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Correlation analysis of the severity and clinical prognosis of 32 cases of patients with COVID-19

Ming Ding [a], Qiang Zhang [a], Qing Li [b], Ting Wu [a], Ying-zi Huang [b,*]

[a] Department of Respiratory Medicine, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, Jiangsu, China
[b] Department of Critical Care Medicine, Zhongda Hospital, School of Medicine, Southeast University, 87 Dingjia Bridge, Nanjing, 210029, Jiangsu, China

1. Background

In December 2019, the pandemic caused by a novel kind of enveloped RNA betacoronavirus took place in Wuhan, Hubei Province, China. This virus was named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. Also, it was declared as coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO) [1]. Because of its extremely strong transmission, this virus has spread to the world in less than 3 months and infected more than 1 million patients [2]. COVID-19 has a higher mortality rate, ranging from 3% to 12% [2]. Also, it is characterized by the high incidence of critical patients, which was reported to about 20% of the total number of patients. Although the clinical characteristics of critical patients defined have been reported in many literatures [3,4], which paid much attention on general characteristics of patients at early-stage, rather than the observation of the whole course of the development of the disease. Judging from both the general law of disease occurrence and development and clinical and laboratory indicators, it is urgent to identify patients with poor prognosis in the early stage and take timely intervention measures so as to prevent patients from turning into further deterioration of the disease. This can help reduce the mortality at the same time.

2. Subjects and methods

This study retrospectively analyzed 32 patients admitted to Huangshi Traditional Chinese Medicine Hospital (Huangshi Infectious Disease Hospital) from January 1st to March 20th, 2020. All cases were positive for nucleic acid detection when admitted to hospital, and characteristic COVID-19 features were shown by chest CT. All of them were in line with the World Health Organization diagnostic criteria and the inclusion criteria for confirmed cases. According to the 7th Trial Version of the ***Guidelines for the Diagnosis and Treatment of COVID-19 by the National Health Commission*** by National Health Commission, COVID-19 patients with pneumonia were categorized as mild, severe and critical patients.

This study excluded those patients whose chest CT showed no characteristic pneumonia features. And this study was approved by the Ethics Committee of Huangshi Traditional Chinese Hospital (HSZYPJ-2020-018-01).

Clinical data of patients, including age, previous medical history and laboratory indexes, were collected. And laboratory indexes include blood routine, liver and kidney function, electrolytes, coagulation function, interleukin-6 (IL-6), C-reactive protein (CRP), blood gas analysis and fibrosis indicators, including hyaluronic acid, type III procollagen, type IV procollagen, and laminin. Fibrosis markers were determined by chemiluminescence method(Maglumi 8000,Shenzhen new industry Co., Ltd). Blood count was examined by flow-cytometry analysis (XN-2000, Meisen, Japan). CRP and IL-6 concentrations were determined by Electrical Chemiluminescence to determine (CobasE602, Roche, Switzerland). All laboratory indexes of patients were collected during the hospitalization. The CT examination was carried out by using Siemens emotion 16 spiral CT. All patients received end-inspiratory scans with the reconstruction layer of 0.7–1.5 mm. All patients' data from admission to discharge were collected and observed. During the hospitalization, necessary laboratory examinations and imaging examinations were performed at any time according to the condition till discharge or death.

3. Statistical methods

The statistical software was SPSS 20.0 (SPSS Inc., Chicago, IL, USA), and the mapping software was Graphpad Prism 5.0 (Graphpad Inc., San Diego, USA). The population data and the percentages obtained from the measurements were expressed by using the form of Mean ± SD. One-way ANOVA or Kruskal-Wallis test was used for the comparison among data groups measured once. One-way repeated measures analysis of variance was used for the comparison among data groups measured multiple times. The correlation test was performed by using Pearson regression or

* Corresponding author.
E-mail address: yz_huang@126.com (Y.-z. Huang).

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Spearman model. Categorical data were expressed in the form of numbers, and Chi-square test was performed. $P < 0.05$ was considered statistically significant.

4. Results

4.1. General information of patients

Patients included in this study can be divided into three groups: the critical group (11 cases), the severe group (10 cases), and the mild group (11 cases). Three patients of the critical group died, and the remaining patients were transferred to the isolation point for medical observation after discharge. Patients in the critical group were older and had more coexistent disorders (Table 1).

A total of 11 patients were admitted to the ICU. And 3 cases received endotracheal intubation and invasive ventilation, including 1 patient who discharged after 11 days of invasive mechanical ventilation and then sequentially noninvasive Bilateral positive airway pressure(BiPAP) ventilation, and 2 patients who died after respectively 13 and 20 days' invasive ventilation. Another dead patient admitted to the ICU was able to maintain oxygen balance by inhaling oxygen through the mask, and did not undergo invasive ventilation because of sudden death. Relevant data were listed in **Attachment 1**.

4.2. Comparisons of laboratory indicators

From the laboratory indicators of the three groups of patients at admission, we discovered that hyaluronic acid and type III procollagen showed the most significant differences among the groups (Table 2, Fig. 1).

Indicators obtained from repeated examinations during the course of the disease were included in the analysis. The indicators that can be analyzed include the lymphocyte count from the 1st to the 7th examination, the interleukin-6 (IL-6) of the 1st and the 2nd examination, and the C-reactive protein (CRP) from the 1st to the 5th examination. The overall comparison of lymphocyte count showed significant difference ($p = 0.018$), which was related to the course of disease ($p = 0.049$). The results of each examination showed significant differences ($p < 0.05$) (Fig. 2); there was no significant difference in the overall CRP comparison ($p = 0.251$). However, significant differences were shown in the comparisons among first five examinations ($p < 0.05$), which were related to the course of disease ($p = 0.02$) (Fig. 2). The above differences were not shown in IL-6 comparisons($p > 0.05$) (Table 3).

5. Discussion

In this study, we analyzed 32 patients diagnosed with COVID-19. All patients had varying degrees of pathological changes in the lung, and the entire course from admission to discharge or death was observed. Although there were many reports on the clinical characteristics of new coronavirus pneumonia (COVID-19), most of them focused on early clinical manifestations rather than the long-term observation of the course of disease, let alone the observation of prognosis.

In this study, we confirmed some characteristics reported previously. For example, most of the critical and severe patients were the elderly, most of whom also suffered from various chronic diseases, especially the cardiovascular and cerebrovascular diseases and malignant tumors (**Attachment 1**). There were 3 cases of death in the critical group, and all of them were the elderly, combined with many coexisting disorders, and their condition changed rapidly, which were consistent with previous reports[3~5]. In terms of symptoms, most patients had a cough, fever or difficulty breathing. Few patients had other symptoms, such as diarrhea or hemoptysis. Although these characteristics were not specific, they were consistent with previous reports (**Attachment 1**) [5–7].

Lung was the main target organ attacked by COVID-19 virus. Bronchus at all levels and pulmonary alveoli were also damaged by this virus. Interstitial alveolar edema and congestion occurred. And a large number of inflammatory cells, such as monocytes and lymphocytes, infiltrated. One of the most important pathological changes was the mechanization of alveolar exudate and the fibrosis of the interstitial lung. Pulmonary fibrosis was closely related to tissue inflammation and damage repair [8]. In this study, we discovered that fibrosis indicators had obvious advantages in predicting the severity and prognosis of patients, which was rarely seen in previous reports. Hyaluronic acid, laminin and collagen were the main components of the extracellular matrix in the lung, which were also the indicators reported a lot in fibrosis studies [8, 9]. These matrix components made the lung tissue a whole, and then performed its physiological function. During the inflammation and repair process, extracellular matrix components were excessively deposited, resulting in pulmonary fibrosis and consolidation in the end. Previous studies reported that the concentration differences of hyaluronic acid and laminin in both patients with idiopathic pulmonary fibrosis(IPF) and rats injected with bleomycin in pulmonary fibrosis model could be observed, suggesting that these indicators can effectively reflect the degree of pulmonary fibrosis [9]. In this study, we discovered that the levels of hyaluronic acid and type III procollagen detected at admission, could clearly distinguish critical patients. The level of laminin differed only between the critical group and the mild group, and

Table 1
Basic Clinical Data of the 32 patients with COVID-19.

	Gender (male/female)	Age (years old)	Complications	Duration of disease (days)
The critical group (n = 11)	7/4	73.5 ± 12.3	4/11	34.2 ± 12.7
The severe group (n = 10)	5/5	61.3 ± 17.9	3/10	36.5 ± 5.6
The mild group (n = 11)	1/10	54.9 ± 11.3	3/11	30.7 ± 7.2

Table 2
Comparisons of fibrosis indicators among the three groups.

	The critical group (n = 11)	The severe group (n = 10)	The mild group (n = 11)
Hyaluronic acid (ng/ml)	667.42 ± 783.78*	86.67 ± 62.71	40.33 ± 30.53
type III procollagen (ng/ml)	23.51 ± 19.26*	10.45 ± 4.77	8.53 ± 2.14
type Ⅳ collagen (ng/ml)	108.56 ± 128.72	73.45 ± 29.52	59.52 ± 19.65
Laminin (ng/ml)	609.04 ± 1501.14#	10.44 ± 8.57	2.73 ± 7.73

"*" indicates there were significant differences among the three groups.
"#" indicates there was significant difference between the critical group and the mild group.

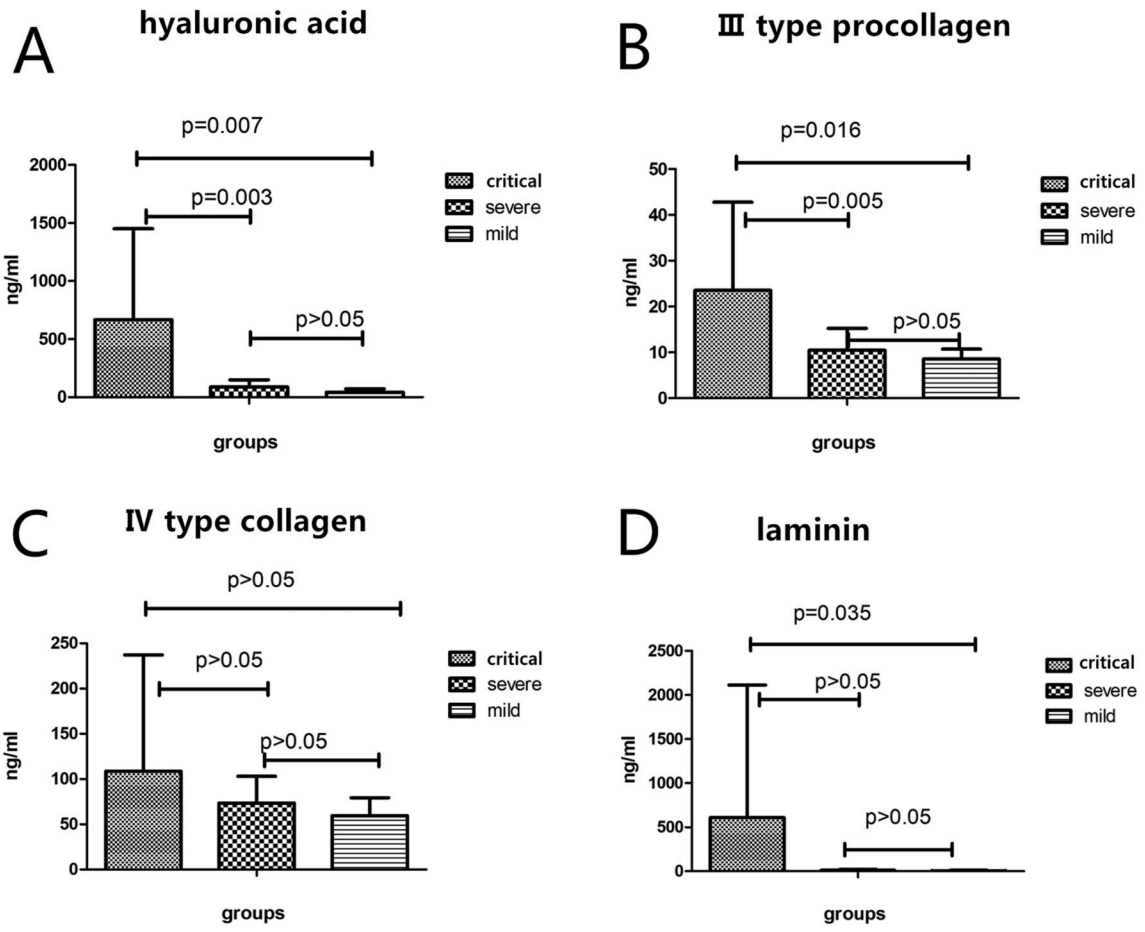

Fig. 1. Both hyaluronic acid and type III procollagen were higher in the critical group than in other two groups (A, B). Laminin was significantly different only between the critical group and the mild group(D). No difference of type Ⅳ procollagen level was shown among all groups(C).

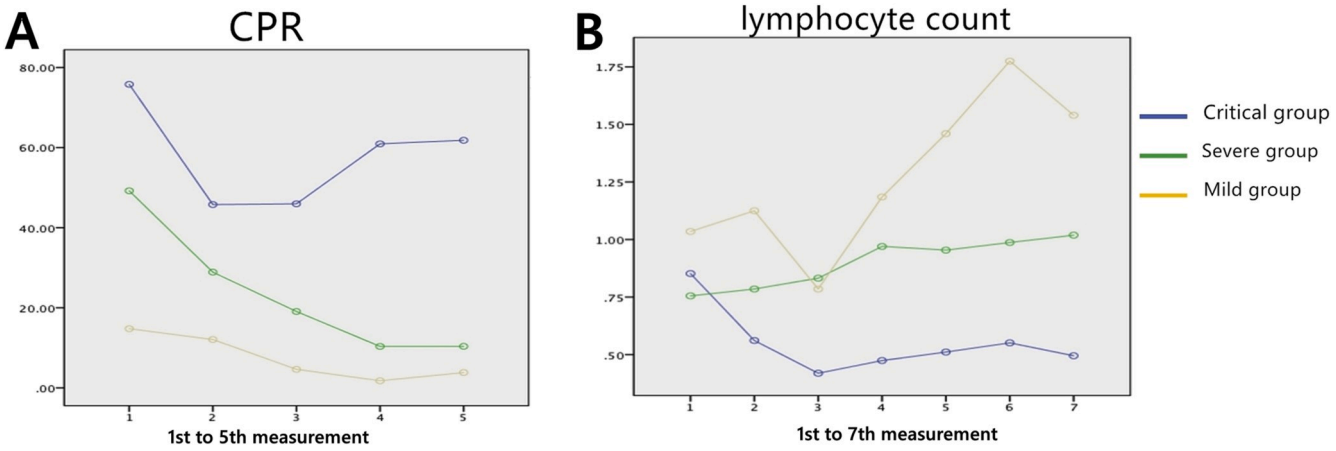

Fig. 2. A. Although no significant difference was observed in the total comparison of CRP (P = 0.251), there were significant differences among first five examinations (P < 0.05), which was correlated with the duration of disease (P = 0.02). **B** Comparisons of lymphocyte counts showed significant differences in each examination (P < 0.05), which was related to the duration of the disease (p = 0.049).

there was no statistical difference between the critical group and the severe group. However, no difference of type Ⅳ procollagen level was shown among all groups (Fig. 1). Fig. 3 shows the transbronchial biopsy pathology and imaging result of a critical patient. We discovered that four indicators, including hyaluronic acid, type III procollagen, type IV procollagen and laminin, were obviously abnormal. CT suggested rapid deterioration. Bronchoscopy biopsy was performed after lung consolidation. It was shown that the inflammation exudates obviously, with

Table 3
Comparisons of total levels of lymphocyte count, CRP and IL-6 in serum.

		F	P
lymphocyte count	**Severity**	2.163	0.018
	Duration	2.163	0.049
CRP	**Severity**	1.365	0.251
	Duration	3.782	0.02
IL-6	**Severity**	1.772	0.197
	Duration	0.837	0.372

CRP:C reactive protein; IL-6: Interleukin −6.

alveolar epithelial hyperplasia, interstitial thickening and local formation of granulomatous nodules. And within the nodules were hyperplastic fibroblasts and lymphocytes. This case visually showed the characteristics of imaging changes and pathological changes in critical patients. It might be due to the small sample size of this study, we did not obtain similar type IV procollagen results to that of previous reports [10, 11]. The results obtained in this study were similar to that of previous studies of both clinical observation of IPF and COPD patients and animal models in the laboratory [8].

Previous reports suggested that, the decline in peripheral blood lymphocytes, as well as the increase of both peripheral inflammatory factors, such as CRP and IL-6, and lactic acid, can be the early warning indicators of poor prognosis for patients [5–7]. The description in previous literature reports was also consistent with this program. However, there were few reports on the changes of these indicators during the whole process of the disease. Besides, few reports analyzed the relationship between dynamic changes of these indicators and the severity of the disease. In this study, we discovered that lymphocyte count at admission can be used as the indicator to distinguish the severity of the disease, also its change was closely related to the prognosis of the disease (Fig. 2, Table 3). CRP also owned a similar trend to lymphocyte count, suggesting the value of lymphocyte count and CRP in diagnosing patients with COVID-19 pneumonia (Fig. 2, Table 3). Fig. 4 shows the dynamic changes in chest CT, lymphocyte count and CRP of a critical patient from admission to discharge. And this provides us an intuitive realization of the relationship between these two indicators and the severity of the disease. It is simple and easy to check both lymphocyte count and CRP. In this way, both of them should be performed as the routine examination on patients with COVID-19. Unlike most previous reports, no statistical differences were shown after comparing all IL-6 levels of patients collected from admission. This might be due to the small sample size (Table 3). In addition, the patients' lactic acid levels were also included in the study. However, not every patient received the blood gas analysis test, and the amount of data was too small. Thus, it was not shown in the results.

The limitations of this study are obvious. First, the small sample size might inevitably lead to biased results, and also, some data cannot be effectively analyzed. Second, because it was a retrospective study, no perfect control group could be found, and we took the mild group as the control group, which might lead to incomplete match. Third, this study did not analyze the intervention effect of different treatment strategies on these indicators, which was more clinically valuable.

In conclusion, fibrosis indicators, especially hyaluronic acid and type III procollagen, can be used as early warning indicators of poor prognosis for critical patients with COVID-19. Dynamic observations of lymphocyte count and CRP levels can better reflect the changes in patients' condition, and were closely related to the prognosis. Given that it is simple and easy to check these two indicators, they should be used as routine tests for COVID-19 patients.

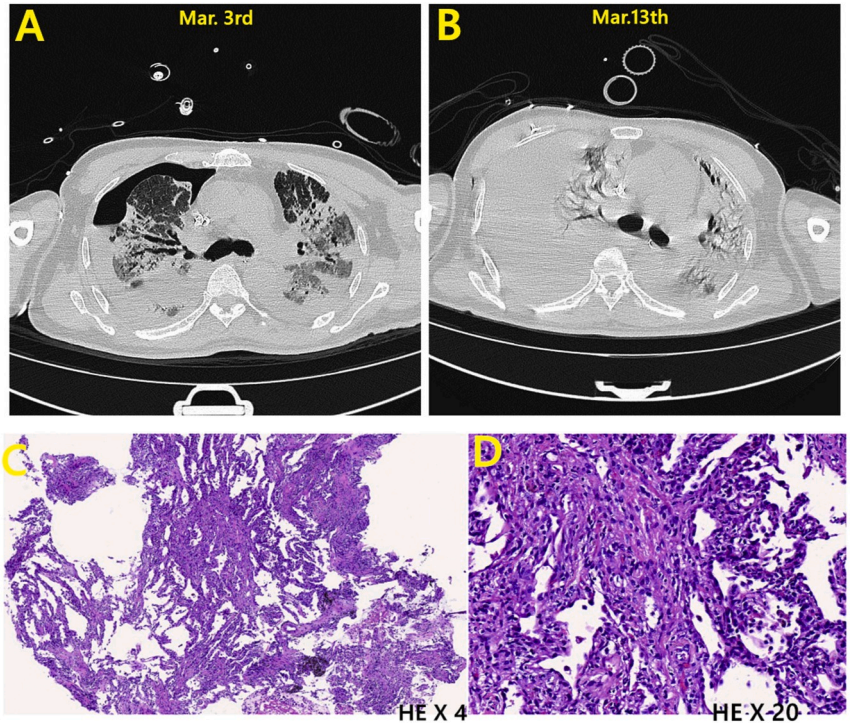

Fig. 3. One of the three deaths in the critically ill patients was a 71-year-old man, whose CT scan showed rapid progression in his lung(**A**, **B**). Two weeks after admission, bronchoscopy was performed when his CT scan showed almost complete pulmonary consolidation. Pathological manifestations included obvious inflammatory exudation, hyperplasia of alveolar epithelium, thickening of interstitial space and local formation of granulomatous nodule. And proliferation of fibroblasts and lymphocytes were found in the nodule(**C**, HEX 4 **& D,** HEX 20).

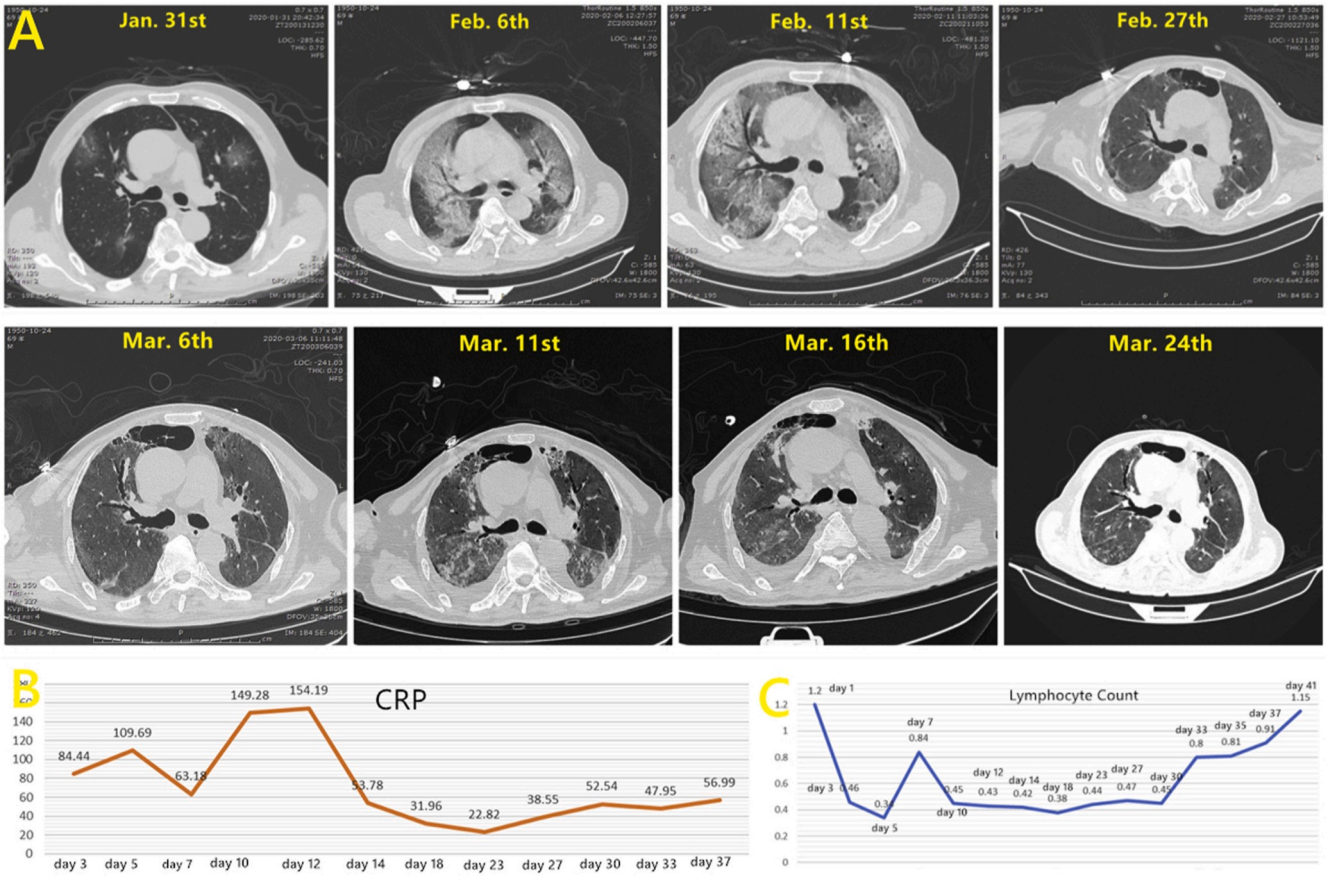

Fig. 4. Fig. 4shows the representative image of a 66-years-old man who had a fever, cough, dyspnea and rapid progressions of both pulmonary ground-glass opacities and consolidation. CT scans were performed once a week to two weeks from admission to discharge. (**A**). With the decline of CRP(**B**) and increase of lymphocyte count(**C**), his chest CT imaging improved. The peaks of both CRP and lymphocyte count emerged almost simultaneously, corresponding with the worst CT images, which occurred in the second scan (**A** Feb.6th) and the third CT scan (**A** Feb. 11th).

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Declaration of competing interest

All other authors declared no potential conflict of interest.

CRediT authorship contribution statement

Ming Ding: Writing - original draft, Writing - review & editing. **Qiang Zhang:** Writing - review & editing. **Qing Li:** Formal analysis. **Ting Wu:** Data curation, Formal analysis. **Ying-zi Huang:** Conceptualization.

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Ming Ding Ph.D. drafted the manuscript; Ting Wu Ph.D. collected and analyzed the data; Qing Li M.D. performed statistical analyses; Qiang Zhang M.D. carried out the following-up; Ying-zi Huang, Ph.D. contributed to studying conception, providing critical review of the manuscript and approving the final submission.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rmed.2020.105981.

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