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ORIGINAL ARTICLE

Association and Prediction of Five Clinical Indexes in COVID-19 Patients with Different Severities

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Abstract

Background: The new and acute febrile respiratory illness outbreak caused by a coronavirus called 'COVID-19' has become a serious global public health, economic, and livelihood concern. Although the diagnosis and treatment of COVID-19 are well established, the altered clinical characteristics of COVID-19 patients have yet to be thoroughly studied.

Materials and methods: We extracted and analyzed some electronic medical records of 178 hospitalized COVID-19 patients with pneumonia, including demographics, clinical manifestations, comorbidities, and laboratory data statistically.

Results: Our study found that the proportion of patients with moderate, severe, and critical severe groups in the group older than 60 was remarkably higher than that in the group younger than 60. Significant differences in the results regarding clinical blood routines among moderate, severe, and critical severe groups. The lymphocyte of critical severe patients was significantly lower than that of moderate patients. However, granulocytes in the critical severe group were considerably higher than those in the moderate and severe groups. We used ROC curve plotting to obtain the best decisive threshold by combined five clinical indexes [lymphocyte, granulocyte, Mean platelet volume (MPV), albumin, and alkaline phosphatase (ALP), AUC= 0.780, 95%CI= 0.69-0.85, $P< 0.0001$]. The expression levels of ACE2, TMPRSS2, DPP4, ANPEP, NRP1, FUNRIN on lung were assessed by single cell sequence analysis.

Conclusion: We found diagnostic power of disease severity improved on a combined panel. Many clinical indexes showed significant differences among the moderate, severe, and critical severe groups.

Keywords: COVID-19, SARS-CoV-2, Clinical characteristic, Infection, Disease severity

Introduction

The new and acute febrile respiratory illness outbreak caused by called coronavirus disease 2019 (COVID-19) has become a serious global public health, economic, and livelihood concern[1]. The World Health Organization (WHO) recently declared COVID-19 and warned it might progress to a pandemic associated with substantial morbidity and mortality. We must unite internationally to respond to the public health emergency of concern that began on 1 February 2020[2-4].

So far, numerous studies have identified six coronaviruses that can infect humans and cause disease. OC43, NL63, 229E, and HKU1 infections are usually mild and mainly cause common cold symptoms[5]. Two previously identified highly pathogenic zoonotic coronaviruses, such as Severe Acute Respiratory

Syndrome (SARS) and Middle East Respiratory Syndrome (MERS), have caused widespread epidemics and deaths in many countries. SARS-CoV-2 was isolated from biologic samples and identified as genusbetacoronavirus, placing it alongside other SARS-CoV, with which it shares more than 79% of its sequence MERS-CoV, 85% homology with SARS-CoV[6]. The viral genome is about 27-32 KB and encodes structural and non-structural proteins. Structural proteins such as membrane protein (M), an envelope protein (E), nucleocapsid protein (N), and spike protein (S) play an essential role in the process of virus entry into host cells and virus replication. Like SARS-CoV, it attaches the S protein to angiotensin-converting enzyme 2 (ACE-2), which enters and infects host cells in the lower respiratory tract, causing alveolar damage leading to progressive respiratory failure[3,5-12]. COVID-19 is the third highly pathogenic human coronavirus infection in the past 20 years, after MERS-CoV and SARS-CoV infection[5,13].

Because of the rapid spread of the disease, we summarize various test data from 178 COVID-19 cases and clinical characteristics of the disease severity. Here, we describe the results of the clinical features of the disease in a selected cohort of Chinese patients, which will provide a comprehensive reference for disease diagnosis and treatment.

Materials and methods

Ethics statement

All hospitalized patients voluntarily provided informed consent for information collection and subsequent analysis. Our study was approved by the ethics committee of Huoshenshan hospital and was conducted by the tenets of the Declaration of Helsinki and its amendments (202018).

Patients involvement and data collection

All participants (n= 178) were hospitalized in Huoshenshan Hospital from January 16 to March 16, 2020, with clinical symptoms (fever or respiratory symptoms) accompanied by typical chest radiographs and clinically diagnosed as "viral pneumonia" and were initially enrolled in our study. A confirmed case of COVID-19 was defined as a positive result on the real-time reverse transcription-polymerase chain reaction (RT-PCR) assay of nucleic acid specimens based on the WHO interim guidance. We only collected and analyzed information on laboratory-confirmed COVID-19 cases.

Laboratory testing

We collected heparin- and EDTA-anticoagulated venous blood samples to perform routine blood examinations, such as coagulation profile, complete blood count, serum biochemical tests, and so on. We ordered some enzymes for each patient, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltranspeptidase (γ-GGT), alkaline phosphatase (ALP), creatine kinase (CK),lactate dehydrogenase(LDH) and its isoenzymes, electrolytes, including sodium, potassium, calcium, magnesium, chlorine, phosphorus, acute response proteins including procalcitonin (PCT), C-reactive protein (CRP), alpha-hydroxybutyric dehydrogenase (α-HBDH), cystatin C, creatinine, uric acid, and so on. Primarily, all patients' plasma samples were analyzed using chemiluminescence immunoassay (CLIA) based on the Modular System SAL 9000 kit provided by Shenzhen Mindray Bio-Medical Electronics Co., Ltd. (Shenzhen, China), following the instructions of the manufacturer.

The microsphere flow immunofluorescence assay detected plasma cytokines and chemokines of COVID-19 patients. Including Interleukin 2 (IL-2), IL-4, IL-5, IL-6, IL-8, IL- 10, IL- 17, IL- 12p70, tumor necrosis factor α (TNF-α) and interferon α (IFN-α), IFN-γ. The test method was the manufacturer's specification of a commercial kit provided by Qingdao Riskon Biotechnology Co., Ltd., China, and was detected by the Beckman Dxflex flow cytometer (Beckman Coulter, USA).

According to the manufacturer's agreement, we use Flow cytometry (CytoFLEX Flow cytometry, Beckman Coulter, Inc.) and commercial kits (Beckman Coulter, Inc.) to detect lymphocyte subsets, including the number and percentage of CD3+T cells, CD4+T cells, CD8+T cells, CD3-CD19+T cells and CD3-(CD16+/CD56+) T cells.

Single-cell RNA sequence data analysis

The single-cell RNA sequencing dataset is based on a meta-analysis of the literature on single-cell RNA sequencing and single-cell databases that include healthy human tissue(https://www.proteinatlas.org/ENSG00000130234ACE2/single+cell+type/lung). To avoid technical bias and ensure the single-cell dataset can best represent the corresponding tissue, We applied the following data selection criteria: (1) Single-cell transcriptomic datasets were limited to those based on the Chromium single-cell gene expression platform from 10X Genomics (version 2 or 3); (2) Single-cell RNA sequencing was performed on single-cell suspension from tissues without pre-enrichment of cell types; (3) Only studies with >4,000 cells and 20 million read counts were included, (4) Only dataset whose pseudo-bulk transcriptomic expression profile is highly correlated with the transcriptomic expression profile of the corresponding HPA tissue bulk sample were included. It should be noted that exceptions were made for the lung (7.3 million reads) to include various cell types in the analysis.

Statistical analysis

We used median and inter-quartile range (IQR) values to describe continuous variables and report categorical variables regarding the frequency of use and percentage (%) of statistical criteria. Kruskal-Wallis H test was used to compare differences between groups. The classified data were analyzed by the Pearson Chi-square test or Fisher exact test[2,14]. Bonferroni correction was used for pairwise comparisons. Perform a receiver operating characteristic (ROC) curve analysis and report the area under the curve (AUC) to determine the ability of individual biomarkers or biomarker panels to diagnose disease severity accurately[15]. During the operation, 0.05 was considered statistically significant[15,16]. We used IBM SPSS Statistics software (version 23; SPSS Research Institute) for all data analyses.

Results

Demographics and essential characteristics of COVID-19 patients

Our study included 178 participants diagnosed with COVID-19, of whom 89 patients (50%) were male. The median interval age for all participants was 64 years (IQR, 53-71), and the majority were over 60 years old (58.99%). Of all patients, 52 (29.21%) cases had a history of primary diseases, including hypertension, hyperlipidemia, hyperuricemia, coronary heart disease, diabetes mellitus, kidney stones, hemangioma, rheumatoid arthritis, gastritis, chronic bronchitis, etc.

We divided the patients into moderate, severe and critical severe groups according to the latest Chinese guidelines. We found that three groups were significantly higher patients older than 60 years than patients younger than 60 (P< 0.0001). However, we found no significant differences in disease severity in gender and primary disease groups (P= 0.445, P= 0.371). The severe and critical severe groups were an observably higher percentage of patients' basic disease scores than the moderate group (P= 0.007). The demographic and clinical characteristics of all patients are shown in Figure 1 and Table 1.

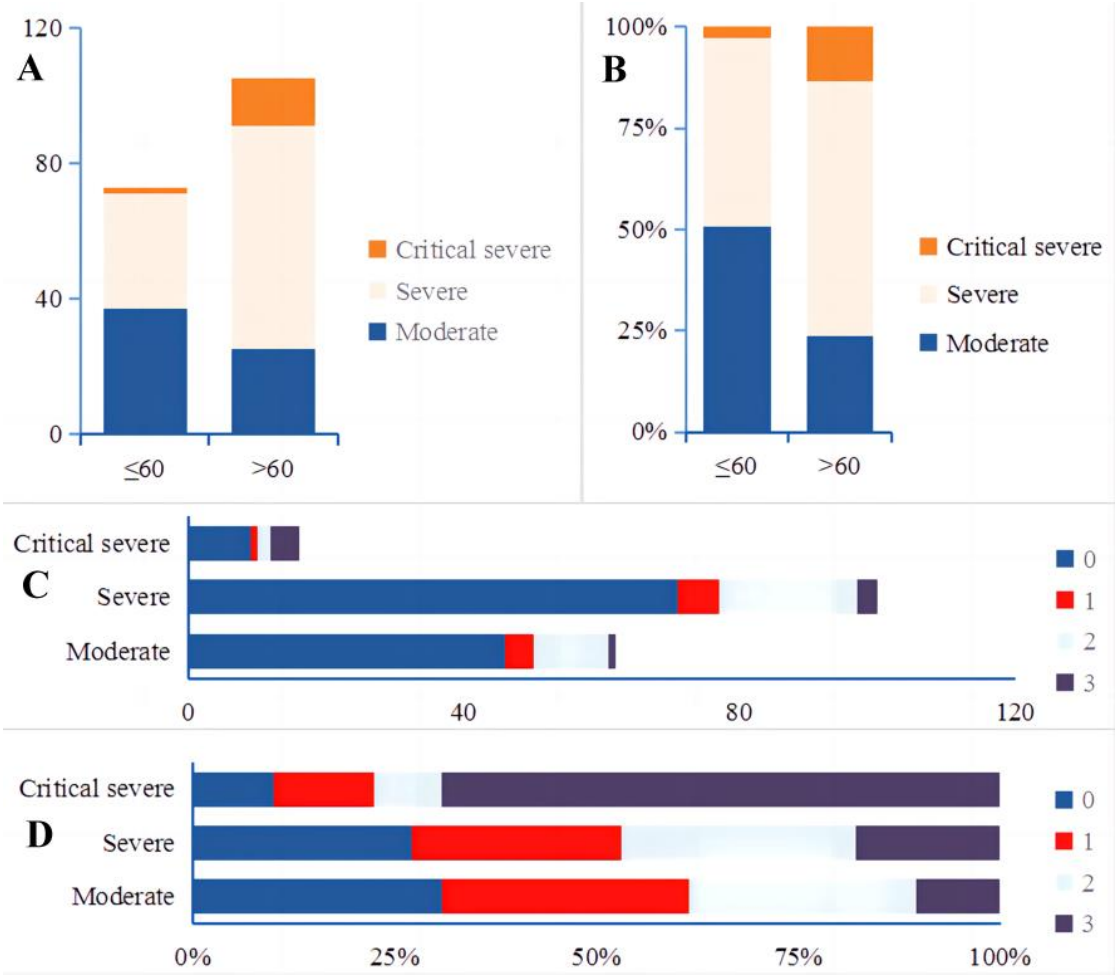


Figure 1 The primary characteristics of all COVID-19 patients. (A) Age groups were correlated with the number of patients in the three different severe groups. (B) Age groups were correlated with the percentage of patients in the three groups. (C) Basic disease score groups were correlated with the number of patients in the three different severe groups. (D) Basic disease score groups correlated with the percentage of patients in the three groups. Basic disease score 0 means no basic disease; basic disease score 1 means there is only one basic disease; basic disease score 2 means there are two basic diseases; basic disease score 3 means having more than two basic diseases.

Table 1. Clinical Characteristics of all COVID-19 patients

Characteristics	All patients (n= 178)	Disease Severity			*P* value
		Moderate(n=62)	Severe(n= 100)	critical severe(n= 16)	
Median age (IQR) - yrs	64 (53-71)	57(46-64.25)	67(57-74)	69.5(63-82.75)	**<0.0001**
Age group no/total (%)					**<0.0001**
<=60	73/ 178(41.01%)	37/73(50.68%)	34/73(46.58%)	2/73(2.74%)	
>60	105/ 178(58.99%)	25/105(23.81%)	66/105(62.86%)	14/105(13.33%)	
Gender group no/total(%)					0.445
Male	89/ 178(50.00%)	28/89(31.46%)	51/89(57.30%)	10/89(11.24%)	
female	89/ 178(50.00%)	34/89(38.20%)	49/89(55.06%)	6/89(6.74%)	
Basic disease no/total(%)					0.371

No	126/ 178(70.79%)	46/126(36.51%)	71/126(56.35%)	9/ 126(7. 14%)	
Y	52/ 178(29.21%)	16/52(30.7%)	29/52(55.77%)	7/52(13.46%)	
Basic disease score no/total(%)					**0.007**
0	126/ 178(70.79%)	46/126(36.51%)	71/126(56.35%)	9/ 126(7. 14%)	
1	11/ 178(6. 18%)	4/ 11(36.36%)	6/ 11(54.54%)	1/ 11(9.09%)	
2	33/ 178(18.54%)	11/33(33.33%)	20/33(60.61%)	2/33(6.06%)	
3	8/ 178(4.49%)	1/8(12.50%)	3/8(37.50%)	4/8(50.00%)	
Total score (score/n%)	92/ 178(51.69%)	20/62(32.26%)	55/100(55.00%)	17/16(106.25%)	

IQR: inter-quartile range. The bold font indicates a*P* value less than 0.05. Basic disease score 0 means no basic disease; basic disease score 1 means there is only one basic disease; basic disease score 2 means there are two basic diseases; basic disease score 3 means having more than two basic diseases.

Laboratory findings of COVID-19 patients

In terms of clinical blood routine, we found that there was significant differences moderate, severe and critical severe groups in WBC (*P*< 0.0001), neutrophil counts (*P*< 0.0001), percentage of neutrophil (*P*< 0.0001), lymphocyte count (*P*= 0.003), percentage of lymphocyte (*P*< 0.0001), percentage of monocyte (*P*= 0.006), eosinophil count (*P*= 0.014), RBC (*P*= 0.007), hematocritg (*P*= 0.041), MCHC (*P*< 0.0001), PLA (*P*= 0.03) and MPV (*P*= 0.001). In terms of sodium, calcium, chlorine and phosphorus, significant differences moderate, severe, and critical severe groups were found.

Similarly, the critical severe group had significant differences percentage of patients and the moderate group and severe groups showing elevated blood glucose (*P*= 0.003), direct bilirubin (*P*< 0.0001), uric acid (*P*< 0.0001), blood urea nitrogen (*P*= 0.001), total protein (*P*= 0.001), albumin (*P*< 0.0001), A/G (*P*= 0.004), AST (*P*= 0.011), ALP (*P*= 0.003), LDH (*P*< 0.0001), CK (*P*= 0.011), CK-MB (*P*= 0.005), PCT (*P*< 0.0001), hs-CRP (*P*< 0.0001), CRP (*P*< 0.0001), cystatin C (*P*= 0.002), α-HBDH (*P*< 0.0001).

However, no prominent differences were observed in the other clinical blood index, including monocyte count, percentage of eosinophil, basophilic granulocyte count, percentage of basophilic granulocyte, MCV, MCH, potassium, magnesium, ALT, total bilirubin, indirect bilirubin, globulin, total bile acid, creatinine, γ-GGT, and total carbon dioxide. The basic information of analysis result is described in Figure 2, Table 2 and Supplementary Table S1-S3. We refer to the normal range values of test reagents in Supplementary Table S4.

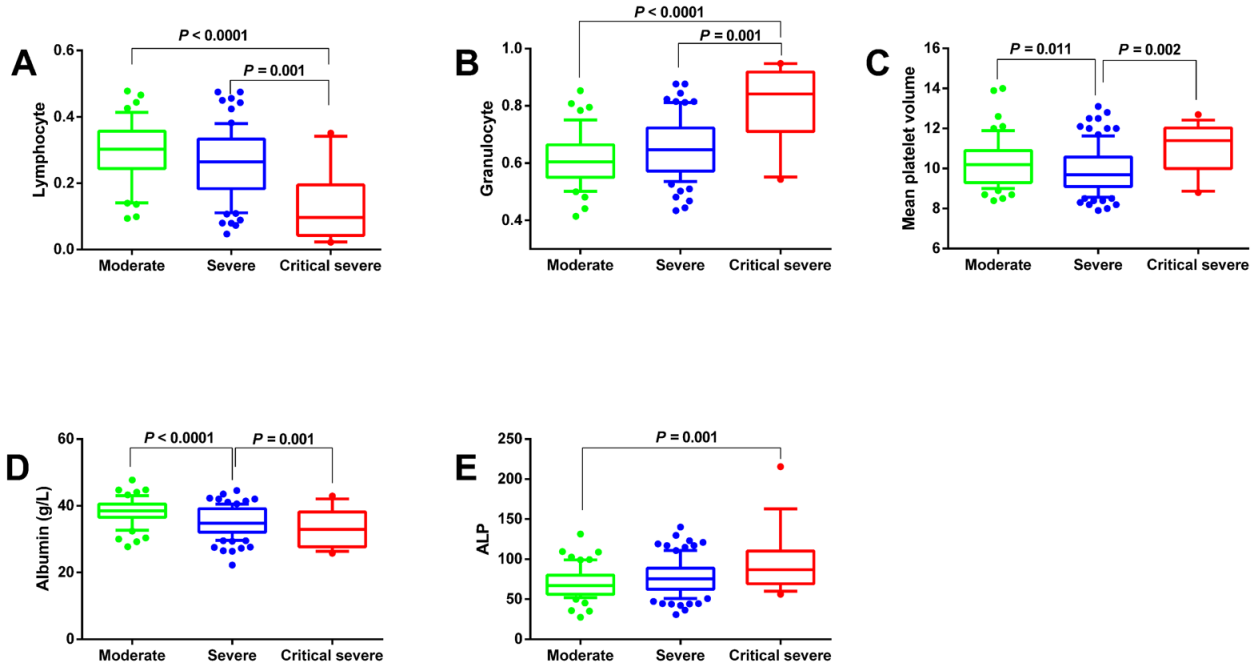


Figure 2 Clinical index showed significant differences among moderate, severe, and critical severe groups. (A) Lymphocytes in three groups. **(B)** Granulocyte in three groups. **(C)** Mean platelet volume in three groups. **(D)** Albumin in three groups **(E)** Alkaline phosphatase in three groups.

Table 2. Laboratory testing results of all COVID-19 patients

Median (IQR)	All(n= 178)			**Disease Severity**			*P*	*P* value
		Moderate(n= 62)	*P*	Severe(n= 100)	*P*	critical severe(n= 16)		
Median age (IQR) years	64(53-71)	57(46-64.25)	**<0.0001**	67(57-74)	0.125	69.5(63-82.75)	**<0.0001**	**<0.0001**
WBC×109/L	6.2(5.08-8)	5.7(4.8-6.975)	0.132	6.35(5.1-7.73)	**0.001**	8.65(6.98-11.4)	**<0.0001**	**<0.0001**
Neutrophil count ×10^9/L	4.03(2.9-5.48)	3.61(2.5575-4.9925)	0.155	4.02(2.91-5.35)	**<0.0001**	7.29(5. 14-10.23)	**<0.0001**	**<0.0001**
Percentage of neutrophil	64.70%(57.70%-74.90%)	61. 15%(56.70%-69.88%)	0.093	64.80%(57.80%-74.70%)	**0.001**	86.75%(69.28%-89.50%)	**<0.0001**	**<0.0001**
Lymphocyte count ×109/L	1.41(0.90-1.83)	1.44(1.075-1.73)	0.627	1.43(0.90-1.92)	**0.002**	0.72(0.51- 1. 13)	**<0.0001**	**0.003**
Percentage of lymphocyte	23.20%(14. 18%-30.28%)	26.30%(20.75%-31.93%)	0.074	24.10%(14.50%-30.05%)	**<0.0001**	7.25%(3.78% - 15.78%)	**<0.0001**	**<0.0001**
Monocyte count	0.44(0.35-0.6)	0.42(0.34-0.52)	0.167	0.46(0.36-0.64)	0.671	0.49(0.22-0.63)	0.919	0.400
percentage of monocyte	7.40%(6.08%-9.20%)	7.30%(6.40%-8.50%)	0.192	7.85%(6.38%-9.40%)	**0.003**	4. 15%(2.53%-8. 13%)	**0.01**	**0.006**
Eosinophil count	0. 12(0.07-0.20)	0.12(0.05-0.18)	0.544	0.12(0.08-0.19)	**0.007**	0.35(0.12-0.49)	**0.005**	**0.014**

Percentage of eosinophil	2.00%(1.10%-3.20%)	1.9%(1.00%-3.08%)	0.642	1.95%(1.20%-3.23%)	0.185	3. 10%(1.30%-5.70%)	0.123	0.302
Basophilic granulocyte count	0.02(0.01-0.03)	0.02(0.01-0.03)	0.881	0.02(0.01-0.03)	0.886	0.02(0.01-0.04)	0.852	0.978
Percentage of basophilic granulocyte	0.30%(0.20%-0.50%)	0.30%(0.20%-0.50%)	0.784	0.30%(0.20%-0.50%)	0.738	0.30%(0.20%-0.50%)	0.075	0.12
MCV	93.15(90.4-95.2)	93.10(90.40-94.68)	0.228	93.10(90.33-95.40)	0.228	93.65()90.58-99.03	0.161	0.257
Hematocrit g	93.15(90.4-95.2)	36.9(32.9-40..2)	0.052	35(32.23-38)	0.231	34.15(26.1-37.95)	0.033	**0.041**
MCH	31.1(30.3-31.9)	31.1(30.4-31.8)	0.488	31.1(30.2-31.9)	0.644	30.8(30.28-31.85)	0.372	0.629
MCHC	335(329-340)	338(333-343)	**0.004**	334.5(329-339)	**0.002**	324.5(316-332)	**<0.001**	**<0.001**
Hb	122(106-132)	126(110-139)	0.023	117.5(106.25-129)	0.062	109(86.25-124.5)	**0.007**	**0.006**
RBC	3.9(3.45-4.27)	4.09(3.79-4.34)	**0.012**	3.795(3.42-4.19)	0.179	3.71(2.72-4.15)	**0.010**	**0.007**
PLA $\times10^9$/L	214(168.75-252.75)	211(166.25-251.25)	0.247	221.5(179.25-271)	**0.012**	169(94.75-220.5)	0.054	**0.030**
MPV	10(9.2-10.98)	10.25(9.35-10.9)	**0.011**	9.7(9.1-10.58)	**0.002**	11.4(10-12.03)	0.075	**0.001**
ALT	21.85(14.33-45.3)	25.8(13.625-47.7)	0.792	21.45(14.15-42.83)	0.343	30.25(17.65-44.03)	0.586	0.680
AST	19.7(15.3-27.85)	18.7(14.5-27.8)	0.731	19.5(15.3-27.40)	**0.004**	27.45(18.98-50.35)	**0.005**	**0.011**
Total bilirubin	9.6(7.3-12.1)	10.4(7.475-12.85)	0.143	8.75(7.1-11.02)	0.060	10.65(8.08-14.75)	0.503	0.109
Direct bilirubin	3.2(2.4-4.5)	3.3(2.675-4.43)	0.106	2.9(2.3-4.03)	**<0.001**	5.55(3.5-7.55)	**0.002**	**<0.001**
Indirect bilirubin	5.68(4.37-7.58)	5.46(4.47-8.15)	0.813	5.81(4.47-7.15)	0.394	4.41(4.16-7.90)	0.333	0.62
Total protein	62.35(57.98-67.65)	65.2(60.78-68.85)	**0.005**	61.75(57.1-66.5)	0.068	54.85(51.08-64.98)	**0.003**	**0.001**
Albumin	36.7(32.7-39.4)	38.55(36.5-40.53)	**<0.001**	34.75(32.08-39.15)	0.110	32.9(27.7-38.1)	**0.001**	**<0.001**
Globulin	26.05(23.13-28.7)	25.25(23.6-28.05)	0.407	26.4(22.88-29.05)	0.173	24(19.98-28.48)	0.343	0.323
A/G	1.42(1.22-1.58)	1.49(1.36-1.63)	**0.001**	1.37(1.17-1.52)	0.885	1.36(1.18-1.62)	0.059	**0.004**
Total bile acid	4.3(2.8-7.55)	4(2.55-5.8)	0.308	4.4(2.85-7.65)	0.240	6.45(3.03-11.4)	0.069	0.193
Blood glucose	5(4.45-6.02)	4.66(4.31-5.63)	0.082	5.03(4.47-5.93)	**0.010**	6.21(5.15-9.06)	**0.002**	**0.003**
Blood urea nitrogen	4.59(3.65-5.66)	4.28(3.32-5.06)	0.081	4.74(3.66-5.87)	**0.005**	6.42(4.48-9.33)	**<0.001**	**0.001**

Uric acid	233(181-310)	254(196.75-326)	0.098	230(181-301)	**0.001**	156.5(101.25-206.5)	**<0.001**	**<0.0001**
Creatinine	62.2(53.4-72.8)	58.15(53.05-73.15)	0.212	62.9(55.2-72.3)	0.790	61.45(52.78-76.13)	0.794	0.478
Sodium	140.95(139.2-142.8)	141.7(140.45-143.35)	**<0.001**	140.1(138.2-142)	0.073	143.2(136.95-145.9)	0.645	**0.001**
Potassium	4.28(3.93-4.61)	4.17(3.86-4.56)	0.141	4.32(3.97-4.6)	0.442	4.42(3.97-4.765)	0.077	0.152
Calcium	2.13(2.02-2.21)	2.14(2.06-2.22)	0.196	2.13(2.04-2.21)	**0.009**	2.01(1.87-2.15)	**0.002**	**0.006**
Magnesium	0.89(0.84-0.94)	0.89(0.84-0.94)	0.905	0.89(0.84-0.94)	0.755	0.87(0.78-0.97)	0.685	0.927
Chlorine	105.75(103.43-107.9)	106.6(104.95-108.55)	**0.013**	105.4(103.1-107.4)	0.204	103.75(100.48-106.5)	0.017	**0.01**
Phosphorus	1.08(0.92-1.24)	1.1(1.01-1.29)	0.200	1.08(0.9-1.23)	0.030	0.97(0.68-1.10)	**0.007**	**0.019**
ALP	72(60.25-88.85)	67(56.2-80.1)	0.025	75.35(62.48-88.63)	0.047	86.8(69.23-110.28)	**0.001**	**0.003**
LDH	186.6(159.85-229.8)	167.9(146.28-212.33)	0.060	185.3(165.9-225.2)	**<0.001**	358.2(258.18-410.25)	**<0.001**	**<0.0001**
CK	41.5(28.3-70.9)	47.3(33.23-80.63)	**0.010**	37.3(22.6-57.4)	0.036	51.7(38.23-86.48)	0.482	**0.011**
CK-MB	9.3(6.8-11.9)	9.15(7.15-10.83)	0.989	8.5(6.40-11.70)	**0.003**	13.1(9.28-20.73)	**0.001**	**0.005**
PCT	0.06(0.03-0.1)	0.04(0.03-0.06)	**0.001**	0.06(0.04-0.09)	**<0.001**	0.295(0.17-0.55)	**<0.001**	**<0.0001**
CRP	3.55(1.04-14.88)	1.92(0.45-6.4)	**0.004**	4.63(1.25-10.69)	**0.001**	58.7(6.14-125.25)	**<0.001**	**<0.0001**
hs-CRP	3.64(1.07-10)	1.99(0.52-6.73)	0.019	4.69(1.34-10)	**0.002**	10(5.88-10)	**<0.001**	**<0.0001**
Cystatin C	0.96(0.83-1.1)	0.92(0.8-1)	**0.013**	0.98(0.86-1.1)	0.037	1.2(0.88-1.44)	**0.002**	**0.002**
a-HBDH	150(128.3-194.25)	139.1(120.25-176.03)	0.082	150(130.6-188.6)	**<0.001**	297.75(212.2-349.6)	**<0.001**	**<0.0001**
Y-GGT	32.8(22.25-59.7)	30.1(19.4-57.9)	0.205	35.8(22.95-65.55)	0.801	37.65(24.75-58.05)	0.688	0.458
Total carbon dioxide	24.2(22.9-25.7)	23.9(22.6-25.1)	0.316	24.2(23.2-25.75)	0.115	25.85(22.58-29.7)	0.075	0.14

IQR: inter-quartile range. The bold font indicates a *P* value less than 0.05. WBC: White blood cell; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; Hb: Haemoglobin; RBC: Red blood cell; PLA: Platelet; MPV: Mean platelet volume; ALT: alanine aminotransferase;AST:aspartate aminotransferase; A/G: The ratio of albumin t globulin; ALP: alkaline phosphatase; LDH: lactate dehydrogenase; CK: Creatine Kinase; CK-MB: creatine kinase-MB; PCT: procalcitonin; CRP: C-reactive protein; hs-CRP: high-sensitivity C-reactive protein; RBC:Red blood cell; α-HBDH: alpha-hydroxybutyric dehydrogenase; γ-GGT: γ-glutamyltranspeptidase.

Supplementary Table S4 Reference range of laboratory values

Laboratory test	Reference Range
White blood cell count	3.5-9.5 $\times 10^9$/L

Neutrophil count	1.80-6.3 $\times 10^9$/L
Percent of neutrophil	40-75%
Lymphocyte count	1.1-3.2 $\times 10^9$/L
Percent of lymphocyte	20-50%
Monocyte count	0.1-0.6 $\times 10^9$/L
Percent of monocyte	3-10%
Eosinophil count	0.02-0.52 $\times 10^9$/L
Percent of eosinophil	0.4-8%
Basophilic granulocyte count	0-0.6 $\times 10^9$/L
Percent of basophilic granulocyte	0-1%
Nucleated red blood cell count	10^9/L
Percent of nucleated red blood cell	0
Red blood cell	4.3-5.8 $\times 10^{12}$/L
Mean corpuscular volume	82-100 fL
Hematocritg	40-50
Mean corpuscular hemoglobin	27-34 pg
Mean corpuscular hemoglobin concentration	316-354 g/L
Haemoglobin	130-175 g/L
Platelet count	125-350 $\times 10^9$/L
Mean platelet volume	8-10 fL
CRP	0-4 mg/L
Hs-CRP	0-4 mg/L
ALT	9-50 IU/L
AST	9-60 IU/L
Total protein	65-85 g/L
Albumin	40-55g/L
Globulin	20-40g/L
A/G	1-2.4
Total bilirubin	0-26 μmol/L
Direct bilirubin	0～8 μmol/L
Indirect bilirubin	0-14 μmol/L
Total bile acid	0-10 umol/L
Blood glucose	3.9-6.11 mmol/L
blood urea nitrogen	3.6-9.5 mmol/L
Creatinine	57-111 μmol/L
Uric acid	202-416 umol/L
ALP	45-125 IU/L
γ-GGT	10-60 IU/L
Sodium	137-147 mmol/L
Potassium	3.5-5.3 mmol/L
Calcium	2.11-2.52 mmol/L
Magnesium	0.75-1.02 mmol/L
Chlorine	99-110 mmol/L
Phosphorus	0.85-1.51 mmol/L
LDH	120-250 IU/L

Creatine Kinase	24-190 IU/L
CK-MB	24-190 IU/L
α-HBDH	72-182 IU/L
Cystatin C	0.63-1.25 mg/L
PCT	0-0.05 ng/mL
Total carbon dioxide	22-29 mmol/L
CD3+cell	59%-85%
CD4+cell	Female: 31%-61%，Male: 29%-57%
CD8+cell	11%-38%
CD4+ cell/CD8+ cell	0.9-3.6
CD3-CD19+ cell	6.4%-23%
CD3-(CD16+/CD56+) cell	5.6%-31%
Lymphocyte	20-40%
Monocyte	3-8%
Granulocyte	50-70%
IL5	≤3.1
IFN-α	≤8.5
IL-2	≤7.5
IL-6	≤5.4
IL-1β	≤12.4
IL-10	≤12.9
IFN-γ	≤23.1
IL-8	≤20.6
IL-17	≤21.4
IL-4	≤8.56
IL-12p70	≤3.4
TNF-α	≤16.5

Immunological findings of COVID-19 patients

The lymphocyte and granulocyte showed a significant difference among the moderate, severe, and critical severe groups ($P< 0.0001$ and $P= 0.001$). Compared with the moderate group, patients in critical have significantly lower lymphocytes. However, granulocyte was substantially higher in the critical severe group than in the moderate and severe groups, respectively. CD8 + T cells showed a different percentage among three moderate, severe, and critical severe groups ($P= 0.027$). However, we failed to observe noteworthy differences in the other clinical and immunological tests, including monocyte, percentage of CD3+ T cell, percentage of CD4+ T cell and the ratio of CD4+ T cell to CD8+ T cell, CD3-CD19+ T cell, and CD3-(CD16+/CD56+) T cell (Table 3).

Table 3. Immunological results of all COVID-19 patients

Median (IQR)	All(n= 178)	Moderate(n =62)	Disease Severity				*P3*	*P* value
			P1	Severe(n= 100)	*P2*	critical severe(n= 16)		
Median age (IQR) - years	64 (53-71)	57(46-64.25)	**<0.0001**	67(57-74)	0.125	69.5(63-82.75)	**<0.0001**	**<0.0001**

Lymphocytes, %	27. 15(17.78-33.33)	30.30 (24.40 - 35.70)	0.069	26.50 (18.35 -33.35)	0.001	9.7 (4.30 - 19.48)	**<0.001**	**<0.001**
Monocytes, %	8.00(7.08-9.90)	7.90 (6.70 - 10.00)	0.765	8. 10 (7.30 - 9.80)	0.087	6.85 (2.68 - 9.08)	0. 102	0.2080
granulocytes, %	64.25(56.63 -72.90)	60.50 (55.00 - 66.40)	0.054	64.70 (57.20 - 72.25)	0.002	84. 15 (71.03 - 91.80)	**<0.001**	**0.001**
CD3+ T cells, %	73.00 (65.20 - 78.30)	69.90 (62.30 - 76.90)	0. 11	73.70 (67.75 - 78.75)	0.247	65.75 (55.83 -79.60)	0.502	0. 184
CD4+ T cellsl, %	43.85 (38.68 - 49.90)	42.90 (39.30 - 49.90)	0.916	44.40 (38.55 - 50.00)	0.287	39.90 (28.25 -49. 10)	0.271	0.531
CD8+ T cellsl, %	22.35 (17.60 - 27.83)	21.60 (17.00 - 25.90)	0.025	23.70 (18.55 -30.40)	0.075	16.50 (12. 15 -30.55)	0.236	**0.027**
CD4+ T cellsl/CD8+ T cellsl	2.00(1.40-2.80)	2. 1(1.5-2.9)	0.093	1.9(1.3-2.5)	0.233	2.5(1.6-3.25)	0.629	0. 163
CD3-CD19+ T cellsl, %	11.35 (7.95 - 15.63)	11.30 (8.70 - 15.20)	0.542	11.30 (7.05 - 15.45)	0.098	17.60 (7.88 - 28. 18)	0.236	0.255
CD3-(CD16+/CD56+), %	12.50 (8. 18 - 18.25)	13. 10 (8.50 -20.80)	0.295	12. 10 (8. 10 - 17.75)	0.456	11.95 (4.88 - 17. 13)	0.248	0.378

IQR: inter-quartile range. The bold font indicates a *P* value less than 0.05.

The cytokine analysis of COVID-19 patients

We analyzed the relationship between the level of cytokines, including IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-17, TNF-α, IFN-α, and IFN-γ, and disease severity. The result revealed that IL-6, IL-8, and IL-10 showed significant differences among moderate, severe, and critical severe groups ($P< 0.0001$, $P= 0.001$, and $P= 0.003$). Compared with the moderate group, patients in the critical severe group have significantly higher IL-6, IL-8, and IL-10. Similarly, there were significant differences between IL-2 and TNF-α among the three groups ($P= 0.01$ and $P= 0.038$). In addition, there is an edge effect between IL-17 and disease severity ($P= 0.063$). The analysis results are described in Table 4.

Table 4. Cytokine detection results of all COVID-19 patients

Median (IQR)	All(n= 178)			Disease severity			*P3*	*P* value
		Moderate(n=62)	*P1*	Severe(n = 100)	*P2*	critical severe(n= 16)		
Median age (IQR) - years	64 (53-71)	57(46-64.25)	**<0.001**	67(57-74)	0. 125	69.5(63-82.75)	**<0.0001**	**<0.0001**
IL-2	2.91(2.67-3.31)	2.91(2.59-3.63)	0.525	2.83(2.59-2.99)	**0.003**	4.26(2.91-4.81)	0.021	**0.01**
IL-4	2.43(2.21-2.91)	2.43(2. 13-2.74)	0.591	2.43(2.28-2.92)	0.7	2.59(2.21-3. 18)	0.454	0.743
IL-5	2.69(2. 10-3.58)	2.48(1.91-4.57)	0.929	2.48(2. 1-2.9)	0.026	3.81(2.69-6.86)	0. 179	0. 111
IL-6	7.85(2.55-30.45)	3.56(2.55-7.85)	0. 119	7.85(2.55-27.22)	**<0.0001**	158.59(23-483.73)	**<0.0001**	**<0.0001**

IL-8	6.94(4.02-9.66)	6.45(5.06-10.86)	0.265	5.06(3.26-8.52)	**0.001**	13.46(8.8-62.54)	**0.006**	**0.001**
IL- 10	3.48(2.98-4.04)	3. 1(2.86-3.48)	0. 135	4. 12(4. 12-11.98)	**0.008**	7.26(3.69-17.99)	**0.001**	**0.003**
IL- 17	2.35(2. 10-2.72)	2.5(1.92-2.67)	0.931	2.27(2.09-2.67)	0.017	2.87(2.345-3.84)	0.078	0.063
IL- 12p70	2.27(1.93 -2.46)	2.09(1.74-2.67)	0. 15	2.27(2.01-2.46)	0.538	2.28(2. 18-2.66)	0. 159	0.238
IFN-α	2.48(2.23-3.08)	2.69(1.97-3.36)	0.983	2.42(2.23-3.08)	0.386	2.81(2.395-2.98)	0.72	0.752
IFN-γ	1.24(0.56-2.93)	2.70(0.27-3.56)	0.479	1.42(0.86-2.82)	0.445	1.21(0.46-2.09)	0.448	0.591
TNF-α	0.78(0.35-1.83)	1.83(0.7-3.95)	0.035	0.7(0.31-1.83)	0.334	0.57(0.09- 1. 12)	0.029	**0.038**
IL- 1 β	5.94(4. 12- 11.98)	7.86(5.94-15.28)	0. 155	4. 12(4. 12-11.98)	0.388	5.94(2.43-5.94)	0.045	0. 15

IQR: inter-quartile range. The bold font indicates a *P* value less than 0.05. IL-2: Interleukin 2; IL-4: Interleukin 4; IL-5: Interleukin 5; IL-6: Interleukin 6; IL-8: Interleukin 8; IL- 10: Interleukin 10; IL- 17: Interleukin 17; IL- 12p70:17p70; TNF-α: tumor necrosis factor α; IFN-α: interferon α; interferon γ: IFN-γ.

Combined diagnostic prediction of ROC curve

Since drawing relative operating characteristic (ROC) curve can only be binary data, we combined the moderate group and the severe group into one group and named it the merge group. Through preliminary screening of data integrity, we included 178 patients with 14 clinical indicators for statistical analysis. Based on binary regression analysis, we found that there are significant differences only seven clinical index including lymphocyte (*P*= 0.010), granulocyte (*P*= 0.006), WBC (*P*= 0.023), neutrophil counts (*P*= 0.025), MPV (*P*= 0.034), albumin (*P*= 0.001), ALP (*P*= 0.018), respectively.

The prognostic accuracy of clinical index for disease severity, as quantified by the area under the ROC curve, was significantly differences among lymphocyte (AUC= 0.651, 95%CI= 0.55-0.76, *P*= 0.005), granulocyte (AUC= 0.659, 95%CI= 0.56-0.76, *P*= 0.003), WBC (AUC= 0.632, 95%CI= 0.53-0.74, *P*= 0.014), neutrophil counts (AUC= 0.644, 95%CI= 0.54-0.75, *P*= 0.008), MPV(AUC= 0.638, 95%CI= 0.53-0.74, *P*= 0.011), albumin (AUC= 0.663, 95%CI= 0.56-0.77, *P*= 0.002) and ALP (AUC= 0.629, 95%CI= 0.53-0.73, *P*= 0.016).

Similarly, with an improvement of diagnostic accuracy of disease severity, we used ROC curve plotting obtained the best decisive threshold by combined panel, including five clinical index (lymphocyte, granulocyte, MPV, albumin and ALP, AUC= 0.780, 95%CI= 0.65-0.84, *P*< 0.0001). The findings of analysis were showed in the Figure 3.

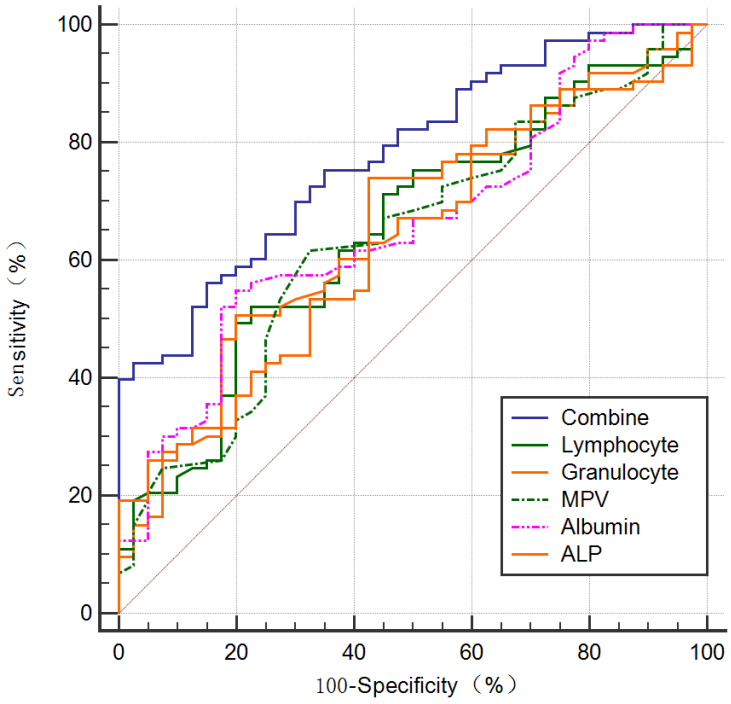


Figure 3 ROC Curve analysis of combine panel compare to six clinical indexes in COVID-19 patients with disease severity. All areas under the curves were significantly different from 0.50. MPV: Mean platelet volume. ALP: Alkaline phosphatase.

Expression of SARS-CoV-2 receptors in the human lung tissue

We investigated the expression of SARS-CoV-2 receptor RNA in different human lung tissue cells. Therefore, we assessed expression levels of six key genes angiotensin-converting enzyme 2 (ACE2), Transmembrane serine protease 2 (TMPRSS2), Dipeptidyl peptidase 4 (DPP4), Alanyl aminopeptidase, membrane (ANPEP), Neuropilin 1 (NRP1), and Furin, paired basic amino acid cleaving enzyme (FURIN), which were related to virus infection at single-cell sequence data (Figure 4 and Supplementary Figure S1-S5). ACE2 and TMPRSS2 were mainly expressed in alveolar epithelial type 2 cells (AT2), basa, and tuft cells. DPP4 and ANPEP were primarily expressed in AT1, AT2, granulocyte, respiratory ciliated cells, and macrophages. NRP1 and FURIN were mainly expressed in endothelial cells and macrophages.

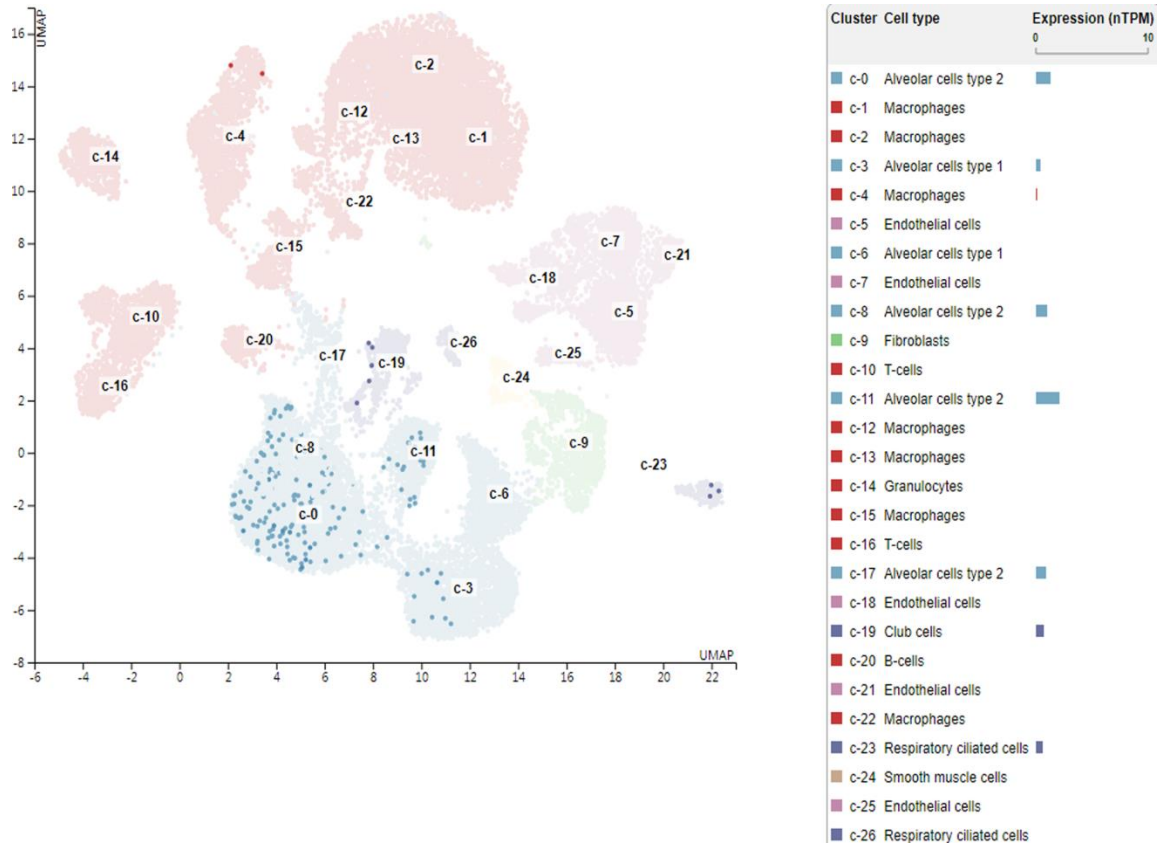
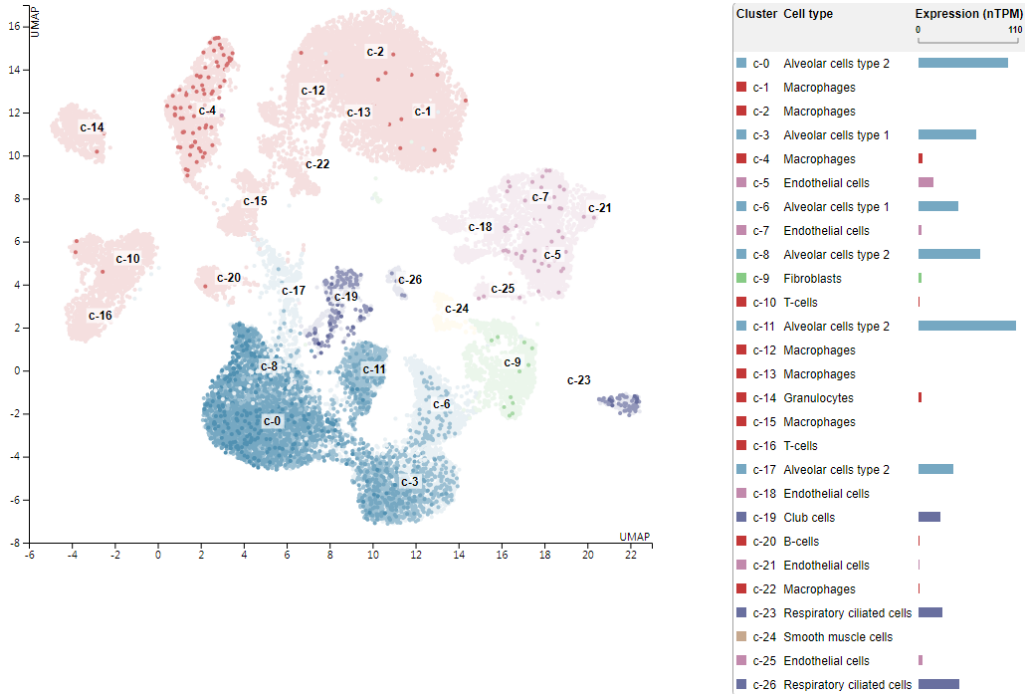
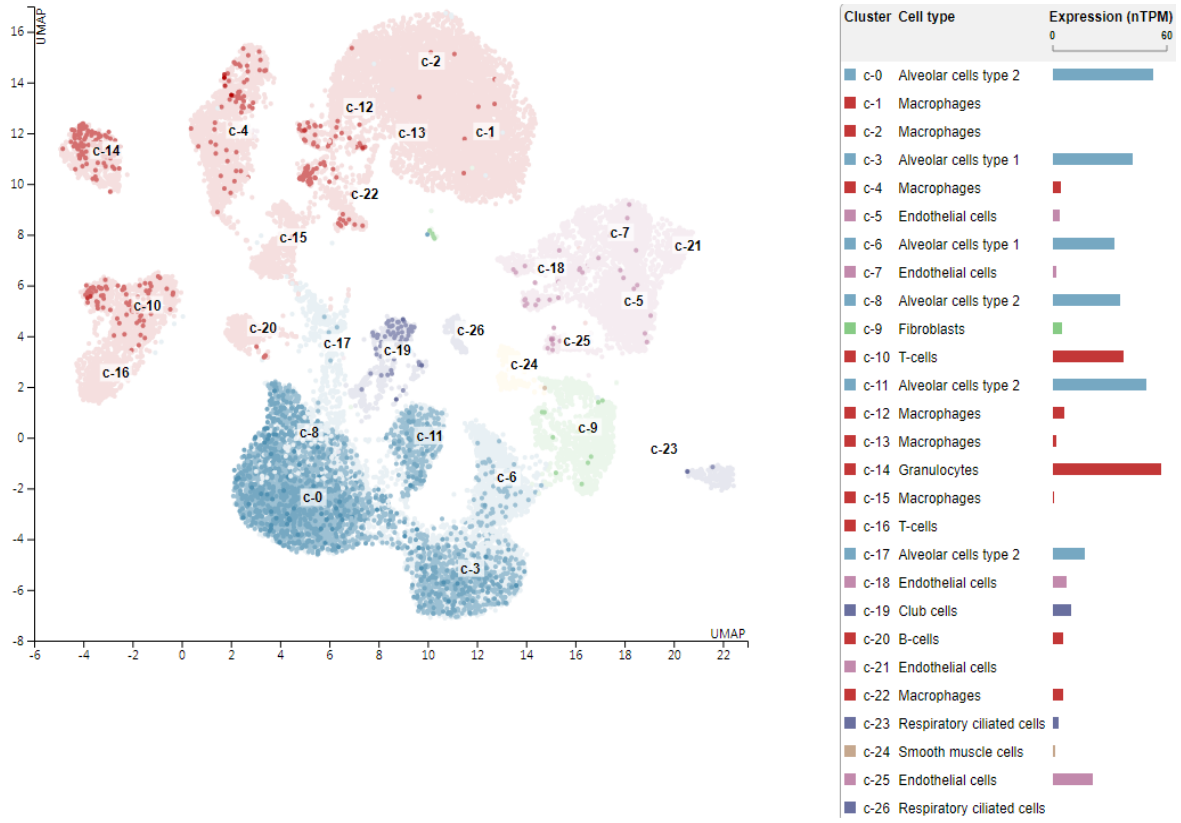


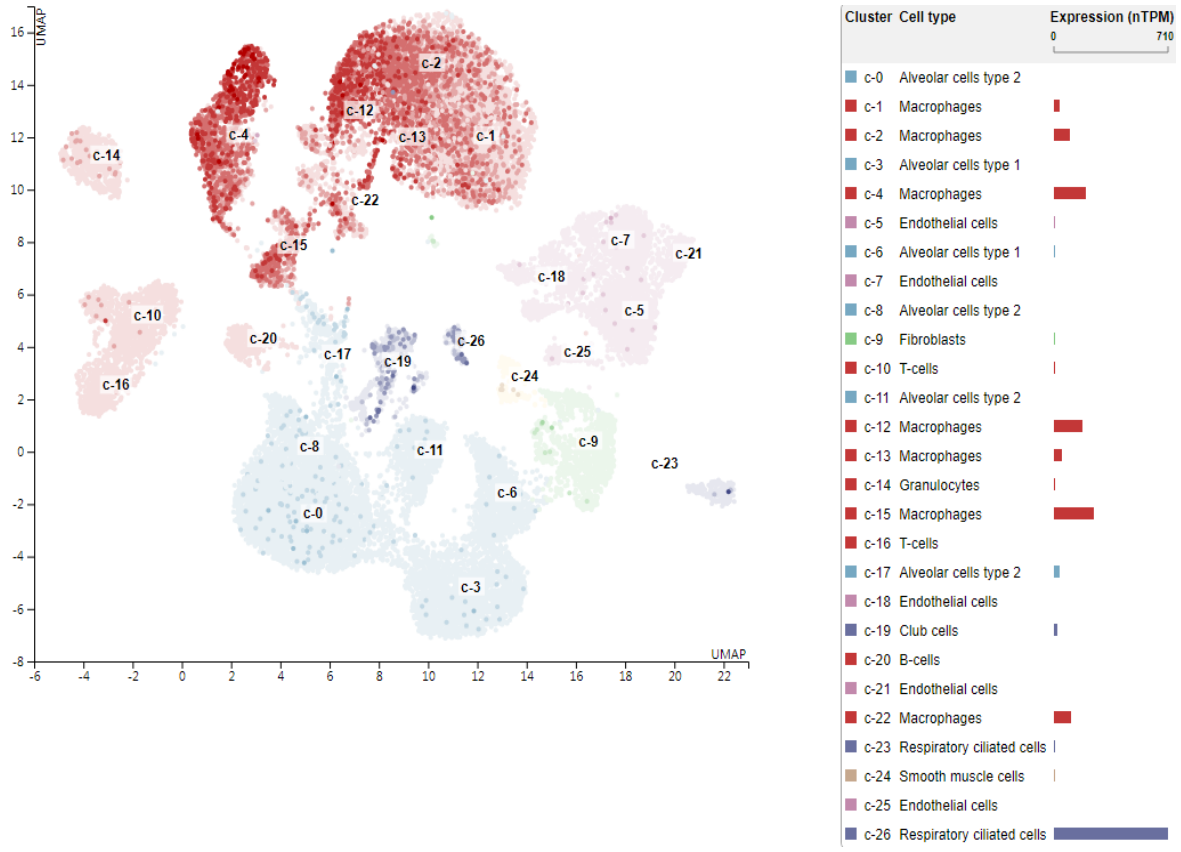
Figure 4 The UMAP plot of ACE2 expression in the human lung tissue by single-cell RNA data UMAP plot visualizes the cells in each cluster; where each dot corresponds to a cell. The color schemes: cell type color, which is based on cell type groups used in the Single Cell Type section. The cell intensities: Intensity, which color the individual cells according to % of max (log2(read_ count +1)/log2(max(read_count)+1)*100) in five different bins (<1%,<25%,<50%,<75%,≥75%). The resulting normalized transcript expression values, denoted nTPM, were calculated for each gene in every sample. nTPM values below 0.1 are not visualized on the Atlas sections.



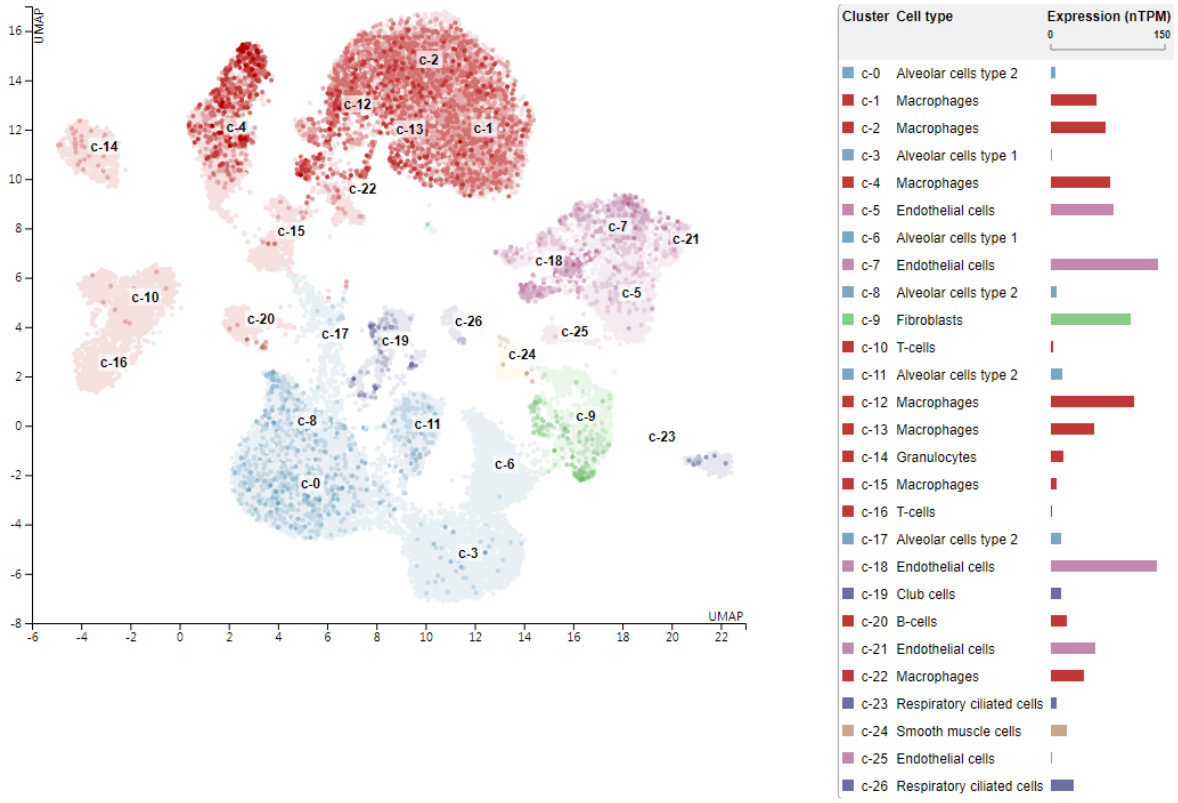
Supplementary Figure S1 The UMAP plot of TMPRSS2 expression in the human lung tissue by single-cell RNA data



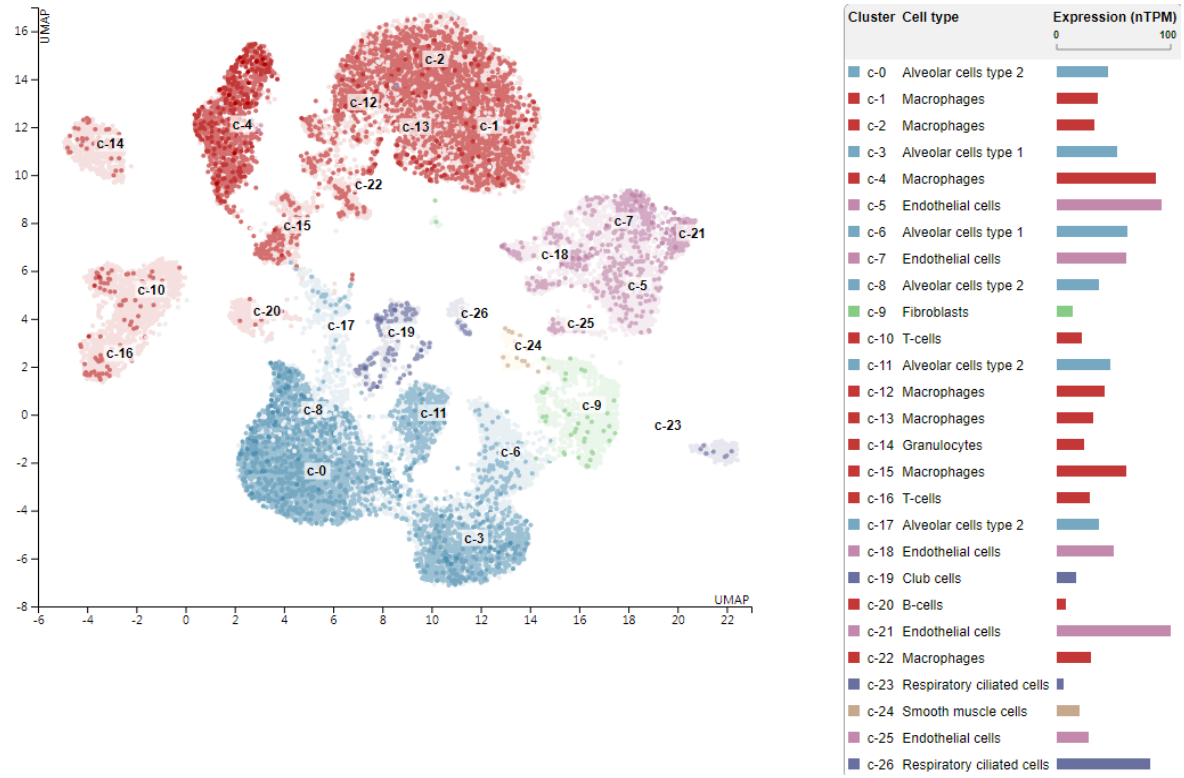
Supplementary Figure S2 The UMAP plot of DPP4 expression in the human lung tissue by single-cell RNA data



Supplementary Figure S3 The UMAP plot of ANPEP expression in the human lung tissue by single-cell RNA data



Supplementary Figure S4 The UMAP plot of NRP1 expression in the human lung tissue by single-cell RNA data



Supplementary Figure S5 The UMAP plot of FURIN expression in the human lung tissue by single-cell RNA data

Discussion

New outbreaks of respiratory disease caused by COVID-19 have become a serious global public health concern[17]. The infection was first declared on 31 December 2019, and the rapid

spread of the virus has heightened fears of a global pandemic. We are pleased that most patients have recovered after timely diagnosis and treatment, thanks to our government's convenient and effective early warning and isolation programs. But incredibly, hundreds of millions of people worldwide have already been diagnosed with COVID-19, and the epidemic is expected to worsen[17,18]. It is significant that the concepts we describe here can be easily extended to other forms of zoonotic diseases, especially those caused by new viral mutations that might otherwise soon overrun our medical systems[19].

SARS-CoV-2 is an RNA virus in the coronavirus family that mainly causes respiratory infections. Transmission is primarily through respiratory droplets and contact. One of the main manifestations of COVID-19 is pneumonia[20]. Previous studies have reported that SARS-CoV-2 has a strong affinity for human respiratory receptors, suggesting that it may pose a potential threat to global public health[13,21]. The natural host of SARS-CoV-2 has failed to be found and is highly suspected to be a bat. Currently, COVID-19 patients are the primary source of infection. More notably, the asymptomatic infections can also be the source of infection[19].

Based on the current epidemiological investigation, the incubation period of COVID-19 is generally 1-14 days, with most being 3-7 days. Clinical symptoms usually include fatigue, fever, and dry cough. Severe symptoms of acute respiratory infection occur in the early stages of COVID-19, and acute respiratory distress syndrome (ARDS), metabolic acidosis, septic shock, and difficult-to-correct coagulation dysfunction occur in exacerbations. However, most patients were mild/moderate, non-pneumonic, and mild (63.1%-85.9%), and even 1.2%-8.3% were asymptomatic carriers[18,20,22-24]. The COVID-19 deaths are most common among the elderly and those with chronic underlying diseases.

The post-COVID-19 syndrome is defined as the persistence of symptoms after viral clearance and the emergence of new symptoms after a few months following recovery from COVID-19. The symptoms may be mild such as fatigue, cough, alopecia, shortness of breath, or severe, leading to acute lower limb ischemia, stroke, renal failure, cardiac abnormalities, etc. During the post-COVID-19 phase, fatigue was the most common persistent symptom, with 34% experiencing fatigue after 60 days and 28.3% even after 90 days from the onset of symptoms[25-28].

We aim to provide an overview of the clinical features of COVID-19, identify areas for improvement in future preparedness plans, and conduct a critical assessment of the risk factors. While the outbreak is still ongoing, early lessons from its spread can help public health officials and medical workers understand its evolution.

Our study found that the moderate, severe, and critical severe groups were significantly higher in patients older than 60 years than in patients younger than 60. We also found that there were striking differences between moderate, severe, and critical severe groups in terms of clinical blood routines, including WBC, neutrophil counts, percentage of neutrophil, lymphocyte count, percentage of lymphocyte, percentage of monocyte, eosinophil count, RBC, hematocrit, MCHC, PLA, MPV, direct bilirubin, uric acid, blood urea nitrogen, total protein, albumin, A/G, AST, ALP, LDH, CK, CK-MB, PCT, CRP, hs-CRP, cystatin C and α-HBDH.

Compared with the moderate and severe groups, lymphocytes were significantly lower in the critical severe group. However, granulocyte in the critical severe group was markedly higher than in the moderate and severe groups. The levels of IL-6, IL-8, and IL-10 in the critical severe group were significantly higher than in the moderate and severe groups. The predictive accuracy of clinical indicators quantifying disease severity by the area under the ROC curve significantly differed among lymphocytes, granulocytes, MPV, albumin, and ALP.

We recalculated the combined panel, including five clinical indicators (lymphocyte*8.46417+granulocyte*11.39640+MPV*-0.47913+albumin*-0.17100+ALP*0.020930). Interestingly, we found two clinical markers of protective factors, MPV and albumin; There are three clinical indicators of risk factors: granulocyte, lymphocyte, and ALP.

Clinicians should focus on these five clinical indicators. If patients show abnormalities in these five clinical indicators, timely intervention measures should be taken to achieve optimal treatment effects and reduce mortality.

It is known that the SARS-CoV-2 Spike protein binds to ACE2 of the host cell, cleaves the Spike protein with the help of proteases such as TMPRSS2 and then forms fusion pores to release RNA into the cytoplasm. The virus is multiplied in infected cells, which sets off an inflammatory reaction. In addition, we found that TMPRSS2 and FURIN could promote the binding of SARS-CoV-2 to ACE2, which were also mainly expressed in AT2 cells.

Several limitations of our study need to be addressed. Firstly, the relatively small total sample size is of limited use in exploring the actual relationship. Secondly, we are limited by a control cohort of COVID-19 patients, not a healthy control group. In addition, our study was determined by the number of subgroup events in the model with a relatively small number of predictors, which may produce an over-fitting of the data. Therefore, we need to further validate this study in an independent discovery and validation cohort before applying the model to clinical decision-making[16,22].

Conclusion

We established the clinical and laboratory characteristics of some patients with COVID-19 in China. Many clinical indexes showed significant differences among three moderate, severe, and critical severe groups. We found diagnostic power of disease severity was improved on a combined panel (lymphocyte, granulocyte, MPV, albumin, and ALP).

Data availability statement

The data sets generated and analyzed during our study are available at the reasonable request of the corresponding author.

Conflict of interest

None of the authors reports any competing financial interest.

Acknowledgment

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Author contributions

All authors contributed significantly to the work reported, whether in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas. All authors took part in drafting, revising, or critically reviewing the article. All authors gave final approval of the version to be published. All authors have agreed on the journal to which the article has been submitted. All authors agree to be accountable for all aspects of the work.

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