

An emerging marker predicting the severity of COVID-19: Neutrophil-Lymphocyte Count Ratio

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Abstract

Background: To analyze clinical features and laboratory indicators and identify the markers of exacerbation in COVID-19. **Methods:** We reviewed clinical histories of 177 patients with confirmed COVID-19. The patients were categorized into mild group (153 patients) and severe group (24 patients). The baseline demographic and laboratory indicators of all patients were collected, including the neutrophil-lymphocyte count ratio (NLCR) and C-reactive protein to albumin ratio (CAR). Receiver operating characteristic curve (ROC) analysis was performed to search for indicators predicting exacerbation in COVID-19 patients, and acquiring the area under the curves (AUCs), sensitivity, specificity and cut-off value. **Results:** The age of the severe group were significantly older than those of the mild group ($P < 0.01$). Fever was the typical symptom in all COVID-19 patients. Cough and fatigue were manifested in mild group, yet severe patients were more prominent in dyspnea. The laboratory indicators showing that the mild group mainly had an elevated C-reactive protein; the severe group had a decreased lymphocyte count and lymphocyte ratio. WBC, neutrophil count, neutrophil ratio, D-dimer, AST, ALT, LDH, BUN, CRP levels increased. Furthermore, compared to mild group, WBC, neutrophil count, neutrophil ratio (Neut%), D-dimer, total bilirubin, albumin, AST, ALT, LDH, BUN, creatine kinase, CRP, CAR, NLCR were significantly higher, the lymphocyte count, lymphocyte ratio, and APTT were significantly lower in severe group ($P < 0.05$). The ROC indicating that NLCR, Neut%, CAR, CRP, and LDH were better at distinguishing mild and severe patients. The AUCs of NLCR was larger than others (NLCR > Neut% > CAR > CRP > LDH: 0.939 > 0.925 > 0.908 > 0.895 > 0.873), which suggested that NLCR was the optimal marker; a cut-off value for NLCR of 6.15 had 87.5% sensitivity and 97.6% specificity for predicting exacerbation in COVID-19 patients. **Conclusions:** The different types of COVID-19 had significant differences in age, clinical symptoms and laboratory indicators, and severe patients might be easier to suffer from the multiple organ damage. An elevated NLCR may indicate that the disease was progressing towards exacerbation. It was essential to dynamically monitor the serum NLCR levels which contributed to evaluate the patient's condition and efficacy. NLCR could be used as a novel, highly specific and sensitive marker for predicting severity of COVID-19 patients.

Background

In late December 2019, several cases of pneumonia of unknown etiology detected in Wuhan, China. So far, the World Health Organization (WHO) has officially named the deadly disease as "COVID-19" [1]. The International Committee on Taxonomy of Viruses (ICTV) named the virus as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) [2]. SARS-CoV-2 virus is primarily transmitted between people through respiratory droplets and contact routes [3-5]. For the virus spreads easily, and the epidemic quickly spread to other parts of China and even the world. As of 28 April 2020, there were 82,858 confirmed cases with COVID-19 reported by national authorities in china, including 4,633 deaths (5.59%) [6]. After the adoption of a series of preventive control and medical treatment measures, the epidemic situation in China has been effectively controlled. However, an alarming acceleration is happening in other countries, such as the United States, Spain, Italy, France. COVID-19 has been constituted as a public

health emergency of international concern. With the deepening of the understanding of the disease and the accumulation of experience in diagnosis and treatment, it has been initially confirmed that COVID-19 is a disease characterized by inflammatory changes in the lungs [7]. However, its pathogenesis, clinical features, pathological changes are not very clear, especially in severe patients with complicated conditions, comorbidities, a long course of disease, and high mortality. Therefore, to identify severe patients in the early stage is of great significance for reducing the clinical morbidity and improving the cure rate. Our study focused on the age, gender, smoking history, epidemiological history, clinical symptoms, laboratory indicators of COVID-19 patients, and combined with peripheral blood neutrophil to lymphocyte count ratio (NLCR), C-reactive protein to albumin ratio (CAR) to explore a convenient, economical, and practical clinical indicator that can predict severity of COVID-19 in the early stage.

Methods

Source of study population

In Hunan Province, China, patients with suspected COVID-19 were admitted and isolated. Collect throat swabs or blood samples and send them to the Hunan Provincial Centers for Disease Control and Prevention as soon as possible after collection. The preventive testing of COVID-19 uses genetic sequence testing or real-time fluorescence polymerase chain reaction (RT-PCR) testing. The diagnosis of COVID-19 was based on at least one positive result of the above two laboratory methods. The confirmed COVID-19 cases were transferred to hospitals designated by the government.

Data Extraction And Collection

We reviewed clinical histories of the patients with confirmed COVID-19 from the designated COVID-19 hospitals in January to February 2020. According to the guideline of COVID-19 (trial version 7) [8], the patients were classified into four types: mild, common, severe, and fatal. The criteria for each clinical type are as follows: (1) Mild type defined as mild clinical symptoms with no evidence of pneumonia on chest CT; (2) Common type defined as fever or respiratory symptoms, with evidence of pneumonia on chest CT; (3) Severe type defined as respiratory distress (respiratory rate ≥ 30 breaths/min), or SpO₂ $\leq 93\%$ in the resting state, or SpO₂/FiO₂ ≤ 300 mmHg; (4) Fatal type defined as respiratory failure and requirement for mechanical ventilation, shock, or complication with other organ failure and requirement for intensive care unit (ICU) care. In our study, the mild and common types were classified as the mild group, and the severe and fatal types were classified as the severe group. The age, gender, smoking history, epidemiological history, temperature, clinical symptoms, laboratory indicators of all patients were collected. Moreover, calculating the neutrophil-lymphocyte count ratio (NLCR), C-reactive protein to albumin ratio (CAR). The clinical and laboratory data were derived from the patient's medical records.

Statistical analysis

Statistical analysis were performed using IBM SPSS Statistics 19.0. Continuous variables were calculated by use of two independent samples t-test when the data were normally distributed. If not, the Mann-Whitney U-test was used. Chi-square test for categorical variables. Receiver operating characteristic (ROC) analysis was performed to search for indicators predicting exacerbation in COVID-19 patients, and acquiring the area under the curves (AUCs), sensitivity, specificity and cut-off value. All data are expressed as mean \pm SD, median or n(%). *P-value* < 0.05 was considered statistically significant.

Results

Characteristics of the study population

There were 177 confirmed cases with COVID-19 reviewed, including 99 males and 78 females, with age distribution ranging from one to eighty-three years old. 85 cases (48%) of the history of exposure in Wuhan, 42 cases (23.72%) of close contact the COVID-19 patients, 10 cases (5.65%) of aggregation, 50 cases (28.25%) did not have any of the above. In our study, there were 153 patients in mild group and 24 patients in severe group. The median ages of mild and severe groups were 40 and 64 years old, respectively (Fig. 1), it suggested that elderly patients might be have a higher risk of severe COVID-19. Fever was the typical symptom in all COVID-19 patients. Otherwise, cough and fatigue were manifested in the mild group, and severe patients were more prominent in dyspnea (Fig. 2). Compared to mild group, the dyspnea and chest tightness symptoms were significantly higher in severe group ($P < 0.01$). In this study, cardiovascular and cerebrovascular diseases (55 patients), lung basic diseases (8 patients), liver diseases (10 patients) were major comorbidities (Fig. 3), and the comorbidities rate in the severe group was significantly higher than mild group ($P < 0.01$). However, there was no difference between the two groups in gender, temperature, fever, cough, diarrhea, anorexia, headache, and fatigue count ($P > 0.05$) (Table 1).

Clinical Laboratory Indicators Analysis

The mild group was characterized by elevated C-reactive protein. Yet, the severe group had a decreased lymphocyte count and lymphocyte ratio. Moreover, white blood cell (WBC), neutrophil count, neutrophil ratio (Neut%), D-dimer, alanine transaminase (ALT), aspartate aminotransferase (AST), lactic dehydrogenase (LDH), blood urea nitrogen (BUN), C-reaction protein (CRP) levels increased. Compared to mild group, WBC, neutrophil count, Neut%, D-dimer, total bilirubin, albumin, ALT, AST, LDH, BUN, creatine kinase, CRP, CAR, NLCR were significantly higher, the lymphocyte count, lymphocyte ratio, and APTT were significantly lower in severe group ($P < 0.05$) (Table 2).

Table 1
Baseline demographic by study group

	Mild(n = 153)	Severe(n = 24)	<i>P</i>
Age(Y)	41.97 ± 14.77	57.92 ± 15.31	< 0.01
Gender			
Male	85(55.56%)	14(58.33%)	NS
Female	68(44.44%)	10(41.67%)	
Smoke	18(11.78%)	2(8.33%)	NS
Complications	45(29.41%)	14(58.33%)	< 0.01
≥ 2 kinds	16(10.46%)	4(16.67%)	NS
cardio-cerebrovascular disease	46(30.07%)	11(45.83%)	NS
pulmonary disease	5(3.27%)	3(12.50%)	NS
hepatic disease	7(4.58%)	3(12.5%)	NS
Temperature(°C)	37.90 ± 0.74	37.96 ± 0.90	NS
Fever	104(67.97%)	21(87.50%)	NS
Dyspnea	12(7.84%)	15(62.50%)	< 0.01
Cough	93(60.78%)	12(50.00%)	NS
Diarrhea	11(7.19%)	2(8.33%)	NS
Anorexia	32(20.92%)	4(16.67%)	NS
Headache	14(9.15%)	3(12.5%)	NS
Chest stuffiness	13(8.50%)	9(37.5%)	< 0.01
Weakness	54(35.29%)	10(41.67%)	NS
Age is expressed as mean ± SD, others data are expressed as n(%).			
P < 0.05 has statistical significance.			

Predictive Markers Of Covid-19 Severity

The ROC curve indicated that NLCR, Neut%, CAR, CRP, and LDH were better at distinguishing mild and severe. Furthermore, the area under the curves (AUCs) of NLCR was larger than that of others, which suggested that NLCR was the optimal maker, a cut-off value for NLCR of 6.15 had 87.5% sensitivity and 97.6% specificity for predicting exacerbation in COVID-19 patients (Fig. 4, Table 3).

Table 2
Comparison of laboratory indicators by study group

	Mild (n = 153)	Severe (n = 24)	P
WBC (10 ⁹ /L)	5.26 ± 1.84	10.22 ± 5.32	< 0.01
Neutrophil count (10 ⁹ /L)	3.22 ± 1.45	9.09 ± 5.03	< 0.01
Neut (%)	60.14 ± 11.02	85.12 ± 12.39	< 0.01
Lymphocyte count (10 ⁹ /L)	1.55 ± 0.75	0.68 ± 0.60	< 0.01
Lymphocyte ratio (%)	30.34 ± 9.83	7.35 ± 6.25	< 0.01
PT (s)	11.69 ± 1.32	11.94 ± 1.45	NS
APTT(s)	33.10 ± 4.74	28.85 ± 5.37	< 0.01
D-dimer(µg/mL)	0.30 ± 0.21	1.93 ± 1.84	< 0.01
Total bilirubin (µmol/L)	12.75 ± 6.65	17.31 ± 9.04	< 0.01
Albumin(g/L)	39.45 ± 4.93	31.25 ± 4.33	< 0.01
ALT(U/L)	28.86 ± 23.22	51.72 ± 31.80	< 0.01
AST(U/L)	25.68 ± 11.67	57.75 ± 29.68	< 0.01
LDH(U/L)	171.25 ± 51.57	312.16 ± 116.09	< 0.01
BUN (mmol/L)	4.58 ± 1.65	8.46 ± 3.42	< 0.01
Creatinine (µmol/L)	60.18 ± 46.28	68.48 ± 45.89	NS
Creatine kinase (U/L)	63.91 ± 46.39	99.13 ± 60.38	< 0.01
CRP(mg/L)	12.15 ± 9.20	45.68 ± 23.07	< 0.01
NLCR	2.34 ± 1.24	17.63 ± 10.84	< 0.01
CAR	0.31 ± 0.24	1.53 ± 0.84	< 0.01
Data are expressed as means ± SD. WBC, white blood cell; Neut%, neutrophil ratio; PT, prothrombin time ; APTT, activated partial thromboplastin time; ALT, alanine transaminase; AST, aspartate aminotransferase ; LDH, lactic dehydrogenase ; BUN, blood urea nitrogen; CRP, C-reaction protein ; NLCR, Neutrophil-Lymphocyte Count Ratio; CAR, C-reactive protein to albumin ratio. P < 0.05 has statistical significance			

Table 3
The markers of predicting severity of COVID-19

	Sensitivity	Specificity	AUCs	Cut-off value
NLCR	0.875	0.976	0.939	6.15
Neut%	0.833	0.976	0.925	79.8
CAR	0.792	0.951	0.908	0.73
CRP(mg/L)	0.75	0.976	0.895	30.05
LDH(U/L)	0.875	0.756	0.873	203.35
AUCs, areas under the curves.				

Discussion

COVID-19 was an acute novel respiratory infectious disease. The disease was first reported in Wuhan, China, and had spread across the world. The source of infection was mainly SARS-cov-2 virus- infected persons, the virus also could be spread by people who had experienced no symptoms, and almost everyone had no immunity to the disease [8]. In our study, the age distribution ranged from one to eighty-three years old; the age of severe patients was older than that of mild patients, and the median ages of mild and severe patients were 40 and 60 years old, respectively, suggesting that elderly patients were at greater risk of severe COVID-19 [9]. With the increase of age, the compensatory ability of various organs of the organism decreases, in addition, the immune system function and resistance were lower [10, 11]. Therefore, we should focus on strengthening the management of elderly COVID-19 patients. The clinical manifestations of different types of COVID-19 were different. Whether it was mild or severe, fever was the typical symptom, cough and fatigue were manifested in mild patients, yet, severe patients were more prominent in dyspnea. As we all had known, fever was an acute response to the infection of the virus in the organism. When infecting the SARS-cov-2 virus, during which the body's metabolism was vigorous and oxygen consumption was increased. Furthermore, the SARS-cov-2 virus could attribute to an overactive immune response, and the cytokine storm in the lungs blocked the exchange of air (oxygen or carbon dioxide) and blood in the lung tissue, causing dyspnea and even respiratory failure. When the patient suffered from dyspnea or exacerbation suddenly, this might indicate that the patient's condition had not been controlled effectively, and even progressing towards exacerbation. In this study, the CRP level of all patients was elevated, which was considered being related to the systemic acute phase inflammatory response induced by SARS-cov-2 virus. Furthermore, the lymphocyte count and lymphocyte ratio decreased in the severe COVID-19 patients, while the WBC, neutrophil count, neut%, D-dimer, ALT, AST, LDH, BUN levels elevated, there were significant differences between the mild and severe patients. This fully revealed that in various stress events of systemic inflammatory response, the physiological response of inflammatory cells was often characterized by an increase in neutrophil count and a decrease in lymphocyte count [12]. At the same time, with the extension of fever time, the aggravation of

hypoxemia, reducing the tolerance of vital organs to hypoxia and lead to the disorder of the internal environment of the organism. The above results indicated that the inflammatory response was aggravated in severe patients, as well as, the liver function and myocardial were damaged, which further suggested that SARS-cov-2 virus could cause multiple organ damage.

The clinical outcomes of COVID-19 were significantly different in different clinical types, and the prognosis might be worse in severe patients. Therefore, the early identification of severe COVID-19 patients was of great value in controlling the incidence of severe diseases, improving the cure rate, and reducing mortality. So, our study analyzed the laboratory indicators of all COVID-19 patients, including NLCR. NLCR defined as peripheral blood neutrophil-lymphocyte count ratio. Neutrophils and lymphocytes were mainly involved in innate and adaptive immunity, respectively. As a combination of them, NLCR was mostly found in the serum of patients with acute inflammation, reflecting the systemic inflammation of the organism and the balance between them. Therefore, NLCR was more sensitive than any of them alone. NLCR was used as a marker to predict the prognosis of pneumonia ^[13, 14] and the risk of death from bacterial infection ^[15] had been reported. When NLCR was elevated, it might be associated with a poor clinical prognosis or an increased risk of death. Our study revealed that NLCR, neut%, CAR, CRP, and LDH were better at distinguishing between mild and severe. Furthermore, NLCR was the optimal maker, a cut-off value for NLCR of 6.15 had 87.5% sensitivity and 97.6% specificity for predicting COVID-19 severity. It is essential to dynamic monitoring the serum NLR levels contribute to evaluate the patient's condition and efficacy. NLCR also was a convenient, economical, and easy-to-obtain clinical inflammatory indicator. Therefore, it was recommended that NLCR could be used as an early predictor of COVID-19 severity.

Limitations

In this study, even though NLCR could predict potentially of COVID-19 severity, there were no the existing NLCR reference values with which to confirm the accuracy of a cut-off value. Studies in large patient cohorts were needed. Moreover, some possible confounder might influence on the NLCR, such as hypertension, diabetes, chronic obstructive pulmonary disease, coronary atherosclerotic heart disease.

Conclusions

The different types of COVID-19 had significant differences in age, clinical symptoms and laboratory indicators, and severe patients might be easier to suffer from the multiple organ damage. An elevated NLCR might indicate that the disease was progressing towards exacerbation. It was essential to dynamically monitor the serum NLCR levels which contributed to evaluate the patient's condition and efficacy. NLCR could be used as a novel, highly specific and sensitive marker for predicting COVID-19 severity.

Abbreviations

WHO, World Health Organization

ICTV, International Committee on Taxonomy of Viruses

COVID-19, coronavirus infected disease 2019

SARS-CoV-2, severe acute respiratory syndrome coronavirus-2

RT-PCR, real-time fluorescence polymerase chain reaction

ICU, intensive care unit

ROC, receiver operating characteristic

AUCs, area under the curves

WBC, white blood cell

Neut%, neutrophil ratio

PT, prothrombin time

APTT, activated partial thromboplastin time

ALT, alanine transaminase

AST, aspartate aminotransferase

LDH, lactic dehydrogenase

BUN, blood urea nitrogen

CRP, C-reaction protein

NLCR ,neutrophil to lymphocyte count ratio

CAR, C-reactive protein to albumin ratio

Declarations

Ethics declarations

Ethics approval and consent to participate

The Medical Ethical Committee (Approved Number, 2020004), which waived the requirement for written informed consent, approved this study. All written informed consents were obtained from all participants and/or their legal guardians.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due individual privacy of patients could be compromised, but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MPZ has analyzed and interpreted the data regarding the COVID-19, and drafted the manuscript. EHX has substantively revised it. JYL, YYC and QZY contribution to the acquisition of datas. All authors read and approved the final manuscript.

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We pay high tribute to the fighters who are fighting COVID-19.

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Figures

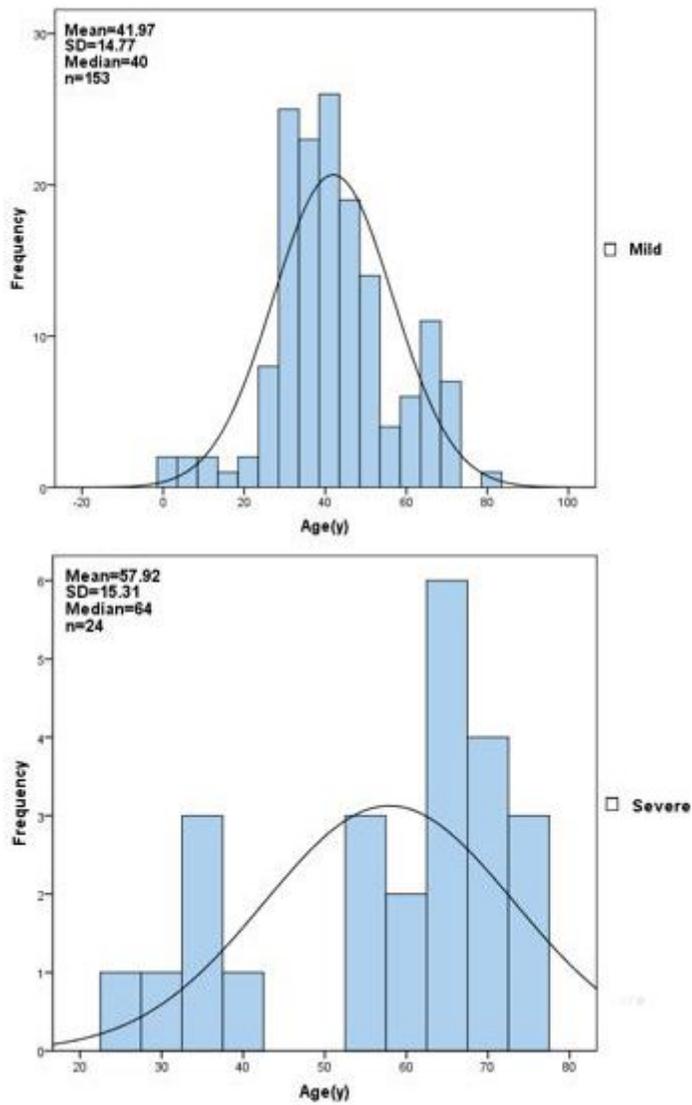


Figure 1

The age distribution of mild and severe COVID-19 patients. The age of severe patients was greater than that of mild patients, and the median ages of mild and severe patients were 40 and 60 years old, respectively.

The main clinical manifestations of COVID-19 patients

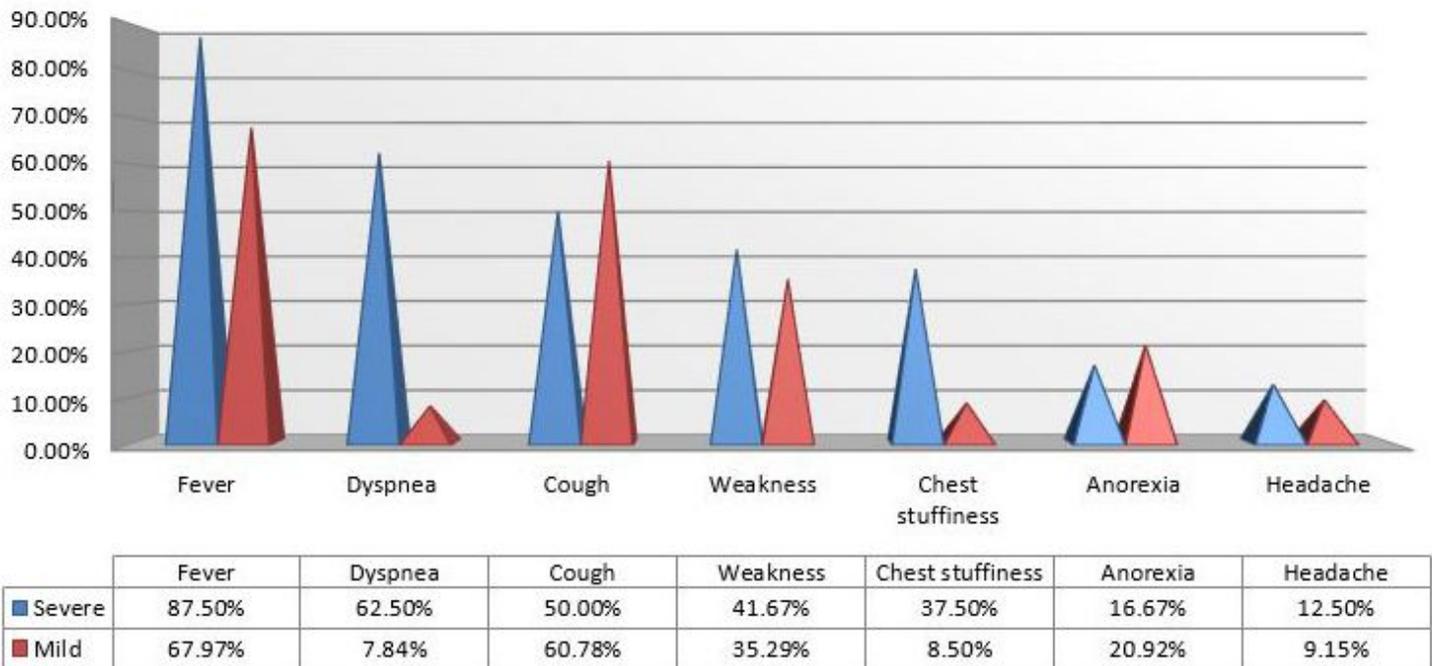


Figure 2

The main clinical symptoms of the mild and severe COVID-19 patients. Fever is the typical symptom in all COVID-19 patients. Otherwise, cough and fatigue were manifested in mild group, and severe patients were more prominent in dyspnea.

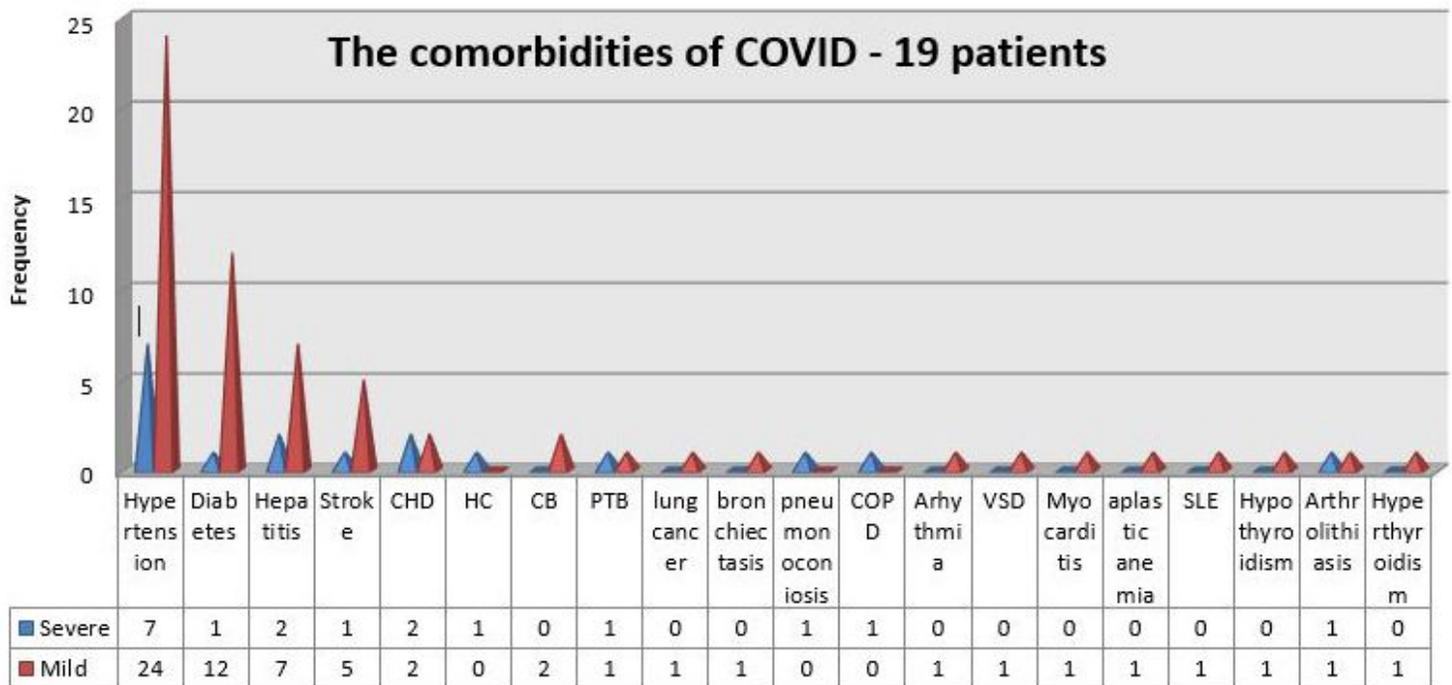


Figure 3

The distribution of comorbidities in mild and severe COVID-19 patients. Hypertension (17.51%) was the most common comorbidity in COVID-19 patients. Diabetes, hepatitis, stroke and CDH are also the major comorbidities. (CHD, coronary atherosclerotic heart disease; HC, Hepatic cirrhosis; CB, Chronic bronchitis; PTB, pulmonary tuberculosis; COPD, chronic obstructive pulmonary disease; VSD, ventricular septal defect; SLE, systemic lupus erythematosus)

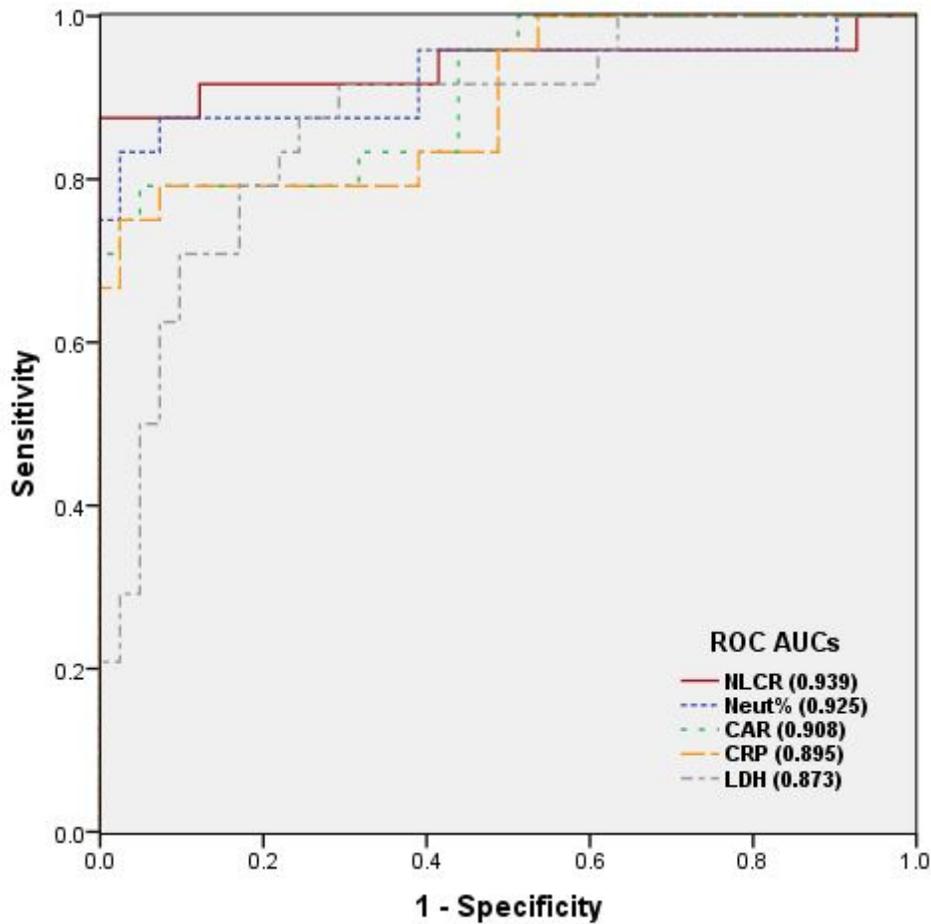


Figure 4

ROC curves of the markers to predict the exacerbation of COVID-19. ROC curves presents the AUCs of four indicators: NLCR > Neut% > CAR > CRP > LDH indicating that NLCR was the optimal marker and provided the greatest sensitivity and specificity to predict exacerbation in COVID-19 patients.