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C-reactive protein correlates with CT **findings and predicts severe COVID-19 early**

Running title: CRP predicts severe COVID-19 early

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ABSTRACT

Background: COVID-19 has developed into a worldwide pandemic; early identification of severe illness is

critical for controlling it and improving the prognosis of patients with limited medical resources. The present

study aimed to analyze the characteristics of severe COVID-19 and identify biomarkers for differential

diagnosis and prognosis prediction.

Methods: In total, 27 consecutive patients with COVID-19 and 75 patients with flu were retrospectively

enrolled. Clinical parameters were collected from electronic medical records. The disease course was divided

into four stages: initial, progression, peak, and recovery stages, according to computed tomography (CT)

progress.

Results: Compared to mild COVID-19, the lymphocytes in the severe COVID-19 progressively decreased at

the progression and the peak stages, but rebound in the recovery stage. The levels of C-reactive protein (CRP)

in the severe group at the initial and progression stages were higher than those in the mild group. Correlation

analysis showed that CRP (R=0.62, P<0.01), erythrocyte sedimentation rate (R=0.55, P<0.01) and

granulocyte/lymphocyte ratio (R=0.49, P<0.01) were positively associated with the CT severity scores. In

contrast, the number of lymphocytes (R=-0.37, P<0.01) was negatively correlated with the CT severity

scores. Receiver-operating characteristic analysis demonstrated that area under the curve of CRP on the first

visit for predicting severe COVID-19 was 0.87 (95% CI 0.10–1.00) at 20.42 mg/L cut-off, with sensitivity

and specificity 83% and 91%, respectively.

Conclusions: CRP in severe COVID-19 patients increased significantly at the initial stage, prior to CT

findings. Importantly, CRP, which was associated with disease development, predicted early severe

COVID-19.

Keywords: COVID-19；SRAS-COVID-2; C-reactive protein;

Abbreviations:

CRP, C-reactive protein; WBC: white blood cell **ICU, intensive c**are unit; NLR: Granulocyte/Lymphocyte

ratio; ESR: erythrocyte sedimentation rate; ROC:Receiver Operating Characteristic; AUC: area under the

receiver-operating curve.

INTRODUCTION

Since the SARS-COVID-2 outbreak in China, a large number of cases have also been reported

worldwide and the disease has become a global epidemic. Although the majority of patients show mild

symptoms, COVID-19 causes mass casualty and poses great challenges to the global healthcare system [1,2].

Early diagnosis of serious illness is critical to early classification and improvement of patients' prognosis.

Additionally, the early identification of patients who will become severely ill could facilitate allocation of the

limited medical resources to patients in need of aggressive treatment. Therefore, further research is urgently

needed on early diagnosis and prognosis. In addition, due to the similarity in the early stages of COVID-19

with the flu, it is not easy to distinguish between them, especially in the winter and spring. Any misdiagnosis

will have disastrous consequences in controlling the epidemic. Changes in the number of lymphocytes,

C-reactive protein (CRP), and erythrocyte sedimentation rates (ESR) have been previously reported in

COVID-19 patients, but little is known about their correlation with disease severity[3,4].

Therefore, it is necessary to determine the clinical laboratory biomarkers that will allow early and

differential diagnosis of SARS-COV-2 infection and predict the severity of the disease. In this study, we

aimed to compare clinical laboratory biomarkers between SARS-COV-2 and influenza infection, and also

between mild and severe COVID-19 patients, to explore the most useful prognostic factors for early, accurate,

and individualized assessment of COVID-19 patients.

MATERIALS AND METHODS

Study Design and Participants

The ethics board of Hunan Provincial People's Hospital approved the present retrospective study

(NO.202005, Changsha, China) and informed consent was waived. In the Hunan Provincial People's

Hospital, a total of 27 consecutive patients were retrospectively enrolled between January 18 and February 10,

2020, who were identified as patients infected with SARS-COV-2. The diagnosis and treatment of

SARS-COV-2 infection followed the Guidelines and Management of COVID-19 of the National Health

Commission of China[5]. According to clinical records, 6 patients (22%) who had progressed into severe illness

were classified as the severity group and the other 21 patients as the mild group. Meanwhile, 75 patients with Influenza A or influenza B infections were enrolled from December 18, 2019 to February 10, 2020. To match the characteristics of COVID-19 infected patients, the following exclusion criteria were applied: (1) <12 years; (2) no history of tumor, severe kidney and liver diseases; (3) no presence of other known infection; (4) no leukemia or other serious blood disease; (5) no pregnancy; (6) no medication before the initial visit.

Clinical Parameters and Data Collection

The exposure history, demographic data, clinical symptoms, comorbidities, laboratory findings, chest computed tomographic (CT) scans and clinical outcomes were collected. The radiographic scores for COVID-19 patients were blindly evaluated by two radiologist doctors. CT scores were recorded following previous research with some modifications[6]. In particular, 5 scores were assigned according to visual assessment of the involvement of each of the 5 lung lobes independently: 0 point, no involvement; 1 point, <5% involvement; 2 points, 25% involvement; 3 points, 26%-49% involvement; 4 points, 50%-75% involvement; 5 points, >75% involvement (Figure 1). The total CT score was the sum of the scores of the individual lobes ranging from 0 to 25. At the same time, according to the CT progress and score of each single patient, the course of the disease was divided into four stages: stage 1 (initial stage), stage 2 (progression stage), stage 3 (peak stage) and stage 4 (absorption stage). Furthermore, the corresponding laboratory parameters per stage including blood count, C-reactive protein, ESR, if any, were retrieved from the clinical database. The last date of follow-up of clinical outcomes including severity and discharges was February 20, 2020.

Statistical Analysis

Categorical variables were analyzed by Pearson's chi-square test or Fisher's exact test as appropriate and are presented by frequencies and percentages. Continuous variables were described as means and standard deviations or medians and interquartile ranges, depending on variable distributions. Continuous variables were analyzed by student's t test or Mann-Whitney U test as appropriate. The receiver-operating characteristic (ROC) curves were generated to evaluate the sensitivity and specificity for prediction of COVID-19 severity. A two-sided P value of < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 19.0 software package (Chicago, Illinois, USA).

RESULTS

Demographics and laboratory characteristics of SARS-COV-2 and influenza

Twenty-seven patients infected by SARS-COV-2 were retrospectively enrolled in our study. As shown in Table 1, 11 (41%) patients had visited Wuhan, 12 (44%) patients had close contact with COVID-19 patients, and 4 patients (15%) denied any obvious history of exposure. There were two patients (7.4%) with no symptoms of pneumonia and six patients (22%) with severe symptoms. The average age of patients was 48.89±18.47. The most common symptoms were fever (88%), cough (44%), and fatigue (33%). Twenty-six patients infected by SARS-COV-2 have been discharged from the hospital, and the remaining ones were still in the hospital. Meanwhile, 75 patients infected by influenza were retrospectively enrolled in our study. Compared to the SARS-COV-2 group, there were higher levels of WBC and granulocytes in influenza

patients. There was no difference in the levels of lymphocytes and other biomarkers between two groups

(Table 1).

Changes of laboratory biomarkers in the course of mild and severe COVID-19

According to CT findings of previous research, COVID-19 patients can be categorized into four stages: initial

stage, progression stage, peak stage and recovery stage. In general, patients had a CT scan at an interval of 3

to 5 days. The median time was 3 days (range 0-8 day) in the initial stage, 7 days (range 4-12 day) in the

progression stage, 12 days (range 8-16 day) in the peak stage, and 16 days (range 11-25 days) in the recovery

stage after the onset of initial symptoms. Because of the non-normal distribution of data, continuous variables

such as lymphocyte number, granulocyte/lymphocyte ratio (NLR), CRP, CT scores, and ESR were

summarized as medians and interquartile ranges. Compared to the mild group, there were no differences in

the levels of WBC and granulocyte numbers among these four stages (Figure 2 A, B). Interestingly, these

results also showed that compared with the mild group, the lymphocyte number progressively decreased at

the progression stage and the peak stage, but increased in the recovery stage (Figure 2 C). Moreover, the NLR

showed changing trends similar to lymphocyte number, which became significantly higher in the severe

group at the recovery stage (Figure 2 D). The CRP in the severe group at the progression stage was higher

than that in the mild group, but was decreased although with no statistically significant differences at the peak

and recovery stages (Figure 2 E). There were significant increases in the CT scores of the severe group at all

four stages (Figure 2 F). Also, there were significant differences in the ESR between the two groups at the

early stage; however, no significant differences were observed at the three later stages (Figure 2 G).

Correlation analysis between clinical laboratory biomarkers and CT scores

At present, CT is the main method to evaluate the disease severity. Correlation analysis showed that CRP (R=0.62, P<0.01), ESR (R=0.55, P<0.01), and NLR (R=0.49, P<0.01) were all positively correlated with the CT scores. Moreover, our results showed that the lymphocyte number (R=-0.37, P<0.01) was negatively associated with the CT scores, and not significantly correlated with CT scores (R=0.14, P=0.15) (Figure 3). Our results suggest that a significant increase in C-reactive protein is a signal of lung deterioration and progression. Three patients in our study confirmed this point.

Receiver Operating Characteristic (ROC) analysis of clinical laboratory biomarkers on admission

ROC analysis was performed to evaluate the clinical laboratory biomarkers on first visit for prediction of COVID-19 severity, which demonstrated that areas under the curve (AUC) of CRP and ESR for predicting severe COVID-19 were 0.87 (95% CI 0.10–1.00) and 0.78 (95% CI 0.39–1.00), respectively (Table 2, Figure 4). ROC analysis also demonstrated at a cut-off value of 20.42 mg/L, the sensitivity, specificity, positive prediction value (PPV) and negative prediction value (NPV) of CRP were 83%, 91%, 71% and 95%, respectively. At a cut-off value of 19.50 mm/h, the sensitivity, specificity, PPV and NPV of ESR were 83%, 81%, 56% and 94%, respectively. Furthermore, ROC analysis also showed that the AUC of CRP to predict disease severity was higher than CT scores, which demonstrated the excellent predictive power for the severity of COVID-19 patients (Table 2).

DISCUSSION

At present, the global outbreak of the SARS-COV-2 epidemic has brought serious burdens to the medical system. It is critical to identify COVID-19 patients who might become severely ill at the early stage, which would greatly facilitate the control of the epidemic and the improvement of the prognosis of patients in the light of short and limited medical resources. Reviewing the whole course of COVID-19, we found that CRP increased significantly at the initial stage, further increased at the progression and peak stage l, but recovered dramatically at the recovery stage. Interestingly, we found that CRP increased significantly at the initial stage in severe COVID-19 patients; while still no significant difference in the CT scores were found between the severe and mild groups. Furthermore, ROC further confirmed that CRP was an early biomarker for predicting the severity COVID-19 with good performance.

In the present cohort of COVID-19 pneumonia, we followed up the patients from the first visit throughout the hospitalization. Their ages ranged from 12 to 84 years. The patients had no history of tumor, severe kidney and liver disease and other serious blood diseases. To match the COVID-19 characteristics, patients with flu were screened under the same conditions and enrolled. Although flu is more common in young people compared with COVID-19 patients, previous research has suggested that both the WBC and granulocyte numbers did not significantly differ with age[7]. The present study showed that patients with flu had higher WBC, granulocyte, and NLR, which were consistent with previous studies showing that few COVID-19 patients had elevated WBC[1,8].

During hospitalization, no significant differences in WBC and granulocyte numbers were observed between the mild and the severe COVID-19 groups. Interestingly, we found that lymphocytes progressively decreased and rebound in the recovery stage. Thus, NLR showed similar trends; they progressively increased and decreased at the recovery stage. In the latest diagnosis and treatment guidelines, progressive decrease of lymphocytes can be regarded as an early warning of critical illness. The pathological results of the latest death cases are consistent with our study. They suggested that a high number of lymphocytes and monocytes infiltrate in the lung, and CD4$^+$T and CD8$^+$ T cells decrease in the spleen and lymph nodes[9]. Moreover, both the CRP and ESR were significantly elevated in the severe COVID-19 group at the early stage; however, CRP changes were more sensitive to the condition of the disease. CT is the main method to judge the severity of the disease. In the present study, another interesting observation was that CRP and ESR levels were significantly elevated at the early stage in severe COVID-19 patients, while still no significant difference of CT scores could be identified between severe and mild patients. These results suggested that CRP and ESR could be used to early identify patients who might become severely ill and prior to the CT finding. The latest research about COVID-19 has also suggested that CRP levels differed significantly between the deceased group and the surviving group and may serve as a potential marker for prognosis[10,11]. Furthermore, compared with CT, another important advantage of CRP and ESR as biomarkers for assessing the severity of COVID-19 is the ease with which they can be measured, especially in developing countries.

Most of the previous studies identified and analyzed the data of patients after hospitalization[3,12,13]; however, this usually takes one or two days from the first visit to admission because of nucleic acid diagnosis. Therefore, in order to predict the severity of COVID-19 as early as possible, we analyzed the laboratory data

of patients' first visit. ROC analysis demonstrated that AUC of CRP for predicting severe COVID-19 were

0.87 (95% CI 0.10–1.00), with sensitivity and specificity 83% and 91% at a cut-off value of 20.42 mg/L.

The present study is not devoid of limitations. First, only 27 COVID-19 patients were included. Although the

small sample size may have resulted in biases, we collected data from multiple stages in the process of

disease, which may help to increase the reliability of conclusions. Second, some biomarkers such as renal

function and myocardial enzymes were not available in every stage during hospitalization.

In conclusion, we found that patients with flu had higher WBC, granulocyte and granulocyte/lymphocyte

ratio, compared with COVID-19 patients. Moreover, CRP and ESR increased significantly at the early stage

in severe COVID-19 patients, prior to identification of any change by the CT scores. Importantly, C-reactive

protein was associated with disease development and showed good performance in predicting severity in

early stage of COVID-19.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval

The ethics board of Hunan Provincial People's Hospital approved the present study (NO.202005, Changsha,

China) and informed consent was waived.

Contributions

Chaochao Tan, Ying Wang,Fengxia Shi and Qionghui Ma were involved in the paper drafting, data acquisition, and analysis; Kui Tan,Xixin Jiang and Yong Chen were responsible for data collection and data analysis; Xiaosong Li involved in the research design and revision of the manuscript.

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Variables	SARS-COV-2	Influenza A or B	P
N	N = 27	N = 75	
Median age, years	48.89±18.47	29.40±13.70	<0.01
Male sex, N (%)	11(41%)	27 (36%)	0.631
Exposure			
Close contact with infected patients, N (%)	12(44%)	NA	
Visit to Wuhan, N (%)	11(41%)	NA	
None, N (%)	4(15%)	NA	
Comorbidities			
Hypertension, N (%)	6(22%)	5(7%)	<0.01
Cardiovascular disease, N (%)	3(11%)	2(2.6%)	0.034
Diabetes, N (%)	2(7%)	4(5.2%)	0.653
Symptoms			
Fever, N (%)	24(88%)	NA	
Fatigue, N (%)	9 (33%)	NA	

Cough, N (%)	12 (44%)	NA	
Headache, N (%)	3(11%)	NA	
Expectoration, N (%)	2(7%)	NA	
muscle soreness, N (%)	4(15%)	NA	
hemoptysis, N (%)	1(4%)	NA	
Diarrhea, N (%)	1(4%)	NA	
Nausea, N (%)	1(4%)	NA	
Disease severity			
Hospitalization, N (%)	27(100%)	15(20%)	<0.01
Clinical outcomes			<0.01
Discharge from hospital, N (%)	26(96%)	14(100%)	
Death, N (%)	0	0	
Staying in hospital, N (%)	1(4%)	0	
Clinical laboratory			
WBC($x10^9$/L)	5.60±1.81	6.18±2.29	0.114
Granulocyte($x10^9$/L)	3.75±1.47	4.26±2.14	0.024

Lymphocyte(x10^9/L)	1.38±0.56	1.17±0.47	0.308
NLR(IQR)	2.58(1.91-3.82)	3.70(2.10-6.04)	0.034
Monocytes	0.44±0.18	0.69±0.33	0.007
RBC(x10^{12}/L)	4.79±0.45	4.58±0.48	0.980
Hemoglobin(g/L)	146.41±13.07	138.52±15.78	0.566
Hematocrit(%)	42.86±3.94	41.22±5.68	0.433
Platelet (x10^9/L)	206.00±68.77	195.68±59.01	0.241

Table 1 Demographics and laboratory characteristics of patients with SRAS-COV2 and with flu

WBC: white blood cell; RBC: red blood cell; IQR, interquartile range; NLR: Granulocyte/Lymphocyte ratio;

Table 2 the receiver operating characteristic curves for severity in COVID-19 patients

Variables	Cut-off	AUC(95% CI)	Sensitivity	Specificity	PPV	NPV	Youden index
WBC(x10^9/L)	4.61	0.51(0.24-0.79)	83%	38%	63%	89%	0.21
N(x10^9/L)	3.15	0.57(0.30-0.85)	83%	43%	29%	90%	0.26
L(x10^9/L)	1.49	0.40(0.13-0.67)	33%	67%	22%	78%	0.10
NLR	2.41	0.61(0.33-0.90)	83%	43%	29%	90%	0.26

CRP(mg/L)	20.42	0.87(0.18-1.00)	83%	91%	71%	95%	0.74
ESR(mm/L)	19.50	0.78(0.39-1.00)	83%	81%	56%	94%	0.64
CT scores	7.00	0.71(0.44-0.98)	50%	91%	60%	86%	0.41

WBC: white blood cell; N: Neutrophils, L: lymphocyte; NLR: Granulocyte/Lymphocyte ratio; CRP:

C-reactive protein; ESR: erythrocyte sedimentation rate; PPV: positive predictive value; NPV: negative

predictive value. The 95% confidence interval is shown in parentheses.

Figures

Figure 1 CT Score example: **0 point**: Five lobes of lungs are not involved, 0 point for every lobe; **1 point**:

Minimal involvement ($< 25\%$) in right middle lobe, right lower lobe, left upper lobe and left lower lobe, 1

point for each lung lobe, 4 points in total; **2 points:** Mild involvement (26-50%) in the left lower lobe, 2

points in the left lower lobe; **3 points**: Moderate involvement (51-75%) in the right upper lobe, and 3 points

in the right upper lobe; **4 points**: Severe involvement ($> 75\%$) in right lower lobe, left upper lobe and left

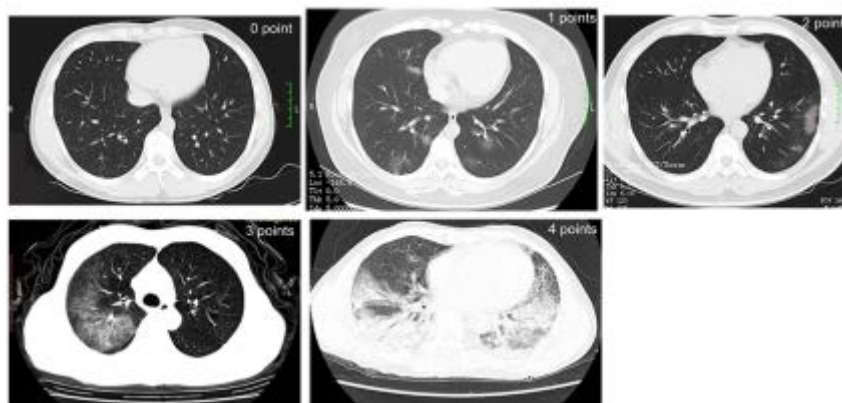lower lobe, 4 points for each lung lobe, 12 points in total.

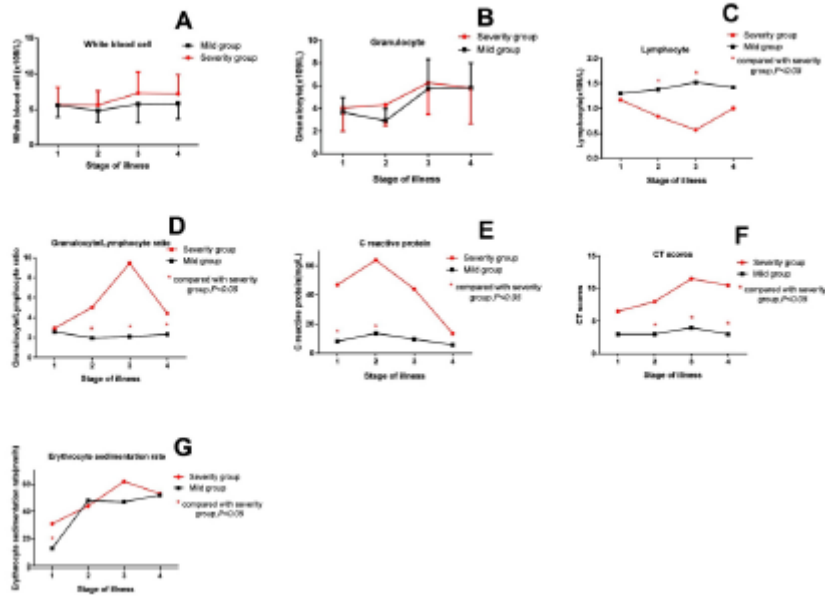Figure 2 the alteration of biomarkers in the disease course

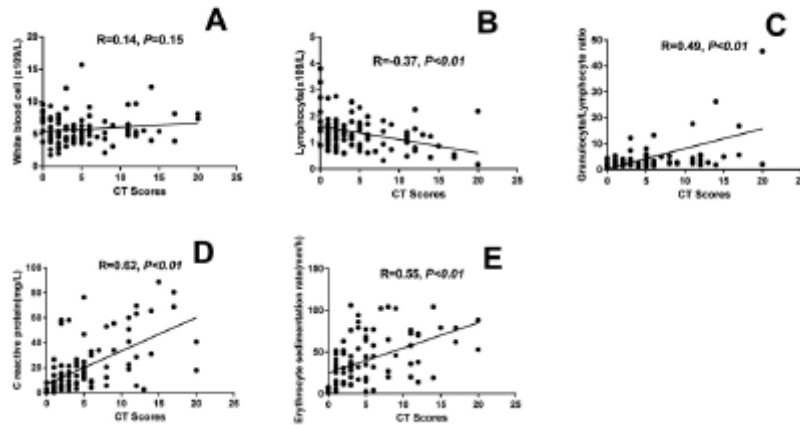Figure 3 Correlation analysis between CT scores and biomarkers

Figure 4 the Receiver Operating Characteristic analysis of biomarkers on first visit