Characteristics of Lymphocyte Subsets and Cytokine Profiles of Patients With COVID-19

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Abstract

Background: Abnormalities of lymphocyte subsets and cytokine profiles have been observed in most patients with coronavirus disease (COVID-19). Here, we explore the role of lymphocyte subsets and cytokines at hospital admission in predicting the severity of COVID-19.

Methods: This study included 214 patients with COVID-19 who were treated at Three Gorges Hospital Affiliated with Chongqing University from January 19, 2020 to April 30, 2020. Patients were divided into the non-intensive care unit (ICU) (mild/moderate) group and the ICU (severe/critical) group, according to the severity of the disease. Clinical and laboratory data, including peripheral lymphocyte subsets and cytokines, were analyzed and compared. Logistic regression was used to analyze the predictive factors for ICU admission. Receiver operating characteristic (ROC) curves were drawn to evaluate the predictive value of selected indicators for the severity of COVID-19.

Results: Of the 214 patients enrolled, 161 were non-ICU patients and 53 were ICU patients. At hospital admission, lymphopenia was observed in nearly all of the ICU patients (96.2%) and 84.5% of the non-ICU patients. The absolute number of lymphocytes, $CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells, $CD19^+$ B cells, and natural killer (NK) cells was lower in the ICU group (659.00×10^6/L, 417.00×10^6/L, 261.00×10^6/L, 140.00×10^6/L, 109.00×10^6/L, 102.00×10^6/L, respectively) than in the non-ICU group (1063.00×10^9/L, 717.00×10^6/L, 432.00×10^6/L, 271.00×10^6/L, 133.00×10^6/L, 143.00×10^6/L, respectively). Interleukin (IL)-6 was significantly higher in the ICU patients than in the non-ICU patients (18.08 pg/mL vs. 3.13 pg/mL). Multivariate logistic regression analysis showed that age (odds ratio: 1.067 [1.034–1.101]), diabetes mellitus (odds ratio: 9.154 [2.710–30.926]), $CD3^+$ T cells (odds ratio: 0.996 [0.994–0.997]), and IL-6 (odds ratio: 1.006 [1.000–1.013]) were independent predictors for the development of severe disease. ROC curve analysis showed that the area under the ROC curve (AUC) of $CD3^+$ T cells and IL-6 was 0.806 (0.737–0.874) and 0.785 (0.705–0.864), respectively, and the cutoff values were 510.5×10^6/L (sensitivity, 71.7%; specificity, 79.5%) and 6.58 pg/mL (77.4%, 74.5%), respectively. There were no statistical differences among all tested indicators of lymphocyte subsets and cytokines between the severe group (n = 38) and the critical group (n = 15) at hospital admission or ICU admission.

Conclusions: The levels of lymphocyte subsets decreased and the level of IL-6 increased significantly in the ICU patients compared with the non-ICU patients. Therefore, the number of $CD3^+$ T cells and the level of IL-6 at hospital admission may serve as powerful factors for identifying patients who will have severe disease.

Introduction

The recent outbreak of severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) has led to the declaration of a pandemic, which has seriously threatened human health and global public health security [1–4]. Initially, the lung was considered to be the organ most commonly damaged by SARS-CoV-2. However, the virus can also affect the nervous system, digestive system, urinary system, blood system,

and other systems. On February 11, 2020, the World Health Organization declared the disease caused by SARS-CoV-2 as coronavirus disease (COVID-19). By June 20, more than 177 million individuals worldwide were infected [5]. Generally, most patients with COVID-19 did not become critically ill and recovered quickly; however, COVID-19 can also lead to death, with a case-fatality rate ranging from 0.7–10.8% [6–8]. It has been reported that the incubation period for COVID-19 is approximately 3–7 days. In the early stages, most patients have mild symptoms, but some of them develop acute respiratory distress syndrome, rapid acute respiratory failure, and even multiple organ failure. Therefore, preparing intensive care units (ICU) to respond to this crisis is of great importance. 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 [9].

SARS-CoV-2 is a novel β-coronavirus that has at least 79.6% genetic sequence similarity with SARS-CoV, the virus responsible for the SARS outbreak in 2003 [10]. SARS-CoV-2 reaches the host cells by the angiotensin-converting enzyme II receptor, which is mainly expressed on pulmonary epithelial cells, but also on lymphocytes and other cell types [11]. Currently, immune disorders and cytokine storms contribute to the pathogenesis and progression of COVID-19. Significant lymphocytopenia has also been observed during the acute phase of COVID-19 [12]. In addition, an increasing number of studies have shown changes in lymphocyte subsets, cytokines, and the dysregulation of the host immune response in patients with different severities of COVID-19 [13–15]. A previous meta-analysis demonstrated that severe COVID-19 is closely associated with a decrease in lymphocytes and lymphocyte subsets, as well as the elevation of C-reactive protein (CRP), procalcitonin (PCT), and cytokines, but not interleukin (IL) −1β and IL-17[16].

The aim of the current study was to describe the changes in peripheral blood lymphocyte subsets and cytokine profiles in patients with COVID-19, and to explore the role of these parameters at hospital admission in predicting the severity of COVID-19.

Methods

Study design and participants

This was a single-center, retrospective, cohort study. The study was approved by the Institutional Ethics Board of Three Gorges Hospital Affiliated with Chongqing University. Laboratory-confirmed patients with COVID-19 who were admitted to the hospital from January 19, 2020 to April 30, 2020 were enrolled in the study. Written informed consent was waived by the Ethics Board of the hospital for emerging infectious diseases, and oral consent was obtained from the patients.

Three Gorges Hospital Affiliated with Chongqing University, located in Wanzhou, Chongqing, is one of the major tertiary teaching hospitals and has been assigned by the government to be responsible for treating patients with COVID-19. All confirmed patients in the northeast area of Chongqing were admitted to our hospital.

In the study, patients who met any of the following criteria were excluded: (1) < 18 years old, and (2) lack of complete cytokine data. The included patients were divided into four groups, according to the Novel Coronavirus Pneumonia Treatment Scheme issued by the National Health Commission of the People's Republic of China (7th edition), at admission or during hospitalization as follows [17]: (1) mild patients, in whom all of the following conditions were met: (i) epidemiological history, (ii) with mild clinical symptoms and normal imaging findings in both lungs, and (iii) positive result of reverse transcription-polymerase chain reaction (RT-PCR) for SARS-CoV-2 RNA; (2) moderate patients, in whom any of the following conditions were met: (i) epidemiological history, (ii) fever or other respiratory symptoms, (iii) typical CT image abnormities of viral pneumonia, and (iv) positive result of RT-PCR for SARS-CoV-2 RNA; and (3) severe patients, in whom any of the following conditions were met in addition to (2), (i) shortness of breath, respiratory rate ≥ 30 times/min, (ii) resting oxygen saturation ≤ 93%, or (iii) oxygenation index (arterial oxygen tension/fractional inspired oxygen) ≤ 300 mmHg (1 mmHg = 0.133 kPa); (3) critical, in whom any of the following conditions were met in addition to (2) or (3), (i) need for mechanical ventilation due to respiratory failure, (ii) shock, or (iii) requiring ICU care, with simultaneous failure of other organs. All of the patients were divided into the non-ICU (mild/moderate) group and the ICU (severe/critical) group. The ICU group was subdivided into the direct-ICU group and the late-ICU group, according to whether or not the ICU patients were admitted directly to the ICU upon hospital admission.

Data collection

Researchers responsible for data collection were trained before the study began so that they could correctly fill out the case report forms and reduce errors. Data including demographic characteristics (age and sex), baseline comorbidities, clinical symptoms, laboratory findings at hospital admission or/and on ICU admission, severity assessment, and outcomes were obtained from the electronic medical records. The two researchers collected data independently and checked each other's forms for mistakes. Clinical outcomes were followed until June 30, 2020.

Statistical analysis

Categorical variables were described as frequency rates and percentages in each category, and compared using χ^2 test or Fisher's exact test. Continuous variables were described as medians with interquartile range (IQR) values, and compared using the Mann–Whitney *U* test or Wilcoxon signed-rank test. For comparisons, a two-sided α value of < 0.05 was considered statistically significant. Multiple logistic regression analysis was used to screen independent risk factors associated with severe/critical condition. The diagnostic values of selected parameters at hospital admission for differentiating the non-ICU and the ICU cases were assessed by receiver operating characteristic (ROC) curves and the area under the area under ROC curve (AUC). Cutoff values were identified following Youden's index of the ROC curve. All statistical analyses were conducted using SPSS 23.0 and GraphPad Prism 8.0.

Results

Baseline characteristics of patients with COVID-19

Patients with confirmed COVID-19 (n = 248) were admitted to our hospital, 31 of whom were excluded due to lack of cytokine data; therefore, 214 patients were included in the final analysis. The median age of the included patients was 49.00 years (IQR, 39.00–56.00), and 117 (54.7%) were men. Diabetes mellitus (24 [11.2%]), hypertension (16 [7.5%]), and underlying pulmonary diseases (10 [4.7%]) were the primary coexisting conditions, and cough (137 [64.0%]) was the most common symptom. Almost half of the patients had a fever (112 [52.3%]). Other symptoms included expectoration (60 [28.0%]), fatigue (49 [22.9%]), shortness of breath (41 [19.2%]), chill (28 [13.1%]), anorexia (27 [12.6%]), myalgia (26 [12.1%]), and other symptoms (Table 1).

Table 1
Comparison of baseline characteristics between the non-ICU and ICU groups

Characteristic	Total (*n* = 214)	Non-ICU group (*n* = 161)	ICU group (*n* = 53)	*P*-value
Sociodemographic				
Age, median (IQR), years	49.00 (39.00–56.00)	46.00 (37.50–54.00)	57.00 (50.50–75.50)	< 0.001
Male/female, n (%)	117 (54.7)/97 (45.3)	88 (54.7)/73 (45.3)	29 (54.7)/24 (45.3)	0.994
Coexisting disorders				
Hypertension, n (%)	16 (7.5)	11 (6.8)	5 (9.4)	0.746
Coronary heart disease, n (%)	7 (3.3)	3 (1.9)	4 (7.5)	0.116
Underlying pulmonary diseases, n (%)	10 (4.7)	4 (2.5)	6 (11.3)	0.023
Chronic liver or kidney disease, n (%)	5 (2.3)	3 (1.9)	2 (3.8)	0.599
Diabetes mellitus, n (%)	24 (11.2)	8 (5.0)	16 (30.2)	< 0.001
Malignancy, n (%)	2 (0.9)	0 (0.0)	2 (3.8)	0.060
Signs and symptoms at hospital admission				
Fever, n (%)	112 (52.3)	79 (49.1)	33 (62.3)	0.095
Chill, n (%)	28 (13.1)	19 (11.8)	9 (17.0)	0.332
Myalgia, n (%)	26 (12.1)	17 (10.6)	9 (17.0)	0.215
Fatigue, n (%)	49 (22.9)	29 (18.0)	20 (37.7)	0.003
Cough, n (%)	137 (64.0)	95 (59.0)	42 (79.2)	0.008
Expectoration, n (%)	60 (28.0)	39 (24.2)	21 (39.6)	0.030
Short of breath, n (%)	41 (19.2)	20 (12.4)	21 (39.6)	< 0.001
Dyspnea, n (%)	7 (3.3)	1 (0.6)	6 (11.3)	0.001
Chest tightness, n (%)	19 (8.9)	11 (6.8)	8 (15.1)	0.120
Chest pain, n (%)	6 (2.8)	2 (1.2)	4 (7.5)	0.034

IQR interquartile range, *NCT* negative conversion time

Characteristic	Total (*n* = 214)	Non-ICU group (*n* = 161)	ICU group (*n* = 53)	*P* value
Anorexia, n (%)	27 (12.6)	18 (11.2)	9 (17.0)	0.270
Nausea or vomiting, n (%)	9 (4.2)	6 (3.7)	3 (5.7)	0.831
Abdominal pain, n (%)	4 (1.9)	2 (1.2)	2 (3.8)	0.257
Diarrhea, n (%)	13 (6.1)	10 (6.2)	3 (5.7)	1.000
Headache, n (%)	21 (9.8)	15 (9.3)	6 (11.3)	0.671
Dizziness, n (%)	21 (9.8)	14 (8.7)	7 (13.2)	0.338
Others				
Time of onset to hospital admission, median (IQR), days	6.00 (3.00–10.00)	6.00 (3.00–10.00)	7.00 (4.50–8.00)	0.612
NCT of SARC-CoV-2 from onset, median (IQR), days	17.00 (13.00–23.00)	17.00 (13.00–23.00)	21.00 (15.50–26.50)	0.004
Hospital stay times, median (IQR), days	14.00 (12.00–22.00)	13.00 (11.00–17.00)	24.00 (14.50–37.00)	< 0.001
Time of onset to hospital discharge or death, median (IQR), days	22.00 (18.00–29.00)	22.00 (18.00–29.00)	30.00 (22.50–43.50)	< 0.001

IQR interquartile range, *NCT* negative conversion time

Of these 214 patients, 1 (0.5%) had mild disease, 160 (74.8%) had moderate disease, 37 (17.3%) had severe disease, and 16 (7.5%) had critical disease. The ICU patients (53 [24.8%]) were significantly older than the non-ICU patients (161 [75.2%]; 57.00 years [50.50–75.50] vs. 46.00 years [IQR, 37.50–54.00], $P < 0.001$). There were more patients with diabetes mellitus in the ICU group than in the non-ICU group (16 [30.2%] vs. 8 [5.0%], $P < 0.001$) and underlying pulmonary diseases (6 [11.3%] vs. 4 [2.5%], $P < 0.01$. 023). There were no significant differences between the two cohorts with regard to hypertension and coronary heart disease. Compared with the non-ICU patients, the ICU patients tended to report fatigue, cough, expectoration, shortness of breath, dyspnea, and chest pain (Table 1).

Laboratory findings of patients with COVID-19 at hospital admission

There were obvious differences in the laboratory findings between the ICU and non-ICU group patients. No abnormalities in white blood cell counts were observed in most patients. Neutrophil counts were significantly higher in the ICU patients than in the non-ICU patients (4.21×10^9/L [IQR, 2.97–5.66] vs. 3.46

× 10^9/L [IQR, 2.51–4.40], $P = 0.006$). Most patients with COVID-19 had a decrease in lymphocyte levels (187 [87.4%]). Lymphocyte counts decreased more significantly in the ICU group than in the non-ICU group (0.75 × 10^9/L [IQR, 0.55–1.04] vs. 1.20 × 10^9/L [IQR, 0.90–1.60], $P < 0.001$). More patients in the ICU group had thrombocytopenia than in the non-ICU group (28.3% vs. 13.0%, $P = 0.028$). The ICU patients had higher direct bilirubin ($P = 0.033$), aspartate aminotransferase (AST) ($P < 0.001$), activated partial thromboplastin time (APTT) ($P < 0.001$), prothrombin time (PT) ($P = 0.019$), and D-dimer ($P < 0.001$) than the non-ICU patients at hospital admission. C-reactive protein (CRP) was significantly higher in the ICU group than in the non-ICU group (81.05 mg/L [IQR, 46.12–131.49] vs. 5.99 mg/L [IQR, 1.72–20.43], $P <$ 0.001). In most patients, the procalcitonin (PCT) level was in the normal range (Table 2).

Table 2
Comparison of laboratory findings between the non-ICU and ICU groups at hospital admission

Characteristic	Normal range	Total (*n* = 214)	Non-ICU group (*n* = 161)	ICU group (*n* = 53)	*P*-value
Blood routine					
White blood cell, median (IQR), $\times10^9$/L	3.50–9.50	5.20 (4.10–6.70)	5.10 (4.10–6.60)	5.40 (4.35–6.95)	0.490
Neutrophil, median (IQR), $\times10^9$/L	1.80–6.30	3.56 (2.58–4.88)	3.46 (2.51–4.40)	4.21 (2.97–5.66)	0.006
Lymphocyte, median (IQR), $\times10^9$/L	1.10–3.20	1.08 (0.80–1.53)	1.20 (0.90–1.60)	0.75 (0.55–1.04)	< 0.001
Monocyte, median (IQR), $\times10^9$/L	3.00–10.00	0.37 (0.29–0.48)	0.39 (0.31–0.48)	0.33 (0.19–0.48)	0.016
Platelet, median (IQR), $\times10^9$/L	125.00–350.00	176.50 (138.00–236.25)	186.00 (146.50–238.00)	150.00 (118.00–229.50)	0.045
Neutrophil, median (IQR),%	40.00–75.00	69.15 (60.68–78.75)	65.80 (58.70–73.45))	79.90 (70.05–84.90)	< 0.001
Lymphocyte, median (IQR),%	20.00–50.00	22.65 (14.30–29.20)	24.80 (17.95–30.65)	13.50 (10.05–21.50)	< 0.001
Monocyte, median (IQR),%	3.00–10.00	7.10 (5.50–9.20)	7.30 (6.00–9.25)	5.70 (3.90–8.45)	0.001
NLR, median (IQR),%	NA	3.05 (2.07–5.34)	2.55 (1.85–4.09)	5.94 (3.26–8.50)	< 0.001
Lymphocytopenia, n (%)	NA	187 (87.4)	136 (84.5)	51 (96.2)	0.025
Thrombopenia, n (%)	NA	36 (16.8)	21 (13.0)	15 (28.3)	0.011
Blood biochemistry					
Urea nitrogen, median (IQR), mmol/L	3.10–8.00	4.00 (3.20–5.20)	3.90 (3.20–4.95)	4.50 (3.05–5.90)	0.160
Creatinine, median (IQR), mmol/L	57.00–97.00	66.00 (55.00–75.25)	66.00 (56.00–76.50)	65.00 (50.00–75.00)	0.435
TBil, median (IQR), umol/L	0.00–26.00	9.60 (6.38–15.93)	9.60 (5.95–16.05)	10.00 (6.80–15.20)	0.768

ICU intensive care unit, *NLR* neutrophil-to-lymphocyte ratio, *IQR* interquartile range, *TBil* total bilirubin, *DBil* direct bilirubin, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *CK* creatine kinase, *CKMB* MB isoenzyme of creatine kinase, *HbA1c* glycated hemoglobin A1c, *CRP* C-reactive protein, *PCT* procalcitonin, *PT* prothrombin time, *APTT* activated partial thromboplastin time

Characteristic	Normal range	Total (*n* = 214)	Non-ICU group (*n* = 161)	ICU group (*n* = 53)	*P*-value
DBil, median (IQR), umol/L	0.00–8.00	4.70 (3.28–6.40)	4.50 (3.10–6.20)	5.10 (3.65–8.15)	0.033
ALT, median (IQR), U/L	9.00–50.00	21.20 (14.68–35.13)	19.70 (14.65–33.35)	26.00 (13.55–39.30)	0.218
AST, median (IQR), U/L	15.00–40.00	23.50 (17.18–34.18)	20.80 (16.50–28.70)	34.00 (25.60–44.50)	< 0.001
CK, median (IQR), U/L	50.00–310.00	62.00 (42.00–90.00)	60.00 (42.00–84.05)	77.00 (40.50–159.50)	0.067
CKMB, median (IQR), U/L	0.00–25.00	12.00 (10.00–16.00)	12.00 (10.00–16.00)	14.00 (9.90–17.00)	0.530
HbA1c, median (IQR),%		5.60 (5.30–5.90)	5.50 (5.30–5.80)	5.90 (5.60–7.15)	< 0.001
Infection related parameters					
CRP, median (IQR), mg/L	0.00–8.00	11.63 (2.12–50.89)	5.99 (1.72–20.43)	81.05 (46.12–131.49)	< 0.001
PCT, median (IQR), ng/mL	< 0.046	0.04 (0.03–0.07)	0.04 (0.03–0.06)	0.09 (0.06–0.14)	< 0.001
Coagulation function					
PT, median (IQR), s	8.00–14.00	11.10 (10.60–11.43)	11.00 (10.55–11.35)	11.30 (10.65–11.80)	0.019
APTT, median (IQR), s	20.00–40.00	26.50 (24.50–29.30)	26.10 (23.90–28.40)	29.00 (26.30–33.50)	< 0.001
D-dimer, median (IQR), mg/L	0.00–0.55	0.37 (0.20–0.60)	0.28 (0.19–0.47)	0.62 (0.44–1.30)	< 0.001

ICU intensive care unit, *NLR* neutrophil-to-lymphocyte ratio, *IQR* interquartile range, *TBil* total bilirubin, *DBil* direct bilirubin, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *CK* creatine kinase, *CKMB* MB isoenzyme of creatine kinase, *HbA1c* glycated hemoglobin A1c, *CRP* C-reactive protein, *PCT* procalcitonin, *PT* prothrombin time, *APTT* activated partial thromboplastin time

Lymphocyte subsets and cytokines of patients with COVID-19 at hospital admission

The lymphocyte, $CD3^+$ T cell, $CD4^+$ T cell, $CD8^+$ T cell, $CD19^+$ B cell (total B cell), and natural killer (NK) cell counts were lower in the ICU group (659.00 × 10^6/L, 417.00 × 10^6/L, 261.00 × 10^6/L, 140.00 × 10^6/L, 109.00 × 10^6/L, 102.00 × 10^6/L, respectively) than in the non-ICU group (1063.00 × 10^9/L, 717.00 × 10^6/L, 432.00 × 10^6/L, 271.00 × 10^6/L, 133.00 × 10^6/L, 143.00 × 10^6/L, respectively). Interleukin (IL)-6 was

significantly higher in the ICU group than in the non-ICU group (18.08 pg/mL vs. 3.13 pg/mL, $P < 0.001$). IL-10 was higher in the ICU group than in the non-ICU group (3.83 pg/mL vs. 2.56 pg/mL, $P < 0.001$), but still in the normal range. There was no significant difference between the two cohorts in terms of the $CD4^+/CD8^+$ ratio, IL-4, IL-17, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ (Table 3, Fig. 1).

Table 3
Comparison of lymphocyte subsets and cytokines between the non-ICU and ICU groups at hospital admission

Characteristic	Normal range	Total (*n* = 214)	Non- ICU group (*n* = 161)	ICU group (*n* = 53)	*P*-value
Lymphocyte classification					
Lymphocyte, median (IQR), $\times10^6$/L	1530.00–3700.00	936.50 (661.50–1323.25)	1063.00 (767.50–1389.50)	659.00 (480.50–885.50)	< 0.001
$CD3^+$, median (IQR), $\times10^6$/L	699.00–2540.00	632.50 (429.50–925.50)	717.00 (544.00–973.00)	417.00 (252.50–571.50)	< 0.001
$CD4^+$, median (IQR), $\times10^6$/L	410.00–1590.00	377.50 (265.75–536.00)	432.00 (308.50–609.50)	261.00 (153.50–351.50)	< 0.001
$CD8^+$, median (IQR), $\times10^6$/L	190.00–1140.00	242.50 (157.75–335.50)	271.00 (193.00–382.50)	140.00 (95.00–205.00)	< 0.001
$CD19^+$, median (IQR), $\times10^6$/L	90.00–660.00	128.00 (86.75–184.75)	133.00 (90.50–209.00)	109.00 (77.50–144.50)	0.012
NK, median (IQR), $\times10^6$/L	90.00–590.00	132.00 (86.75–211.00)	143.00 (96.50–230.50)	102.00 (65.50–166.00)	0.002
$CD3^+$, median (IQR),%	55.00–84.00	68.09 (61.74–75.00)	70.26 (64.23–75.48)	62.59 (54.61–68.75)	< 0.001
$CD4^+$, median (IQR),%	31.00–60.00	39.30 (34.58–44.40)	40.05 (35.87–44.93)	37.78 (29.18–43.56)	0.030
$CD8^+$, median (IQR),%	13.00–41.00	25.02 (20.28–30.41)	25.96 (21.03–30.75)	22.42 (17.52–29.01)	0.009
$CD19^+$, median (IQR),%	6.00–25.00	14.54 (10.46–18.09)	13.94 (9.99–17.14)	17.48 (13.29–22.21)	< 0.001
NK, median (IQR),%	5.00–27.00	15.00 (9.59–21.97)	13.98 (9.11–20.85)	18.64 (10.10–25.99)	0.038
$CD4^+/CD8^+$, median (IQR)	0.70–2.87	1.54 (1.20–2.05)	1.54 (1.24–1.97)	1.53 (1.12–2.43)	0.649
Cytokine profiles					

ICU intensive care unit, *IQR* interquartile range, *NK* natural killer, *IL* interleukin, *TNF* tumor necrosis factor, *IFN* interferon

Characteristic	Normal range	Total (*n* = 214)	Non- ICU group (*n* = 161)	ICU group (*n* = 53)	*P*-value
IL-4, median (IQR), pg/mL	0.00–8.56	1.70 (1.33–2.38)	1.70 (1.34–2.37)	1.68 (1.26–2.45)	0.573
IL-6, median (IQR), pg/mL	0.00–5.40	4.15 (0.00–11.75)	3.13 (0.00–6.69)	18.08 (6.69–46.45)	< 0.001
IL-10, median (IQR), pg/mL	0.00–12.90	2.72 (2.33–3.76)	2.56 (2.23–3.08)	3.83 (2.98–5.16)	< 0.001
IL-17, median (IQR), pg/mL	0.00–21.40	1.21 (1.04–1.38)	1.21 (1.04–1.39)	1.18 (1.02–1.37)	0.393
TNF-α, median (IQR), pg/mL	0.00–16.50	3.39 (1.82–5.93)	3.48 (1.86–6.25)	3.05 (1.61–5.42)	0.390
IFN-γ, median (IQR), pg/mL	0.00–23.10	4.15 (1.77–8.28)	4.05 (1.70–7.88)	4.32 (2.32–8.72)	0.295

ICU intensive care unit, *IQR* interquartile range, *NK* natural killer, *IL* interleukin, *TNF* tumor necrosis factor, *IFN* interferon

Multivariate logistic regression model and ROC curve analysis for ICU admission

Twelve univariate variables, including age, sex, underlying pulmonary diseases, diabetes mellitus, the counts of all tested lymphocyte subsets, and IL-6, were selected to perform multivariate logistic regression analysis to identify independent predictors of ICU admission. Stepwise forward method was used. The results showed that age (*OR* = 1.067; *95% CI*, 1.034–1.101; *P* < 0.001), diabetes mellitus (*OR* = 9.154; *95% CI*, 2.710–30.926; *P* < 0.001), $CD3^+$ T cell counts (*OR* = 0.996; *95% CI*, 0.994–0.997; *P* < 0.001), and IL−6 (*OR* = 1.006; *95% CI*, 1.000–1.013; *P* = 0.039) at hospital admission were risk factors of the ICU cases with COVID-19. The following equation was obtained: Probability (severe/critical COVID-19) = 1/1 + exp − [−2.458 + (0.065 × age) + 2.214 × diabetes mellitus + (−0.004 × $CD3^+$ T cell count) + (0.006 × IL−6) (Table 4).

Table 4
Multivariate logistic regression model of predictors for ICU admission

Variables	β	*OR*	*95% CI*	*P*-value
Age (years)	0.065	1.067	1.034–1.101	< 0.001
Diabetes mellitus (1 = yes, 0 = no)	2.214	9.154	2.710–30.926	< 0.001
$CD3^+$ ($\times 10^6$/L)	−0.004	0.996	0.994–0.997	< 0.001
IL-6 (pg/ml)		1.006	1.000–1.013	0.039
Constant	−2.458	0.086	-	0.014

ICU intensive care unit, *IL* interleukin.

Table 5
Summary of ROC curve parameters for predicting ICU admission

Predictor	AUC	*P*-value	95% *CI*	Cutoff value	Jordan index	Sensitivity	Specificity
Age	0.766	< 0.001	0.689–0.844	> 53.50	0.437	69.8%	73.9%
Diabetes mellitus	0.626	0.006	0.532–0.720	> 0.50	0.252	30.2%	95.0%
Lymphocyte	0.780	< 0.001	0.709–0.851	< 840.00	0.456	73.6%	72.0%
$CD3^+$	0.806	< 0.001	0.737–0.874	< 510.50	0.512	71.7%	79.5%
$CD4^+$	0.776	< 0.001	0.705–0.847	< 357.50	0.426	77.4%	65.2%
$CD8^+$	0.796	< 0.001	0.721–0.872	< 173.50	0.505	67.9%	82.6%
$CD19^+$	0.616	0.012	0.533–0.699	< 148.00	0.196	81.1%	38.5%
NK	0.639	0.002	0.553–0.725	< 134.50	0.270	71.7%	55.3%
IL-4	0.526	0.573	0.433–0.618	< 1.27	0.096	26.4%	83.2%
IL-6	0.785	< 0.001	0.705–0.864	> 6.58	0.519	77.4%	74.5%
IL-10	0.760	< 0.001	0.681–0.838	> 2.95	0.494	77.4%	72.0%
IL-17	0.539	0.393	0.451–0.627	< 1.27	0.096	69.8%	39.8%
TNF-α	0.539	0.390	0.452–0.626	< 8.40	0.099	96.2%	13.7%
IFN-γ	0.548	0.295	0.460–0.636	> 7.73	0.116	37.7%	73.9%
Combined predictor	0.887	< 0.001	0.837–0.937	> 0.309	0.631	77.4%	85.7%

ROC receiver operating characteristic, *ICU* intensive care unit, *NK* natural killer, *IL* interleukin, *TNF* tumor necrosis factor, *IFN* interferon

Next, ROC curve analysis was performed to further evaluate the predictive accuracy of different variables. The results showed that the AUC of the $CD3^+$ T cell count and IL-6 at hospital admission were 0.806 (*95% CI*, 0.737–0.874; $P < 0.001$) and 0.785 (*95% CI*, 0.705–0.864; $P < 0.001$); the cutoff values were 357.5 ×

10^6/L (sensitivity, 77.4%; specificity, 65.2%) and 6.58 pg/mL (77.4%, 74.5%), respectively. Moreover, the ROC curve of the model, combining age, diabetes mellitus, $CD3^+$ T cell count reduction, and IL−6 elevation, had a larger AUC (0.887 [*95% CI*, 0.837–0.937; $P < 0.001$) with the cutoff value of 0.309 (sensitivity 77.4%, specificity 85.7%) (Fig. 2).

Lymphocyte subsets and cytokines of ICU COVID-19 patients

Of the 53 patients in the ICU group, there were no statistical differences among all tested indicators of lymphocyte subsets and cytokines between the severe group ($n = 37$) and the critical group ($n = 16$) at hospital or ICU admission (Tables 6, 7).

Table 6
Comparison of lymphocyte subsets and cytokines between severe and critical groups at hospital admission

Characteristic	Total (n = 53)	Severe group (n = 37)	Critical group (n = 16)	Z value	P value
Lymphocyte classification					
Lymphocyte, median (IQR), $\times 10^{6}$/L	659.00 (480.50–885.50)	743.00 (517.00–965.50)	547.00 (473.00–704.75)	−1.792	0.073
$CD3^+$, median (IQR), $\times 10^{6}$/L	417.00 (252.50–571.50)	430.00 (327.00–602.00)	327.00 (211.75–476.75)	−1.482	0.138
$CD4^+$, median (IQR), $\times 10^{6}$/L	261.00 (153.50–351.50)	280.00 (173.00–380.50)	211.00 (131.50–294.75)	−1.782	0.075
$CD8^+$, median (IQR), $\times 10^{6}$/L	140.00 (95.00–205.00)	148.00 (95.00–234.50)	120.00 (90.75–160.25)	−1.298	0.194
$CD19^+$, median (IQR), $\times 10^{6}$/L	109.00 (77.50–144.50)	116.00 (86.00–146.00)	99.00 (46.00–128.75)	−1.579	0.114
NK, median (IQR), $\times 10^{6}$/L	102.00 (65.50–166.00)	103.00 (77.50–175.00)	87.00 (49.50–133.50)	−1.298	0.194
$CD3^+$, median (IQR),%	62.59 (54.61–68.75)	62.59 (56.71–68.28)	61.37 (49.94–70.59)	−0.019	0.985
$CD4^+$, median (IQR),%	37.78 (29.18–43.56)	38.54 (30.89–43.93)	30.14 (26.93–40.06)	−1.376	0.169
$CD8^+$, median (IQR),%	22.42 (17.52–29.01)	21.13 (17.32–27.49)	22.86 (18.58–36.27)	0.872	0.383
$CD19^+$, median (IQR),%	17.48 (13.29–22.21)	17.00 (14.65–21.74)	18.87 (11.86–22.82)	−0.194	0.846
NK, median (IQR),%	18.64 (10.10–25.99)	16.68 (12.29–25.77)	19.39 (9.23–26.18)	0.039	0.969
$CD4^+/CD8^+$, median (IQR)	1.53 (1.12–2.43)	1.82 (1.20–2.34)	1.24 (0.84–2.55)	−1.066	0.287
Cytokine profiles					
IL-4, median (IQR), pg/mL	1.68 (1.26–2.45)	1.55 (1.20–2.38)	1.99 (1.32–2.50)	1.027	0.304

NK natural killer, *IL* interleukin, *TNF* tumor necrosis factor, *IFN* interferon

Characteristic	Total (*n* = 53)	Severe group (*n* = 37)	Critical group (*n* = 16)	*Z* value	*P* value
IL-6, median (IQR), pg/mL	18.08 (6.69–46.45)	17.35 (6.69–43.62)	21.89 (7.20–85.51)	1.077	0.281
IL-10, median (IQR), pg/mL	3.83 (2.98–5.16)	3.55 (2.81–4.94)	4.79 (3.23–6.25)	1.705	0.088
IL-17, median (IQR), pg/mL	1.18 (1.02–1.37)	1.15 (1.00–1.33)	1.20 (1.04–1.47)	0.349	0.727
TNF-α, median (IQR), pg/mL	3.05 (1.61–5.42)	3.12 (1.36–5.59)	2.93 (2.19–5.25)	0.281	0.779
IFN-γ, median (IQR), pg/mL	4.32 (2.32–8.72)	5.18 (2.00–10.04)	4.08 (2.35–7.44)	−0.562	0.574

NK natural killer, *IL* interleukin, *TNF* tumor necrosis factor, *IFN* interferon

Table 7
Comparison of lymphocyte subsets and cytokines between the severe and critical groups on ICU admission

Characteristic	Total (*n* = 53)	Severe group (*n* = 37)	Critical group (*n* = 16)	*Z* value	*P* value
Lymphocyte classification					
Lymphocyte, median (IQR), $\times 10^{6}$/L	597.00 (420.50–870.00)	695.00 (423.00–902.00)	535.00 (367.50–704.75)	–1.395	0.163
$CD3^{+}$, median (IQR), $\times 10^{6}$/L	399.00 (196.50–547.50)	430.00 (211.00–590.00)	327.00 (175.25–476.75)	–1.085	0.278
$CD4^{+}$, median (IQR), $\times 10^{6}$/L	243.00 (118.00–341.00)	261.00 (131.50–377.00)	211.00 (86.50–294.75)	–1.279	0.201
$CD8^{+}$, median (IQR), $\times 10^{6}$/L	140.00 (87.50–209.00)	157.00 (89.00–227.50)	117.50 (84.75–160.25)	–1.143	0.253
$CD19^{+}$, median (IQR), $\times 10^{6}$/L	103.00 (73.50–144.50)	103.00 (80.00–146.00)	99.00 (46.00–131.00)	–1.163	0.245
NK, median (IQR), $\times 10^{6}$/L	103.00 (60.50–166.00)	109.00 (67.50–168.50)	87.00 (49.50–130.00)	–1.424	0.154
$CD3^{+}$, median (IQR),%	61.33 (50.67–68.25)	61.33 (50.96–67.90)	61.37 (49.94–70.59)	0.446	0.656
$CD4^{+}$, median (IQR),%	35.84 (28.26–42.69)	36.04 (29.87–42.89)	30.14 (26.93–40.06)	–0.911	0.363
$CD8^{+}$, median (IQR),%	21.98 (16.61–28.62)	21.10 (16.61–27.49)	22.77 (16.09–36.27)	0.581	0.561
$CD19^{+}$, median (IQR),%	17.92 (13.04–22.91)	17.73 (14.38–22.93)	18.87 (11.86–22.82)	–0.271	0.786
NK, median (IQR),%	19.28 (10.66–26.77)	19.18 (13.41–27.32)	19.39 (9.23–26.18)	–0.542	0.587
$CD4^{+}/CD8^{+}$, median (IQR)	1.53 (1.09–2.33)	1.71 (1.12–2.08)	1.23 (0.83–2.55)	0.794	0.416
Cytokine profiles					
IL-4, median (IQR), pg/mL	1.73 (1.33–2.45)	1.68 (1.32–2.45)	1.98 (1.32–2.50)	0.523	0.607

ICU intensive care unit, *NK* natural killer, *IL* interleukin, *TNF* tumor necrosis factor, *IFN* interferon

Characteristic	Total (*n* = 53)	Severe group (*n* = 37)	Critical group (*n* = 16)	*Z* value	*P* value
IL-6, median (IQR), pg/mL	16.39 (4.53–49.63)	11.61 (2.48–46.45)	21.89 (7.20–85.51)	1.437	0.151
IL-10, median (IQR), pg/mL	3.76 (2.85–5.17)	3.44 (2.70–5.17)	4.79 (3.23–6.25)	1.744	0.081
IL-17, median (IQR), pg/mL	1.19 (1.04–1.40)	1.18 (1.04–1.36)	1.20 (1.04–1.47)	–0.058	0.954
TNF-α, median (IQR), pg/mL	3.52 (2.14–5.42)	3.54 (1.92–5.59)	2.93 (2.19–5.25)	–0.203	0.839
IFN-γ, median (IQR), pg/mL	5.18 (2.27–8.72)	5.42 (1.95–9.81)	4.08 (2.35–7.44)	–0.668	0.504

ICU intensive care unit, *NK* natural killer, *IL* interleukin, *TNF* tumor necrosis factor, *IFN* interferon

Moreover, among the patients in the ICU group, 33 patients (62.3%) were admitted directly to the ICU, and 20 (37.7%) had delayed admission to the ICU by 2 days from the point of hospital admission (IQR, 1–4). There were no statistical differences among all tested indicators of lymphocyte subsets and cytokines between the direct-ICU group and the late-ICU group at hospital admission (Table 8).

Table 8
Comparison of lymphocyte subsets and cytokines between the direct-ICU and late-ICU groups at hospital admission

Characteristic	Direct-ICU group (n = 33)	Late-ICU group (n = 20)	Z value	P value
Lymphocyte classification				
Lymphocyte, median (IQR), $\times 10^6$/L	681.00 (480.50–965.50)	578.50 (489.50–820.25)	–0.706	0.480
$CD3^+$, median (IQR), $\times 10^6$/L	430.00 (220.50–571.50)	357.00 (264.00–573.50)	–0.505	0.614
$CD4^+$, median (IQR), $\times 10^6$/L	282.00 (145.00–350.50)	236.00 (154.50–367.75)	–0.422	0.673
$CD8^+$, median (IQR), $\times 10^6$/L	145.00 (95.00–225.50)	135.00 (90.75–185.00)	–0.340	0.734
$CD19^+$, median (IQR), $\times 10^6$/L	119.00 (77.50–156.50)	102.00 (72.25–130.75)	–1.000	0.317
NK, median (IQR), $\times 10^6$/L	86.00 (60.00–175.00)	103.00 (82.25–124.75)	0.284	0.776
$CD3^+$, median (IQR),%	61.22 (53.58–67.36)	66.93 (55.12–69.16)	1.229	0.219
$CD4^+$, median (IQR),%	37.78 (29.39–42.93)	38.36 (27.61–46.54)	0.440	0.660
$CD8^+$, median (IQR),%	20.60 (17.11–28.12)	23.09 (17.65–29.87)	0.706	0.480
$CD19^+$, median (IQR),%	17.98 (15.78–22.67)	15.35 (11.19–20.80)	–1.596	0.110
NK, median (IQR),%	16.68 (9.62–26.46)	18.95 (12.06–22.85)	0.037	0.971
$CD4^+/CD8^+$, median (IQR)	1.53 (1.15–2.43)	1.55 (1.10–2.51)	–0.303	0.762
Cytokine profiles				
IL-4, median (IQR), pg/mL	1.68 (1.31–2.47)	1.64 (1.20–2.36)	–0.725	0.468
IL-6, median (IQR), pg/mL	10.12 (2.52–43.99)	20.48 (14.27–51.22)	1.517	0.129
IL-10, median (IQR), pg/mL	3.76 (2.79–5.17)	3.85 (3.09–4.90)	0.339	0.734
IL-17, median (IQR), pg/mL	1.15 (1.04–1.36)	1.21 (0.96–1.43)	0.073	0.941
TNF-α, median (IQR), pg/mL	3.12 (1.88–5.04)	2.97 (1.30–5.84)	–0.028	0.978

ICU intensive care unit, *NK* natural killer, *IL* interleukin, *TNF* tumor necrosis factor, *IFN* interferon

Characteristic	Direct-ICU group (n = 33)	Late-ICU group (n = 20)	Z value	P value
IFN-γ, median (IQR), pg/mL	5.18 (1.77–8.72)	3.73 (2.61–8.73)	0.009	0.993

ICU intensive care unit, *NK* natural killer, *IL* interleukin, *TNF* tumor necrosis factor, *IFN* interferon

Of the 20 late-ICU patients, 14 patients (70.0%) had delayed admission to the ICU by > 2 days from hospital admission; all of them had their lymphocyte subsets and cytokines measured at hospital admission and at ICU admission. There were no statistical differences among most of the tested indicators of lymphocyte subsets and cytokines from hospital admission to ICU admission, but the lymphocyte, $CD3^+$ T cell, $CD4^+$ T cell, $CD8^+$ T cell, and $CD19^+$ B cell counts showed downward trends (Table 9).

Table 9
Comparison of lymphocyte subsets and cytokines of the late-ICU group from hospital admission to ICU admission ($n = 14$)

Characteristic	Hospital admission	ICU admission	Z value	P value
Lymphocyte classification				
Lymphocyte, median (IQR), $\times 10^{6}$/L	561.00 (477.00–767.00)	498.50 (301.00–712.50)	−1.682	0.093
$CD3^{+}$, median (IQR), $\times 10^{6}$/L	347.00 (254.25–520.50)	267.00 (117.50–499.25)	−1.784	0.074
$CD4^{+}$, median (IQR), $\times 10^{6}$/L	236.00 (152.25–369.25)	174.00 (56.25–273.50)	−1.784	0.074
$CD8^{+}$, median (IQR), $\times 10^{6}$/L	125.00 (86.75–185.00)	101.00 (51.25–190.75)	−1.580	0.114
$CD19^{+}$, median (IQR), $\times 10^{6}$/L	108.50 (85.75–136.00)	98.50 (58.50–122.50)	−1.887	0.059
NK, median (IQR), $\times 10^{6}$/L	99.50 (77.25–124.25)	97.00 (57.75–125.25)	−0.866	0.386
$CD3^{+}$, median (IQR),%	67.31 (54.31–69.75)	54.72 (40.50–70.09)	−2.191	0.028
$CD4^{+}$, median (IQR),%	40.69 (26.76–49.50)	33.60 (22.43–39.50)	−2.293	0.022
$CD8^{+}$, median (IQR),%	21.88 (17.25–26.61)	19.91 (14.78–26.61)	−1.479	0.139
$CD19^{+}$, median (IQR),%	16.82 (12.84–23.31)	19.32 (11.66–26.93)	−1.478	0.139
NK, median (IQR),%	16.25 (8.95–24.06)	19.40 (10.79–29.06)	2.293	0.022
$CD4^{+}/CD8^{+}$, median (IQR)	1.90 (1.14–2.67)	1.74 (1.09–1.92)	−2.191	0.028
Cytokine profiles				
IL-4, median (IQR), pg/mL	1.42 (1.23–2.21)	1.81 (1.34–2.43)	1.992	0.046
IL-6, median (IQR), pg/mL	19.01 (13.26–57.87)	16.87 (4.13–69.75)	−0.314	0.753
IL-10, median (IQR), pg/mL	3.69 (3.12–4.81)	3.63 (2.75–6.00)	−0.734	0.463
IL-17, median (IQR), pg/mL	1.17 (0.95–1.39)	1.24 (1.03–1.53)	1.572	0.116
TNF-α, median (IQR), pg/mL	2.89 (1.22–5.93)	3.75 (2.66–5.66)	0.943	0.345
IFN-γ, median (IQR), pg/mL	3.37 (2.08–11.80)	4.12 (2.08–10.70)	0.943	0.345

ICU intensive care unit, *NK* natural killer, *IL* interleukin, *TNF* tumor necrosis factor, *IFN* interferon

Discussion

COVID-19 is a novel infectious disease that has led to a worldwide pandemic. According to the report from the Chinese Centers for Disease Control and Prevention, the mortality rate of COVID-19 is 2.3%[8]; however, this figure increased to 49.0% among critical cases[8]. Thus, it is of great significance to study the laboratory data and clinical development of the disease to guide management.

In this study, the clinical manifestations and laboratory data of the ICU and non-ICU 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 the ICU patients were older than the non-ICU patients. In addition, the ICU group had more patients with basic diseases than the non-ICU group; this indicates that older patients, in particular those with basic diseases, such as hypertension and diabetes mellitus, may be more likely to develop severe COVID-19. These findings are consistent with several previous studies [12, 18, 19]. 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 only had symptoms in the digestive system or nervous system.

Cellular immunity is an important part of the human immune system in a viral infection. Increasing evidence suggests that lymphocytes play a crucial role in airway diseases [20, 21]. Marked lymphocytopenia occurred in most patients during the acute phase of SARS and Middle East respiratory syndrome (MERS), with $CD4^+$ and $CD8^+$ T cells particularly affected. In addition, the degree of decrease in the T lymphocytes was associated with disease severity [22–24]. However, the mechanisms by which the viruses cause lymphocyte changes are different. In COVID-19, a growing number of studies have found that lymphocytopenia, particularly in T lymphocyte subsets, is common, especially in severe/critical cases [16, 18, 25–27], while results concerning $CD19^+$ B and NK cells are inconsistent [28]. In this study, lymphocytopenia occurred in 96.2% of the ICU patients and in 84.5% of the non-ICU patients. Specifically, the number of $CD3^+$ T cells, $CD4^+$ T cells, and $CD8^+$ T cells was significantly lower in the ICU group than the non-ICU group. While $CD8^+$ T cells are vital for the elimination of virus-infected cells as a result of the secretion of perforins, granzymes, and interferons, $CD4^+$ T cells participate via co-stimulating $CD8^+$ T cells and $CD19^+$ B cells [28]. $CD4^+$ T and $CD8^+$ T cells have been reported as powerful predictors of COVID-19 severity and clinical outcome in different studies, respectively [13, 15, 29]; however, there were no significant differences between the two T lymphocyte subsets in our study. The $CD4^+/CD8^+$ T cell ratio in the non-ICU group and the ICU group were similar, which may indicate that $CD4^+$ T and $CD8^+$ T cells were equally reduced in both groups [16]. $CD3^+$ T cells are composed of $CD4^+$ and $CD8^+$ T cells, and $CD3^+$ T cells may be a more reasonable and valuable parameter for severe disease and death. Differences in immune profiles can help better understand the pathogenesis and clinical expression of COVID-19 [28]. Currently, the pathophysiological mechanism of lymphocyte reduction in patients with COVID-19 remains unclear and further investigations are required.

Early studies have documented that cytokine storms, also known as inflammatory storms, have occurred in a large number of patients with COVID-19. In patients with SARS, an increased number of proinflammatory cytokines in the serum, such as IL-1β, IL-6, IL-12, IFN-γ, IFN-γ-inducible protein-10, and C-

C motif chemokine ligand 2, was observed and was considered to be related to pulmonary inflammation, extensive lung damage, and even multiple organ failure [30]. A previous study showed that patients with MERS also had increased concentrations of proinflammatory cytokines (IFN-γ, TNF-α, IL-15, and IL-17) [31]. 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[12, 18]. In clinical work, it was found that the course of disease and lung lesions progressed rapidly, and that multiple organ failure developed over 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, the serum IL-6 concentration was significantly higher in the ICU patients than in the non-ICU patients, which is in agreement with the concept of a cytokine storm [32]. 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 cytokines remains to be elucidated, a higher concentration of serum cytokines seems to be associated with poor outcomes. Therefore, monitoring the changes in cytokines is of a certain significance for the early detection and management of critically ill patients.

For patients with COVID-19, it is important to determine who has inherent susceptibility to develop severe or even critical disease. Monitoring COVID-19 severity is helpful in clinical decision making [33]. Early screening of critically ill patients may improve clinical outcomes. Searching for potential predictors of the severity of disease and disease outcome could help us to identify patients requiring special care, i.e., early ICU admission, intense monitoring, and more aggressive therapy [28].

In this 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 the ICU and non-ICU groups. Multivariate logistic regression analysis and ROC curve analysis were further performed. In addition, AUC and cutoff values were calculated. It was found that age, diabetes mellitus, $CD3^+$ T cells < 510.5×10^6/L at hospital admission, and IL-6 > 6.58 pg/mL at hospital admission were the predictive factors for the development of severe disease. ROC curve analysis showed that the AUC of $CD3^+$ T cells and that of IL-6 were 0.806 and 0.785, respectively, and the AUC of combined predictor (combining age, diabetes mellitus, $CD3^+$ T cell count reduction, and IL-6 elevation) was 0.887.

Among the 53 patients in the ICU group, 33 patients were admitted straight to the ICU due to the severity of their condition, while the remaining 20 patients were admitted to general isolation ward and then transferred to the ICU following deterioration of the disease. The value of lymphocyte subsets and cytokines in predicting severe conditions is in the identification of patients with less severe disease at hospital admission. We found that there was no significant difference in any of the indicators of lymphocyte subsets and cytokines between the direct-ICU group and the late-ICU group at hospital admission. For the late-ICU group, there was no significant difference in the lymphocyte subsets and cytokines from hospital admission to ICU admission, but the lymphocyte subsets showed a downward trend with disease progression. This finding also confirmed the value of lymphocyte subsets and cytokines in predicting severe illness at hospital admission. We also compared the changes in

lymphocyte subsets and cytokines at hospital admission and ICU admission between severe and critical patients, and found no significant difference in all indicators.

In summary, the characteristics of lymphocyte subsets and cytokine profiles between ICU and non-ICU patients with COVID-19 were compared in this study. As a result, predictive factors for patients developing a severe condition were identified. This is helpful to identify high-risk patients as early as possible, which allows intensive monitoring and treatment to be provided at an early stage, and ultimately reduces the mortality associated with COVID-19.

This study has several potential limitations. First, the retrospective single-center design leads to missing data and unavoidable biases. However, the researchers responsible for data collection were trained before the study began so that they could correctly fill out the case report forms 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, consequently, the trend of these clinical and laboratory indicators could not be described. Fortunately, all of the data were recorded in our electronic medical record system, and we plan to extract and collect parameters required for further study in the future.

Conclusions

The counts of lymphocyte subsets decreased and the level of IL-6 increased in the ICU patients compared with the non-ICU patients. $CD3^+$ T cells and IL-6 in peripheral blood may serve as independent predictors of the early identification of severe patients with COVID-19.

Abbreviations

APTT: Activated partial thromboplastin time, AUC: Area under curve, CK: Creatine kinase, CKMB: Creatine kinase-MB, COVID-19: Coronavirus disease, CRP: C-reactive protein, DBil: Direct bilirubin, HbA1c: Glycated hemoglobin A1c, ICU: Intensive care unit, IFN-γ: Interferon-γ, IL: Interleukin, IQR: Interquartile range, MERS: Middle East respiratory syndrome, NLR: Neutrophil-to-lymphocyte ratio, PCT: Procalcitonin, PT: Prothrombin time, ROC: Receiver operating characteristic curve, RT-PCR: Reverse transcription-polymerase chain reaction, SARS: Severe acute respiratory syndrome, SARS-CoV-2: Severe acute respiratory syndrome-coronavirus 2, TBil: Total bilirubin, TNF-α: Tumor necrosis factor-α

Declarations

Acknowledgment

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Authors' contributions

Conceived and designed the experiments: Weihua Shi and Xiangyou Yu. Collected clinical data: Qilong Zhou, Xiaochun Deng, Chao Liu, and Jianguo Chen. Analyzed and interpreted the data: Xinxin Du, Zongjun Hu, and Yong Cui. Drafted the manuscript: Pengfei Pan, Xinxin Du, and Qilong Zhou. All authors read and approved the final manuscript.

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Availability of data and materials

All the data of this study is available on request from corresponding author.

Ethics approval and consent to participate

This study was approved by the Institutional Ethics Board of Three Gorges Hospital Affiliated with Chongqing University (No. 2020-74).

Consent to publication

All authors approved the manuscript for publication.

Competing interests

The authors disclose no conflicts of interest.

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Figures

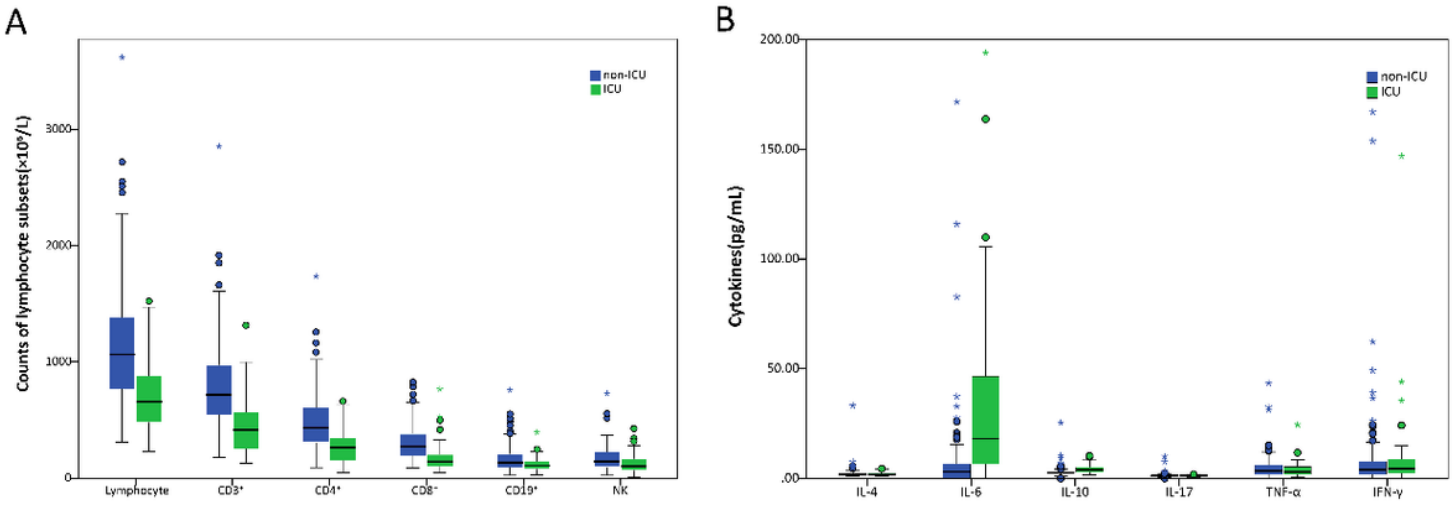

Figure 1

Comparison of lymphocyte subsets and cytokine profiles between the ICU and non-ICU groups. (A) Counts of lymphocyte subsets. (B) Cytokines. NK natural killer, IL interleukin, TNF tumor necrosis factor, IFN interferon

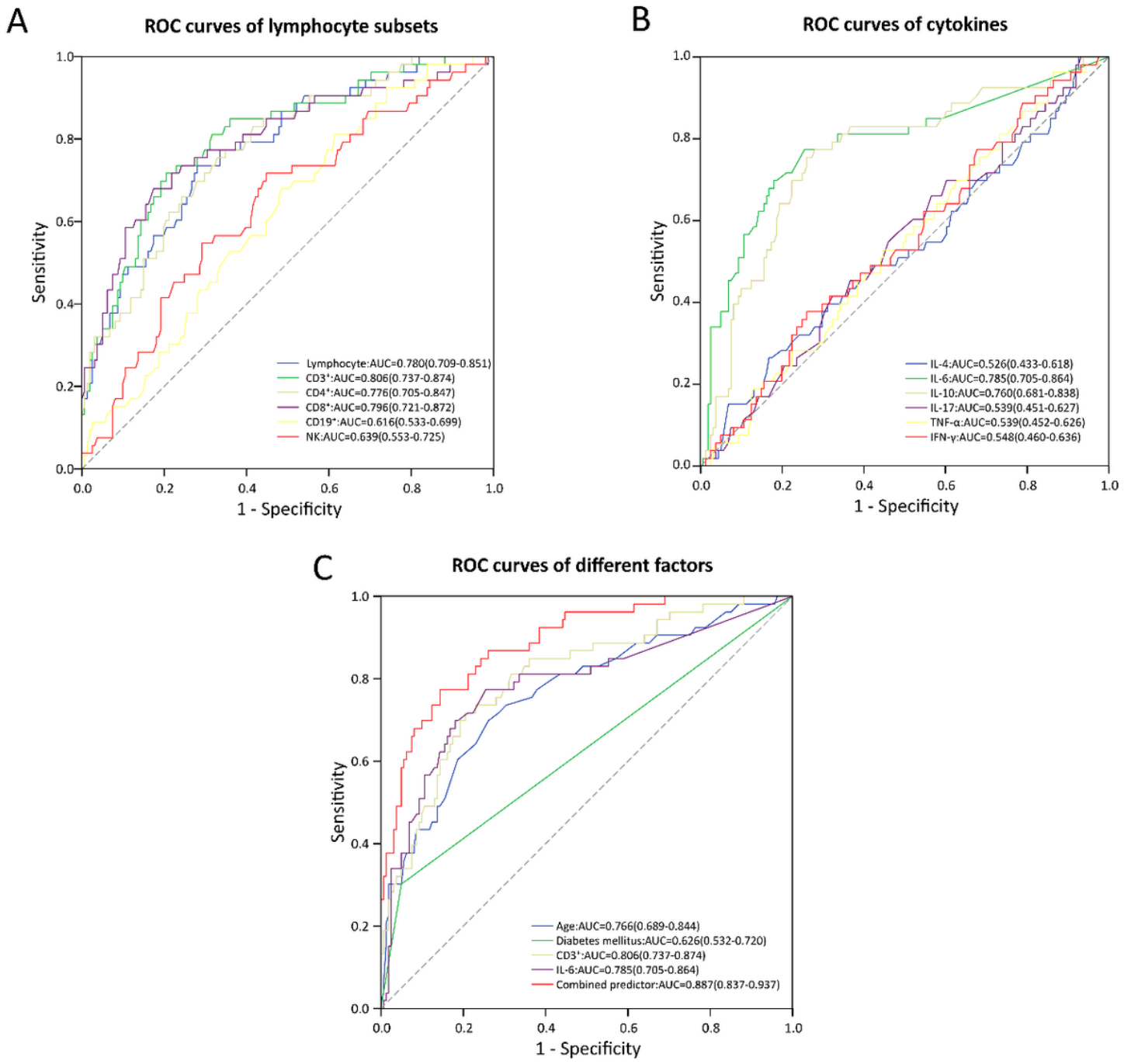

Figure 2

ROC curve analysis of different predictors for ICU admission. (A) ROC curves of lymphocyte subsets. (B) ROC curves of lymphocyte cytokines. (C) ROC curves of different factors. ROC receiver operating characteristic, AUC area under ROC curve, NK natural killer, IL interleukin, TNF tumor necrosis factor, IFN interferon