Results: Lymphocyte counts, absolute number of total T lymphocytes, CD^{4+}T cells, CD^{8+}T cells, and total B lymphocytes were significantly lower in severe patients (0.8×10^9/L, 424.5×10^6/L, 266×10^6/L, 145.5×10^6/L, 109.5×10^6/L, respectively) than in mild patients (1.2×10^9/L, 721×10^6/L, 439.5×10^6/L, 281.5×10^6/L, 135×10^6/L, respectively). A multivariate logistic regression analysis showed that age, C-reactive protein (CRP) and the neutrophil-to-lymphocyte ratio (NLR) were independent risk factors for developing into severe condition. The lymphocyte subsets decreased and cytokine profiles increased more significantly in severe patients than in mild patients.

Conclusions: CRP, NLR, and age may serve as powerful factors for early identification of severe patients.

Background

An epidemic caused by the 2019 novel coronavirus (2019-nCoV) has been seriously threatening human health and global public health security [1–3]. Initially the lungs were considered to be the commonly damaged organs. Therefore, the disease caused by 2019-nCoV was named as novel coronavirus pneumonia in China. However, the virus can also affect the nervous system, digestive system, urinary system, blood system, and other systems. On February 11, the World Health Organization (WHO) declared the disease caused by 2019-nCoV as coronavirus disease 2019 (COVID-19) as a consequence. COVID-19 is a new emerging infectious disease that quickly spreads and poses significant threats to global public health [4–6]. In general, most patients with COVID-19 did not become critically ill and recovered quickly. However, it can lead to death, with a 1.4% case fatality rate [7]. Severe patients may die from respiratory failure due to massive alveolar damage. Recently, the Surviving Sepsis Campaign COVID-19 panel has issued several recommendations to help support healthcare workers caring for critically ill ICU patients with COVID-19 [8].

Coronaviruses belong to the virus family Coronaviridae and are enveloped, positive-sense RNA viruses [9]. They can infect various kinds of host species, including humans and other vertebrates [10]. The viruses primarily cause respiratory infections and lead to a series of clinical manifestations. The 2019-nCoV is a β CoV of group 2B and has 79.5% similarity, at least in its genetic sequence, with SARS-CoV [11]. Currently, it is commonly believed that angiotensin converting enzyme II (ACE2) is the cell receptor for SARS-CoV [11]. It has been reported that the incubation period of COVID-19 is approximately three to seven days. In the early stages, most of patients have mild symptoms, but some of them develop acute respiratory distress syndrome (ARDS), rapid acute respiratory failure, and even multiple organ failure. Therefore, preparing intensive care units to respond in crisis is also of great importance [12].

Immune disorders and cytokine storm were considered to be the main causes of damage (Fig. 1). Lymphocyte and cytokine changes in peripheral blood have been noted as one of the prominent characteristics in patients with COVID-19. Significant lymphocytopenia has been observed during the acute phase of COVID-19, and the degree of lymphocyte decrease and increase in cytokines has seemed to be associated with disease severity [13]. In the present study, the aim is to describe the changes in the peripheral blood lymphocyte subsets and cytokine profiles in patients with COVID-19 and to compare their features between severe and mild patients.

Methods And Materials

Study Design, Setting, and Population

This was a single-center, retrospective, cohort study. The study was approved by the institutional ethics board of Chongqing Three Gorges Central Hospital, Chongqing, China. The Chongqing Three Gorges Central Hospital is one of the major tertiary teaching hospitals and is responsible for the treatment of COVID-19 assigned by the government. Clinically and laboratory diagnosed patients with COVID-19 admitted to Chongqing Three Gorges Central Hospital from January 1, 2020 to February 20, 2020 were analyzed in this study. Two cohorts were generated according to diagnosis and treatment of pneumonia caused by novel coronavirus infection issued by National Health Commission of the People's Republic of China, severe group and mild group. Mild patients met all following conditions: (1) Epidemiological history, (2) Fever or other respiratory symptoms, Typical CT image abnormities of viral pneumonia, and (4) Positive result of RT-PCR for SARS-CoV-2 RNA. Severe patients additionally met at least one of the following conditions: (1) Shortness of breath, respiratory rate ≥30 times/min, (2) Oxygen saturation (Resting state) ≤93%, or (3) PaO_2/FiO_2 ≤300mmHg.Written informed consent was waived by the ethics board of the hospital for emerging infectious diseases and oral consent was obtained from the patients.

Data Collection

Researchers responsible for data collecting were trained before the study began so that they could correctly fill out the case report form and reduce errors. Patient demographic characteristics, such as age, gender, baseline comorbidities, epidemiological history, clinical, and laboratory data, were obtained on the day of admission. Symptoms, vital signs, treatment measures, and radiological data were also collected. The two researchers collected data independently and checked each other's form for mistakes. Clinical outcomes were followed until February 29, 2020.

Statistical Analysis

Categorical variables were described as frequency rates and percentages, and continuous variables were described using the mean, median, and interquartile range (IQR) values. Means for continuous variables were compared using independent group t-tests when the data were normally distributed; otherwise, the Mann-Whitney test was used. For comparisons, a two-sided α of less than 0.05 was considered to be

statistically significant. A univariate analysis was used to compare the risk factors for developing to critical condition. A multiple logistic regression analysis was used to screen independent risk factors that affect outcomes. The diagnostic values of selected parameters for differentiating severe and mild patients were assessed by the receiver operating characteristic (ROC) and the area under the ROC curve (AUC). Cut-off values were identified following the Youden's index of the ROC curve. All of the statistical analyses were conducted using SPSS, Version 17.0.

Results

Baseline Characteristics

A total of 259 patients with confirmed COVID-19 were included, 31 of whom were excluded due to lack of cytokine data. Finally, our cohort consisted of 228 patients (Fig. 2). The median age was 48.5 years (IQR, 37–56), and 127 (55.7%) were men. Of the 228 patients, 62 (27.2%) had one or more coexisting medical conditions. Hypertension (16 [7.2%]), diabetes (11 [4.8%]), cardiovascular disease (7 [3.1%]), and chronic gastritis (11 [4.8%]) were the primary coexisting conditions. Cough (142 [62.3%]) was the most common symptom. Almost half of the patients had a fever (117 [51.3%]). Other symptoms included expectoration (61 [26.8%]), fatigue (49 [21.5%]), shortness of breath (42 [18.4%]), chill (28 [12.3%]), anorexia (27 [11.8%]), myalgia (26 [11.4%]), and other symptoms (Table 1).

Baseline characteristics comparison of severe and mild patients

Of these 228 patients, 48 (21.1%) were severe patients and 180 (78.9%) were mild patients. Compared with the mild group, patients in the severe group were significantly older (median age, 58.5 years [IQR, 50.3–74.8] vs 46 years [IQR, 34–54]; $p < 0.001$) and were more likely to have underlying comorbidities (22 [45.8%] vs 37 [20.6%], $p = 0.002$). There was no significant difference between the two cohorts in hypertension (4 [4.8%] vs 14 [6.7%] and cardiovascular disease (3 [6.3%] vs 4 [2.2%]). There were more diabetes patients in severe group than in mild group (9 [8.6%]) vs 2 [1.1%], $p < 0.001$). Compared with mild patients, the severe patients were more likely to report fever, fatigue, cough, dyspnea, chest pain, and shortness of breath (Table 1).

Table 1
Baseline Characteristics of Patients with COVID-19

	No. (%)			*p* Value
	Total(n = 228)	**Severe(n = 48)**	**Mild (n = 180)**	
Age, median (IQR), yr	**48.5(37,56)**	**58.5(50.3,74.8)**	**46.0(34.0,54.0)**	< 0.001
Sex				0.745
Female, n (%)	101(44.3)	20(41.7)	81(45.0)	
Male, n (%)	127(55.7)	28(58.3)	99(55.0)	
Comorbidities	62(27.3)	22(45.8)	37(20.6)	0.002
Hypertension, n (%)	16(7.2)	4(8.3)	12(6.7)	0.750
Cardiovascular disease, n (%)	7(3.1)	3(6.3)	4(2.2)	0.164
Diabetes, n (%)	11(4.8)	9(8.6)	2(1.1)	< 0.001
Malignancy, n (%)	2(0.9)	2(4.2)	0(0)	0.044
Cerebrovascular disease, n (%)	3(1.3)	1(2.1)	2(1.1)	0.510
COPD, n (%)	3(1.3)	2(4.2)	1(0.6)	0.113
Asthma, n (%)	1(0.4)	1(2.1)	0(0)	0.211
Emphysema, n (%)	3(1.3)	2(4.2)	1(0.6)	0.113
Tuberculosis, n (%)	4(1.8)	1(2.1)	3(1.7)	1.000
Chronic liver disease, n (%)	4(1.8)	1(2.1)	3(1.7)	1.000
Chronic kidney disease, n (%)	1(0.4)	1(2.1)	0(0)	0.211
Chronic gastritis, n (%)	11(4.8)	2(4.2)	9(5.0)	1.000
Signs and symptoms				
Fever, n (%)	117(51.3)	31(64.6)	86(47.8)	0.008
Fatigue, n (%)	49(21.5)	18(37.5)	31(17.2)	0.005
Cough, n (%)	142(62.3)	39(81.3)	103(57.2)	0.002
Expectoration, n (%)	61(26.8)	18(37.5)	43(23.9)	0.068
Anorexia, n (%)	27(11.8)	7(14.6)	20(11.1)	0.615
Myalgia, n (%)	26(11.4)	9(18.8)	17(9.4)	0.079
Dyspnea, n (%)	7(3.1)	5(10.4)	2(1.1)	0.005
Chest distress, n (%)	19(8.3)	7(14.6)	12(6.7)	0.085

	No. (%)			*p* Value
	Total(n = 228)	Severe(n = 48)	Mild (n = 180)	
Chest pain, n (%)	6(2.6)	4(8.3)	2(1.1)	0.019
Diarrhea, n (%)	14(6.1)	3(6.3)	11(6.1)	1.000
Short of breath, n (%)	42(18.4)	18(37.5)	24(13.3)	0.001
Chill, n (%)	28(12.3)	9(18.8)	19(10.6)	0.139
Nausea, n (%)	9(3.9)	3(6.3)	6(3.3)	0.402
Vomiting, n (%)	6(2.6)	2(4.2)	4(2.2)	0.609
Dizziness, n (%)	22(9.6)	6(12.5)	16(8.9)	0.421
Headache, n (%)	21(9.2)	5(10.4)	16(8.9)	0.780
Abdominal pain, n (%)	4(1.8)	2(4.3)	2(1.1)	0.196

Laboratory Findings

There were obvious differences in the laboratory findings between the severe and mild patients. Abnormalities in the white blood cell (WBC) counts were not observed in most patients. Neutrophil counts were significantly higher in severe patients (median 4.4×10^9/L, IQR [3.1–5.9] $\times 10^9$/L) than in mild patients (median 3.5×10^9/L, IQR [2.5–4.4] $\times 10^9$/L, $p = 0.001$). A majority of patients had a decrease in lymphocytes (191 [83.8%]), and the lymphocyte decrease in the severe group (median 0.8×10^9/L, IQR [0.6–1.1] $\times 10^9$/L) was more significant than that in the mild group (median 1.2×10^9/L, IQR [0.9–1.6] $\times 10^9$/L, $p < 0.001$). The absolute number of total T lymphocytes, CD4 + T cells, CD8 + T cells, and total B lymphocytes were significantly lower in severe patients (0.8×10^9/L, 424.5×10^6/L, 266×10^6/L, 145.5×10^6/L, 109.5×10^6/L, respectively) than in mild patients (1.2×10^9/L, 721×10^6/L, 439.5×10^6/L, 281.5×10^6/L, 135×10^6/L, respectively). IL-6 was significantly higher in severe patients (19 pg/mL) than in mild patients (3.1 pg/mL) (Fig. 3)

More patients in the severe group (27.1%) had thrombocytopenia than those in the mild group (13.3%, $p = 0.028$). The severe patients had a higher activated partial thromboplastin time ($p < 0.001$), d dimer ($p < 0.001$), direct bilirubin ($p = 0.003$), and AST ($p < 0.001$) than mild patients. C-reactive protein (CRP) was significantly higher in severe patients (81.6 mg/L) than in mild patients (5.3 mg/L). The procalcitonin (PCT) of the majority of patients was in the normal range **(**Table 2**).**

Table 2
Laboratory findings of patients with COVID-19

Normal Range		No. (%)			*p* Value
		Total (n = 228)	Severe (n = 48)	Mild (n = 180)	
Blood routine and lymphocyte classification					
WBC, median (IQR), $X10^9/L$	3.5–9.5	5.2(4.1,6.7)	5.5(4.5,7.2)	5.2(4.1,6.7)	0.152
Neutrophil,$\times10^9/L$	1.8–6.3	3.5(2.6,4.8)	4.4(3.1,5.9)	3.5(2.5,4.4)	0.001
Lymphocyte,$\times10^9/L$	1.1–3.2	1.1(0.8,1.6)	0.8(0.6,1.1)	1.2(0.9,1.6)	< 0.001
NLR	NA	2.9(2.0,5.3)	6.1(3.4,8.2)	2.5(1.8,4.0)	< 0.001
Lymphocytopenia, n (%)	NA	191(83.8)	47(97.9)	144(80)	0.03
Monocyte, $\times10^9/L$	0.1–0.6	0.4(0.3,0.5)	0.3(0.2,0.5)	0.4(0.3,0.5)	0.040
Platelet count,$\times10^9/L$	125–350	182.0(140,241)	161.5(118.5,239.3)	186.5(147.3,241.0)	0.136
Thrombopenia, n (%)	NA	37(16.2)	13(27.1)	24(13.3)	0.028
Total T lymphocytes,$\times10^6/L$	699 – 2.540	649(430.3,942.5)	424.5(250.8,594)	721(526.3,1000)	< 0.001
CD4 + T cells,$\times10^6/L$	410–1590	390(272.3,590.8)	266(153.3,370.5)	439.5(303.5,636.5)	< 0.001
CD8 + T cells,$\times10^6/L$	190–1140	250.5(162,381.5)	145.5(100.5,223.5)	281.5(189.3,418.8)	< 0.001
CD4+/CD8+	0.7–2.87	1.6(1.2,2.0)	1.6(1.1,2.4)	1.6(1.2,2.0)	0.896
Total B lymphocytes,$\times10^6/L$	90–660	131.5(88,199.8)	109.5(72.3,146.5)	135(92,218.8)	0.011
NK,$\times10^6/L$	90–590	134.5(86.3,216.0)	101.0(63.8,169.8)	141.5(92.8,235.8)	0.008
Cytokine profiles					

Normal Range		No. (%)			*p* Value
		Total (n = 228)	Severe (n = 48)	Mild (n = 180)	
IL-6, pg/mL	0-5.4	4.3(0,11.4)	19(5.4,46.5)	3.1(0,6.7)	< 0.001
IL-4, pg/mL	0-8.56	1.7(1.3,2.5)	1.7(1.3,2.5)	1.7(1.4,2.5)	0.461
IL-10, pg/mL	0-12.9	2.7(2.4,3.7)	3.8(3,5.2)	2.6(2.3,3.1)	< 0.001
IL-17, pg/mL	0-21.4	1.2(1.0,1.4)	1.2(1.0,1.4)	1.2(1.0,1.4)	0.258
TNF-α, pg/mL	0-16.5	3.5(1.9,6.4)	3.0(1.4,5.6)	3.7(2.0,6.6)	0.202
IFN-γ, pg/mL	0-23.1	4.2(1.9,8.3)	4.8(2.0,8.8)	4.2(1.8,7.9)	0.449
Blood biochemistry					
Urea nitrogen, mmol/L	3.1-8	4.0(3.1,5.2)	4.0(2.9,5.9)	4.0(3.2,5.0)	0.966
Creatinine, mmol/L	57–97	65.5(53,75)	63(50,74.5)	66(55,75.3)	0.274
TBil, umol/L	0–26	9.6(6.2,15.8)	10.7(6.9,15.3)	9.4(5.7,15.8)	0.259
DBIL, umol/L	0–8	4.5(3.2,6.4)	5.3(4.1,8.6)	4.3(3.0,6.2)	0.003
ALT, U/L	9–50	20.8(13.8,33.8)	23.3(12.7,36.5)	19.3(13.8,33.1)	0.313
AST, U/L	15–40	23.1(17.2,33.5)	33.9(23.9,44.9)	20.9(16.6,28.5)	< 0.001
CK, U/L	50–310	61(42,90.8)	77.8(40,154.8)	60(42.8,86)	0.081
CKMB, U/L	0–25	12.5(10,16)	14(9.5,15.5)	12(10,16)	0.904
Infection related parameters					
CRP, mg/L	0–8	10(2,48.9)	81.6(47.9,130.0)	5.3(1.5,20)	< 0.001
PCT, ng/mL	< 0.046	0.04(0.03,0.07)	0.09(0.06,0.15)	0.04(0.03,0.06)	< 0.001
Coagulation function					
PT, s	8–14	11.1(10.6,11.5)	11.3(10.7,11.9)	11.1(10.6,11.4)	0.006

Normal Range		No. (%)			*p* Value
		Total (n = 228)	Severe (n = 48)	Mild (n = 180)	
APTT, s	20–40	26.6(24.7,29.4)	29(26.5,33.6)	26.1(24,28.7)	< 0.001
D-dimer, mg/L	0-0.55	0.3(0.2,0.6)	0.6(0.4,1.2)	0.3(0.2,0.5)	< 0.001

Multivariate logistic regression model for disease severity

The multivariate logistic regression analysis showed that age (odds ratio 1.053 [1.018–1.090]), CRP (odds ratio 1.043 [1.043–1.057]), and the neutrophil-to-lymphocyte ratio (NLR) (odds ratio 1.200[1.076–1.338]) were independent risk factors for developing to severe condition. (Table 3). The receiver operating characteristic (ROC) curve and area under the ROC curve (AUC) were calculated. The results of this analysis identified CRP with a higher AUC (0.90) than NLR (0.81) and age (0.77). The cutoff values were calculated from the ROC curves, with a value of 32.9 for CRP (Specificity: 83.1%, Sensitivity: 85.4%), 2.9 for NLR (59%, 87.5%), and 53.5 for age (74.2%, 70.8%) (Fig. 4).

Table 3
Multivariate logistic regression model for developing to severe condition

	OR	95% *CI*	*p* value
Age	1.053	1.018, 1.090	0.003
C-reactive protein	1.043	1.043, 1.057	< 0.001
NLR	1.200	1.076, 1.338	0.001

Discussion

COVID-19 is a novel infectious disease that is seriously threatening human health and global public health security. According to the report from the Chinese Center for Disease Control and Prevention, the mortality was 2.3%. However, this figure increased to 49.0% among critical cases [14]. Thus, it is of great significance to study the laboratory data and the clinical development of the disease in order to guide management.

In this study, the clinical manifestations and laboratory data of severe and mild patients with COVID-19 were compared. In addition, the characteristics of lymphocyte subsets and cytokine profiles of peripheral blood in the enrolled patients were analyzed. It was found that most of severe patients were older than mild patients. In addition, the severe group had more patients with basic diseases than the mild group, which means that older patients, in particular those with basic diseases, such as hypertension and diabetes, may be more likely to develop severe COVID-19. These findings are consistent with several previous studies [13, 15]. Fever, cough, and expectoration were found to be the most common symptoms. However, the above symptoms did

not appear in some patients. In addition, some patients had symptoms in the digestive system or nervous system only.

In terms of laboratory findings, a majority of COVID-19 patients suffered from lymphocytopenia. This study showed that lymphocytopenia occurred in 97.9% of severe patients and 80% of mild patients. Specifically, lymphocyte counts, absolute number of total T lymphocytes, CD4+T cells, CD8+T cells, and total B lymphocytes were significantly lower in severe patients than mild patients. The decrease in lymphocyte and lymphocyte subsets was related to the severity of the disease. Cellular immunity is an important part of the human immune system in a viral infection. It has been confirmed that cell-mediated specific cellular immunity plays a crucial role in the process of virus elimination and killing [16].

Increasing evidence suggests that lymphocytes play a crucial role in airway diseases [17, 18]. Previous studies have shown that a marked lymphocytopenia also occurred in a majority of the patients during the acute phase of severe acute respiratory syndrome (SARS) and middle east respiratory syndrome (MERS), with CD4+ and CD8+T cell subsets particularly affected. In addition, the degree of decrease in the T lymphocytes was found to be associated with disease severity [19-23]. However, the mechanisms by which they cause lymphocyte changes were different. Previous data and the results of this study found that there was a remarkable decrease in lymphocyte subsets in COVID-19 patients, especially in severe patients. Currently, the pathophysiological mechanism of lymphocyte decline in COVID-19 patients remains unclear and further investigations are required. Although there is insufficient knowledge to understand the mechanism, cellular immunodeficiency may be one of the primary immunopathologic changes in COVID-19 patients.

Early studies have documented that cytokine storms, also known as inflammatory storms, have occurred in a large number of patients with COVID-19. In SARS patients, increased amounts of proinflammatory cytokines in the serum, such as IL1B, IL6, IL12, IFN-γ, IP10, and MCP1, were observed and were considered to be related to the pulmonary inflammation and extensive lung damage, and even multiple organ failure [24]. Study showed that MERS patients also had increased concentrations of proinflammatory cytokines (IFN-γ, TNF-α, IL15, and IL17) [25]. Recent data have indicated that patients with COVID-19 also had high concentrations of serum cytokine profiles, such as TNF-α, IL-1, IL-6, and IFN-γ [13, 15]. In clinical work, it was found that the course of disease and lung lesions progressed rapidly, and even multiple organ failure developed in a short time in some patients with a high concentration of cytokines. In this study, it was noted that patients typically had increased concentrations of serum IL-6. Moreover, concentrations of serum IL-6 was significantly higher in severe patients than in mild patients, and this agreed with the concept of a cytokine storm [26]. However, IL-4, IL-10, IL-17, TNF-α, and IFN-γ were all nearly in the normal range. Although the exact mechanism of changes in the cytokines remains to be elucidated, a higher concentration of serum cytokine seems to be associated with poor outcomes. Therefore, monitoring the changes in cytokines is of certain significance for the early detection and management of critically ill patients.

Early screening of critically ill patients may improve clinical outcomes. In the current study, the clinical and laboratory features of patients with COVID-19 were explored. The enrolled patients were divided into two cohorts based on disease severity. Baseline characteristics, clinical presentation, and laboratory data were

compared between severe and mild patients. A multivariate logistic regression analysis and a ROC curve analysis were further performed. In addition, AUC and cutoff values were calculated. It was found that patients with CRP > 32.9 mg/L, NLR > 2.9, and age > 53.5 years tended to develop into a severe condition. CRP is an acute-phase reactant that increases in the circulation in response to a variety of inflammatory stimuli. CRP has traditionally been considered a biomarker of bacterial infection. However, evidence is needed to distinguish bacterial and viral infections with CRP. A study indicated that COPD patients with lower plasma CRP and IL-6 levels had lower grade systemic inflammation and better physical activity [23]. A systematic review suggested that the average CRP levels upon diagnosis were significantly higher in patients who developed severe H1N1 influenza compared to their counterparts with a no severe disease. Furthermore, levels of CRP have been associated with the degree of H1N1 severity [27]. It was noted in this study that a majority of patients with COVID-19 had increased levels of CRP. In this study, CRP was significantly higher in severe patients than mild patients, which indicated that CRP may be associated with disease severity. The results of this study showed that CRP was the most significant factor that affected the incidence of severe illness, and it had a significant predictive value. The AUC of CRP was 0.90, and the cutoff value was 32.9 (Specificity: 83.1%, Sensitivity: 85.4%). A recent study showed that the NLR was the most useful prognostic factor that affected the prognosis for severe patients with COVID-19. In this study, NLR was significantly higher in severe group than mild group. NRP can also be considered as one of the warning indexes for critically ill patients. Our study revealed that patients with a CRP > 32.9 mg/L, NLR > 2.9, and age > 53.5 years tended to develop into the severe condition.

In summary, the characteristics of lymphocyte subsets and cytokine profiles between severe and mild patients with COVID-19 were compared in this study. On the basis of the results, risk factors for patients developing a severe condition were identified. This is helpful to identify high-risk patients as early as possible and to provide intensive monitoring and treatment, ultimately to reduce the mortality rate of COVID-19 patients.

This study has several potential limitations. First, the retrospective single-center design leads to missing data and unavoidable biases. However, researchers responsible for data collection were trained before the study began so that they could correctly fill out the case report form and reduce errors. In addition, two researchers collected data independently and checked each other's forms for mistakes so as to minimize the bias as much as possible. Second, data were not collected continuously during the patients' hospitalization, and as a consequence, the trend of these clinical and laboratory indicators could not be described. Fortunately, all of the data was recorded in our electronic medical record system, and we will extract and collect parameters needed for further study in the future.

Conclusions

The degree of lymphocyte subsets decreased and cytokine profiles increased in severe patients as compared to mild patients. The CRP, NLR, and age may serve as powerful prognostic factors for the early identification of severe patients.

Abbreviations

ACE, angiotensin converting enzyme; APTT, activated partial thromboplastin time; ARDS, acute respiratory distress syndrome; AUC, area under curve; CK, creatine kinase; CKMB, creatine kinase-MB; COVID-19, coronavirus disease 2019; COPD, chronic obstructive pulmonary disease; CRP, C-reactive protein; DBil, direct bilirubin; ICU, intensive care unit; IFN-γ, interferon-γ; IQR, inter quartile range; MERS, Middle East respiratory syndrome; NLR, neutrophil-to-lymphocyte ratio; PCT, procalcitonin; PT, prothrombin time; ROC, receiver operating characteristic curve; SARS, severe acute respiratory syndrome; TBil, total bilirubin; TNF-α, tumor necrosis factor-α; WBC, white blood cell; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; L, lymphocyte; Total T, Total T lymphocytes; CD4+, CD^{4+} T cells; CD^{8+}, CD^{8+} T cells; Total B, Total B lymphocytes; NK, natural killer; APTT, activated partial thromboplastin time; OR, odds ratio; CI, confidence interval.

Declarations

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Authors' contributions

Conceived and designed the study: Xiangyou Yu, Yong Cui. Performed the experiments: Qilong Zhou, Xiaochun Deng, Chao Liu. Analyzed and interpreted the data: Xinxin Du, Li Li. Contributed reagents/materials/analysis tools: Chao Liu, Zongjun Hu, Jianguo Chen. Drafted the manuscript: Pengfei Pan, Xinxin Du, Weihua Shi. All authors reviewed and approved the final version of the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study has been approved by the Ethics Committee of Chongqing Three Gorges Central Hospital, Chongqing, China. (number: 2020–023).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020;579:265–9.
2. Lu H, Stratton CW, Tang YW. Outbreak of pneumonia of unknown etiology in Wuhan, China: The mystery and the miracle. J Med Virol. 2020;92:401–2.
3. Coronavirus Infections-More Than Just the Common Cold
   Paules CI, Marston HD, Fauci AS. Coronavirus Infections-More Than Just the Common Cold. JAMA 2020.
4. Hui DS, Azhar EI, Madani TA, et al. The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health-The latest 2019 novel coronavirus outbreak in Wuhan, China. Int J Infect Dis. 2020;91:264–6.
5. Mahase E. Coronavirus covid-19 has killed more people than SARS and MERS combined, despite lower case fatality rate. BMJ. 2020;368:m641.
6. Ronco C, Navalesi P, Vincent JL. Coronavirus epidemic: preparing for extracorporeal organ support in intensive care. Lancet Respir Med. 2020;8:240–1.
7. Guan WJ, Ni ZY, Hu Y, et al., Clinical Characteristics of Coronavirus Disease 2019 in China, N Engl J Med 2020.
8. Alhazzani W, Møller MH, Arabi YM, et al. Surviving Sepsis Campaign: Guidelines on the Management of Critically Ill Adults with Coronavirus Disease 2019 (COVID-19). Crit Care Med. 2020;48:e440–69.

9. Yin Y, Wunderink RG. MERS, SARS and other coronaviruses as causes of pneumonia. Respirology. 2018;23:130–7.
10. Su S, Wong G, Shi W, et al. Epidemiology, Genetic Recombination, and Pathogenesis of Coronaviruses. Trends Microbiol. 2016;24:490–502.
11. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579:270–3.
12. Daugherty EL, Rubinson L. Preparing your intensive care unit to respond in crisis: considerations for critical care clinicians. Crit Care Med. 2011;39:2534–9.
13. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020;323:1061–9.
14. Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention, JAMA 2020. [published online ahead of print, 2020 Feb 24].
15. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395:497–506.
16. Channappanavar R, Zhao J, Perlman S. T cell-mediated immune response to respiratory coronaviruses. Immunol Res. 2014;59:118–28.
17. Kato A. Group 2 Innate Lymphoid Cells in Airway Diseases. Chest. 2019;156:141–9.
18. Moldaver DM, Larché M, Rudulier CD. An Update on Lymphocyte Subtypes in Asthma and Airway Disease. Chest. 2017;151:1122–30.
19. Cui W, Fan Y, Wu W, et al. Expression of lymphocytes and lymphocyte subsets in patients with severe acute respiratory syndrome. Clin Infect Dis. 2003;37:857–9.
20. Peiris JS, Chu CM, Cheng VC, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. Lancet. 2003;361:1767–72.
21. de Wit E, van Doremalen N, Falzarano D, et al. SARS and MERS: recent insights into emerging coronaviruses. Nat Rev Microbiol. 2016;14:523–34.
22. Li T, Qiu Z, Zhang L, et al. Significant changes of peripheral T lymphocyte subsets in patients with severe acute respiratory syndrome. J Infect Dis. 2004;189:648–51.
23. Moy ML, Teylan M, Weston NA, et al. Daily step count is associated with plasma C-reactive protein and IL-6 in a US cohort with COPD. Chest. 2014;145:542–50.
24. Wong CK, Lam CW, Wu AK, et al. Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. Clin Exp Immunol. 2004;136:95–103.
25. Mahallawi WH, Khabour OF, Zhang Q, et al. MERS-CoV infection in humans is associated with a pro-inflammatory Th1 and Th17 cytokine profile. Cytokine. 2018;104:8–13.
26. Channappanavar R, Perlman S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. Semin Immunopathol. 2017;39:529–39.
27. Vasileva D, Badawi A. C-reactive protein as a biomarker of severe H1N1 influenza. Inflamm Res. 2019;68:39–46.

Figures

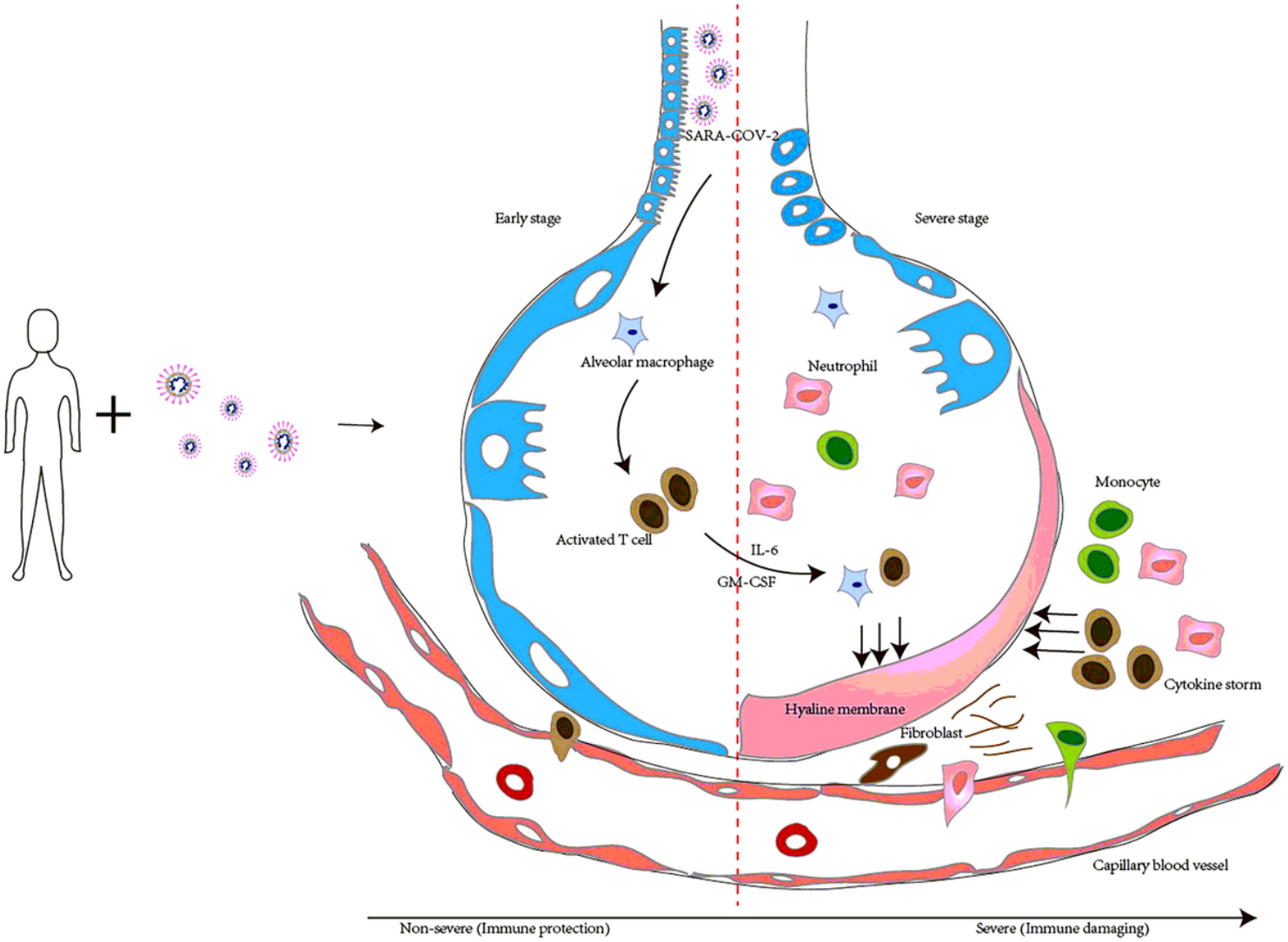

Figure 1

Immune disorders and cytokine storm were considered to be the main causes of damage

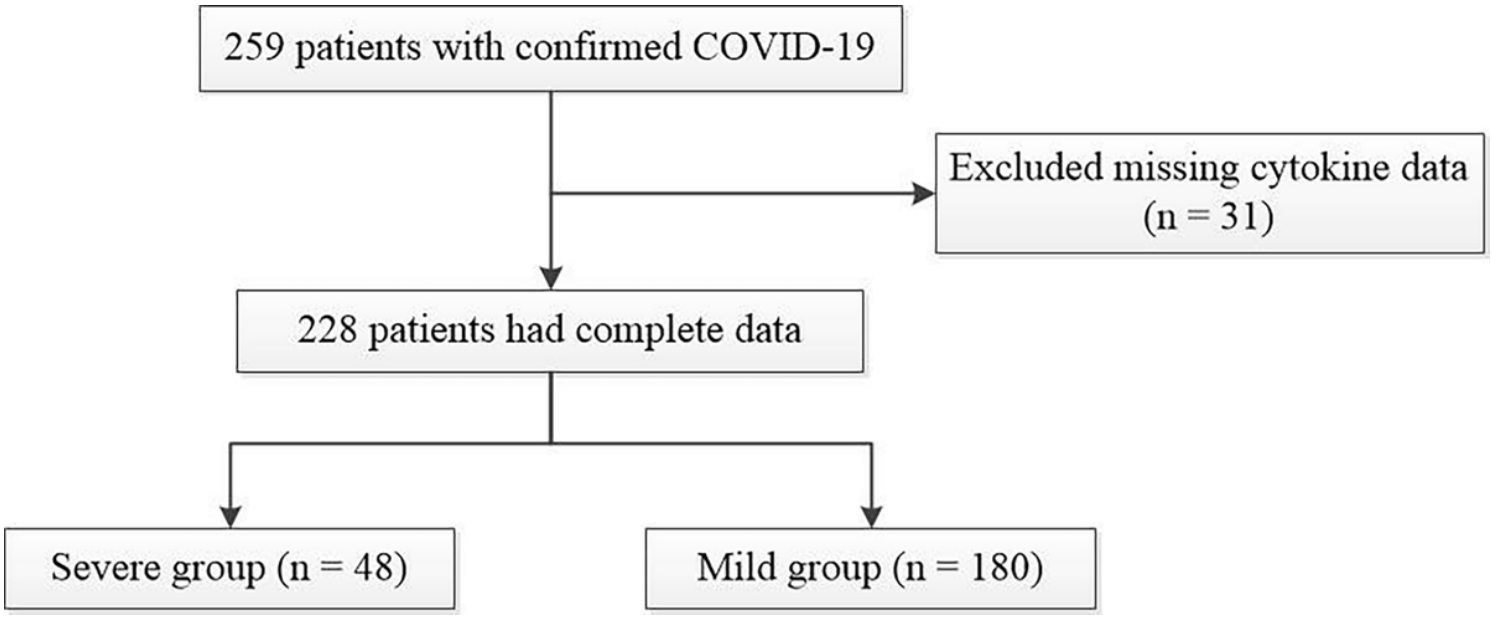

Figure 2

Flowchart of the study population

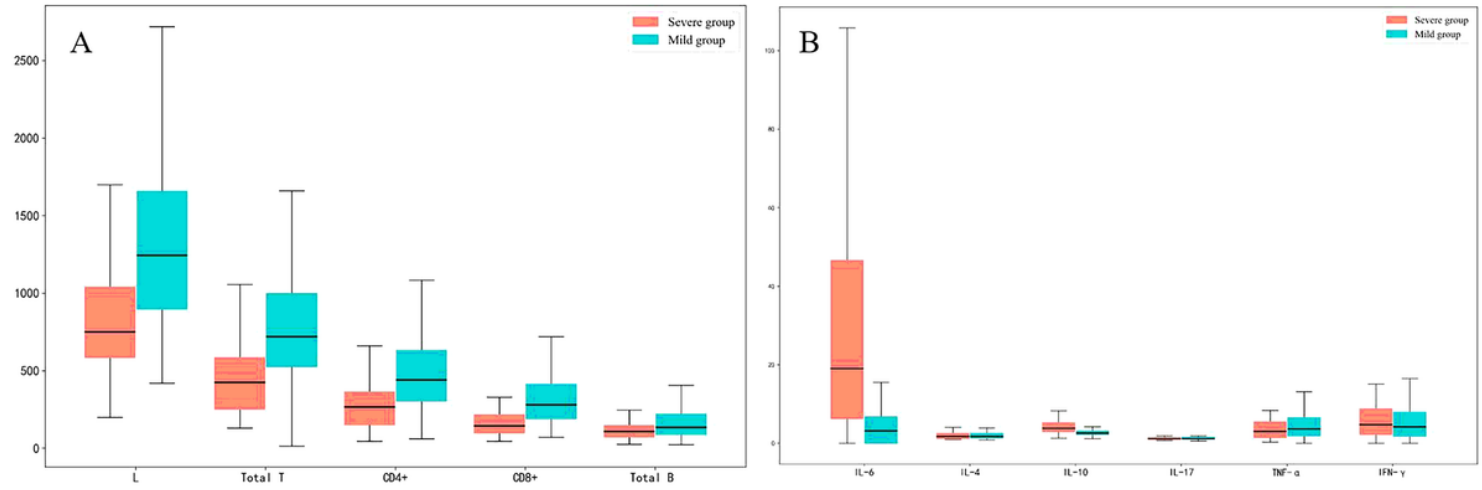

Figure 3

Comparison of lymphocyte subsets and cytokine profiles between severe and mild patients with COVID-19 A) Comparison of lymphocyte subsets. B) Comparison of cytokine profiles

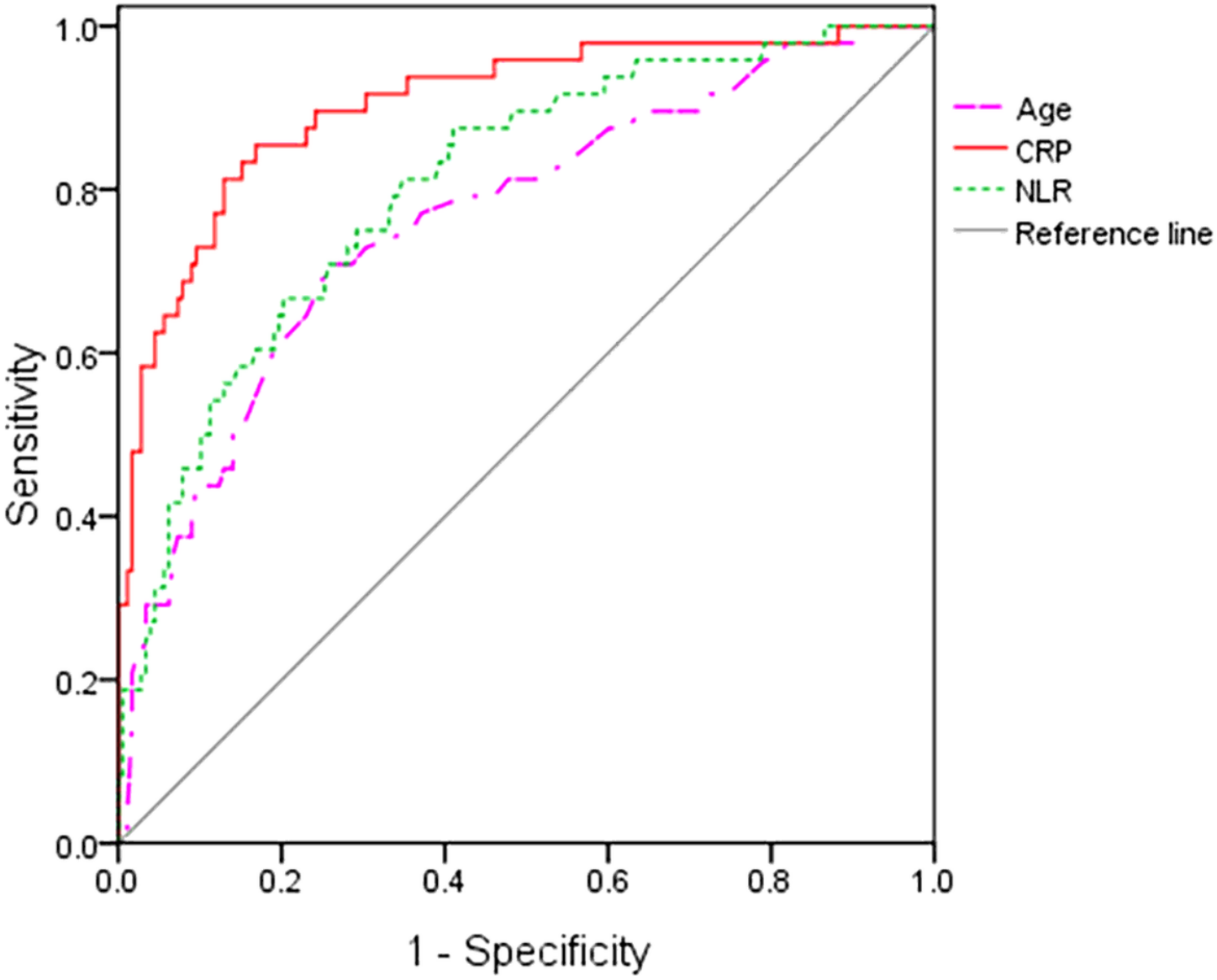

Figure 4

The ROC curve for predicting disease severity using CRP, NLR, and age