Clin. Lab. 2021;67:XXX-XXX

ORIGINAL ARTICLE

Study on Laboratory Indicators Related to Lung Injury of Corona Virus Disease 2019

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SUMMARY

Background: **Since December 2019, a series of pneumonia cases caused by COVID-19 emerged in Wuhan, Hubei Province, China. People are generally susceptible to COVID-19 because people lack immunity to this new virus. With the spread of this epidemic disease from Wuhan, a national outbreak soon appeared, and now many countries have this disease. Unfortunately, no effective drug for COVID-19 treatment has been found so far.**
Methods: **We designed a retrospective study based on patients admitted to The Affiliated Infectious Hospital of Soochow University from January 22, 2020, to February 25, 2020, with diagnosed COVID-19. We analyzed correlations between RT-PCR negative time and laboratory indicators, then divided all cases into 2 groups according to oxygenation index, data of RT-PCR negative time and related laboratory indicators of the two groups were compared.**
Results: **We collected 84 confirmed patients whose RT-PCR had turned negative, including 23 patients with the lowest oxygenation index ≤ 300 mmHg and 61 patients had > 300 mmHg. There was a positive correlation between the RT-PCR negative time and age, WBC count, LDH, SCr. There were statistically significant differences in fever numbers, WBC count, lymphocyte count, CRP, ALT, AST, albumin, LDH, SCr, D-dimer, and fibrinogen between the two groups based on the oxygenation index.**
Conclusions: **Age, WBC count, LDH, and SCr may be related to the duration of COVID-19 disease. Fever, WBC count, lymphocyte count, CRP, ALT, AST, albumin, LDH, SCr, D-dimer, and fibrinogen are related to the severity of acute lung injury.**
(Clin. Lab. 2021;67:xx-xx. DOI: 10.7754/Clin.Lab.2020.200606)

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Manuscript accepted July 10, 2020

KEY WORDS

COVID-19, lung injury, laboratory indicators, retrospective analysis

INTRODUCTION

Since December 2019, a series of pneumonia cases caused by novel coronavirus infection emerged in Wuhan, China. The corona virus belongs to the genus β, which mainly causes human respiratory infections. World Health Organization (WHO) named the pneumonia caused by the virus as Corona Virus Disease 2019 (COVID-19) [1-5]. Most patients had mild symptoms and good prognosis. A few COVID-19 patients had severe pneumonia, acute respiratory distress syndrome (ARDS), multiple organ dysfunction (MODS), and even death. People are generally susceptible to COVID-19 because people lack immunity to this new virus. Subsequently, more and more COVID-19 patients who were found in other cities over China had sojourn records in Wuhan, suggesting the possibility of human-to-human transmission [6]. By 17:55 on February 25, 2020, the cumulative number of confirmed cases in China has reached 77,785, and the cumulative death toll was 2,666, and there were 9,126 severe cases. Additionally, COVID-19 patients had been confirmed in more than thirty countries including Japan, Thailand, South Korea, and the United States, some of which were imported cases [7-10]. The concern is that up to now there are still no specific treatments for COVID-19, and the effects of recommended drugs such as lopinavir/ritonavir and arbidol are still unclear [11]. In this study, we analyzed the differences between laboratory indictors in patients and RT-PCR turned negative time, compared the data of two groups according to oxygenation index, for the purpose of identifying the factors relating to the disease duration and the severity of lung injury.

MATERIALS AND METHODS

Study population

All the patients were from The Affiliated Infectious Hospital of Soochow University as appointed medical institutions for confirmed patients in Suzhou. We collected COVID-19 cases who were admitted to the hospital from January 22, 2020, to February 25, 2020, with throat swabs that had already been negative for two consecutive times after treatment. All selected patients met the diagnostic criteria set out in the Diagnosis and Treatment of Pneumonia Infected by Novel Coronavirus (5th trial edition) issued by the General Office of the National Health Commission and the Office of the State Administration of Chinese Tradition Medicine in 2020 [12]. Throat swabs of pathogenic examinations were confirmed by real-time fluorescent RT-PCR of coronavirus nucleic acid test by Suzhou CDC. The study has obtained the approval from the Ethics Committee of The Affiliated Infectious Hospital of Soochow University.

Methods

We reviewed the clinical electronic medical records and laboratory data of all patients with diagnosed COVID-19 whose throat swabs had been negative for two consecutive times, recorded days from admission to first RT-PCR negative as RT-PCR negative time (or throat swab negative time), and collected the related data on age, gender, history of underlying diseases, RT-PCR negative time, patient numbers with fever (armpit temperature $\geq$ 37°C), oxygenation index, WBC count, LYM count, CRP, bilirubin, ALT, AST, albumin, LDH, SCr, myoglobin, D-D, and fibrinogen. All cases were divided into 2 groups according to the oxygenation index, among which cases with oxygenation index values $\leq$ 300 mmHg were in Group I, while those values > 300 mmHg were in Group II. The data were followed up to 23:59 January 25, 2020.

Major equipment

The instrument for detecting blood gas index was a Roche Cobas b 123 automatic blood gas analyzer, and the reagents were the company's supporting products. The instrument for detecting blood routine was a Sysmex-XN3000 blood analyzer of Japan Sysmex Corporation, and the reagents were supporting products of the company. The instrument for detecting CRP was the Jet-iStar 3000 immunoassay analyzer of Zhonghan Shengtai Biotechnology Co., Ltd, and the reagents were supporting products of the company. The instrument for detecting blood biochemical indicators was a Johnson & Johnson VITROS350 automatic dry biochemical analyzer, and the reagents were the company's supporting products. The instrument for detecting the coagulation index is a CA1500 Hemagglutination Apparatus of Japan Sysmex Corporation, and the reagents were supporting products of the company. The instrument for detecting myoglobin was a Roche 411, and the reagents were supporting products of the company.

Data processing

SPSS 22.0 statistical software was used for data processing. All the quantitative variables were described as mean ± SD, and the correlation of variables was performed by Pearson's correlation analysis. Comparison between the two groups was performed by *t*-test. Categorical variables were tested by Fisher's exact test. The difference was statistically significant with $p < 0.05$.

RESULTS

According to our statistics, this study included a total of 84 diagnosed COVID-19 patients with throat swabs that had been negative for two consecutive times, including 45 (54%) males, 39 (46%) females, patient's ages

Table 1. Correlation between throat swab negative time and indicators.

	Pearson's correlation	p-value
Age	**0.324** **	**0.003**
WBC	**0.270** *	**0.013**
LYM	**-0.033**	**0.763**
CRP	**-0.006**	**0.96**
Oxygenation index	**-0.181**	**0.1**
Bilirubin	**0.124**	**0.259**
ALT	**0.044**	**0.688**
AST	**0.144**	**0.193**
Albumin	**-0.115**	**0.298**
LDH	**0.216** *	**0.048**
SCr	**0.277** *	**0.011**
Myoglobin	**0.023**	**0.835**
D-dimer	**-0.011**	**0.921**
Fibrinogen	**0.068**	**0.538**

*** - Significant correlation at p < 0.05 (both sides), ** - significant correlation at p < 0.01.**

Table 2. Basic information of two groups of patients.

		Group I	Group II
		23 (100%)	**61 (100%)**
Gender	**male**	**15 (65.22%)**	**30 (49.18%)**
	female	**8 (34.78%)**	**31 (50.82%)**
Age (years)	**0 - 18**	**0 (0.00%)**	**3 (4.92%)**
	19 - 35	**3 (13.04%)**	**16 (26.23%)**
	36 - 55	**11 (47.83%)**	**27 (44.26%)**
	56 - 80	**9 (39.13%)**	**15 (24.59%)**
	median	**49**	**41**
Underlying chronic diseases	**hypertension**	**1 (4.35%)**	**3 (4.92%)**
	diabetes	**1 (4.35%)**	**2 (3.28%)**
	others	**4 (17.39%)**	**5 (8.19%)**
	total	**6 (26.09%)**	**9 (14.75%)**
Sojourn records in Hubei		**8 (34.78%)**	**22 (36.07%)**
No sojourn records in Hubei		**15 (65.22%)**	**39 (63.93%)**

ranged from 6 to 77, with a median of 43.5. There were 57 cases discharged, and 0 deaths, also there were 15 cases with an underlying chronic disease history, and 1 pregnant woman. There was a positive correlation between the time when the throat swab turned negative and age, WBC count, LDH, and SCr in 84 patients, with respective correlation coefficients of 0.324, 0.270, 0.216, and 0.277 (Table 1).

As divided by the oxygenation index level during the disease course, there were 23 patients with the lowest oxygenation index ≤ 300 mmHg and 61 patients had > 300 mmHg. The median ages of Group I and Group II were 49 and 41, respectively, among which those aged 56 - 80 years accounted for 39.13% and 24.59% of their

Table 3. Comparison of clinical indicators between the two groups.

	Group I	Group II	p-value
n	23	61	-
Age (years)	49.304 ± 11.679	42.803 ± 16.071	0.081
Fever (%)	23 (100%)	43 (70.5%) **	0.002
Time of throat swabs negative (days)	13.696 ± 7.690	12.262 ± 5.531	0.346
Oxygenation index (mmHg)	212.982 ± 83.232	474.019 ± 135.402 **	< 0.001
WBC (x 10^9/L)	6.506 ± 3.530	5.142 ± 2.050 *	0.031
LYM (x 10^9/L)	0.882 ± 0.369	1.589 ± 0.999 **	0.001
CRP (mg/L)	31.448 ± 29.773	8.864 ± 10.970 **	< 0.001
Bilirubin (μmol/L)	15.570 ± 11.557	11.687 ± 14.829	0.261
ALT (U/L)	48.652 ± 36.354	35.033 ± 19.913 *	0.031
AST (U/L)	38.087 ± 28.615	27.836 ± 12.058 *	0.023
Albumin (g/L)	35.613 ± 5.095	38.738 ± 3.996 **	0.004
LDH (U/L)	587.609 ± 336.624	417.213 ± 143.285 **	0.002
SCr (μmol/L)	71.809 ± 27.035	59.356 ± 20.278 *	0.025
Myoglobin (ng/mL)	32.112 ± 24.254	52.750 ± 239.097	0.682
D-D (μg/L)	509.565 ± 566.589	277.213 ± 328.761 *	0.022
Fibrinogen (g/L)	4.520 ± 1.556	3.642 ± 0.917 **	0.002

* - Significant statistical difference at $p < 0.05$, ** - significant statistical difference at $p < 0.01$.

Table 4. Comparison of hemagglutination indexes between the two groups.

	Group I	Group II	p-value
n	23	61	-
D-D	6 (26.1%)	5 (8.2%)	0.063
Fibrinogen	13 (56.5%)	23 (37.7%)	0.143

* - Significant statistical difference at $p < 0.05$, ** - significant statistical difference at $p < 0.01$.

groups, and those with combined underlying diseases accounted for 26.09% and 14.75% of their groups, respectively. Detailed information was set out as below (Table 2).

There were significant statistical differences between the two groups in the percentage of patients with fever, WBC count, LYM count, CRP, oxygenation index, ALT, AST, albumin, LDH, SCr, d-dimer, and fibrinogen. The percentage of patients with fever, WBC count, CRP, ALT, AST, LDH, SCr, d-dimer, and fibrinogen in Group I were higher than those in Group II, while LYM count, oxygenation index, and albumin were lower than those in Group II. There was no statistical significance in age, time of throat swab turned negative, bilirubin, myoglobin (Table 3).

We analyzed the data of D-dimer and fibrinogen in two groups and found that a total of 11 patients (13.1%) had an increase in D-dimer and 36 patients (42.9%) had an increase in fibrinogen, there were 6 cases of D-dimer increase and 13 cases of fibrinogen increase in Group I, 13 cases of D-dimer increase and 23 cases of fibrinogen increase in Group II. But there was no statistical difference between the two groups (Table 4).

DISCUSSION

COVID-19 is a single-stranded, positive-strand RNA virus, which belongs to the ß genus Coronavirus. Its genetic characteristics are different from SARS-CoV and

MERS-CoV. The sources of COVID-19 infections so far are mainly from patients with novel coronavirus infection, and those asymptomatic infectors may also become the source of infection. The main transmission route is through respiratory droplets and close contact. Aerosol transmission is possible under the condition of prolonged exposure to high concentrations of aerosols in a relatively closed environment. The disease is an acute, self-limiting disease that mainly causes respiratory tract infections in humans. Most symptoms are mild but this disease can also be fatal, with a fatality rate of about 2% in China. The current findings reveal that severe patients may die mainly from diffuse alveolar damage and progressive respiratory failure [13].

Many studies are currently looking for factors related to disease severity, especially lung damage, with quite different results. Studies have shown that the severity of lymphocytopenia reflects the severity of novel coronavirus infection [5]. In the MuLBSTA viral pneumonia mortality risk assessment model, low lymphocyte count can be used as a simple and reliable predictor of viral pneumonia risk [14]. However, there are some studies suggesting that there was no significant correlation between lymphocyte count and disease severity [15]. Other studies have shown that reduced albumin levels and lymphocyte counts, as well as the increased levels in CRP and LDH, may be used to predict the severity of acute lung injury in pneumonia patients infected with novel coronavirus [16].

Our study showed that there was a positive correlation between throat swab negative time and age, WBC count, LDH, SCr in 84 patients, with respective correlation coefficients of 0.324, 0.270, 0.216, and 0.277, suggesting age, WBC count, LDH, and SCr may be related to the disease duration of COVID-19. The older the patient, the longer the time for the throat swab to turn negative, and kidney damage may also affect the negative time.

We used the minimum oxygenation index of 300 mmHg in the course of disease as the threshold for grouping patients, and found that the proportion of patients with age distribution of 56 - 80 years or with underlying diseases in Group I was higher than that in the Group II, indicating that elderly patients with underlying diseases are more likely to have sever lung injury, which is related to the decline of autoimmunity. There were no patients less than 18 years old in Group I, indicating that infants, young children, and adolescents were hardly observed with lung injury, which may be related to the gradual improvement of immune function during the growth period of the children. However, in this study, the sample number of children was relatively small. We still need more data to support our investigation.

We also found that the proportion of patients with fever, WBC count, CRP, ALT, AST, LDH, SCr, D-dimer, and fibrinogen in Group I were higher than those of Group II, while lymphocytes and albumin were significantly lower in Group I, all have statistically significant differences, while there was no statistical significance in time of throat swabs turned negative, bilirubin, and myoglobin between the two groups. Although there was no significant correlation between throat swabs negative time and D-dimer or fibrinogen, the relevant data in Group I were significantly higher than that of Group II, with statistical significance, indicating that D-dimer and fibrinogen level could not be used to predict the disease duration, but their elevation level could reflect patients' oxygenation status, that is, the degree of lung injury. It is our view that patients with COVID-19 often have accompanying changes in indicators such as fever, WBC count, LYM count, CRP, ALT, AST, albumin, LDH, SCr, D-dimer, and fibrinogen. Furthermore, the decrease level of lymphocytes and albumin as well as the increase level of CRP, LDH, and fibrinogen are more significantly correlated with the decrease in oxygenation, that is, the severity of lung injury, so they are more accurate indicators to predict the severity and prognosis of lung injury.

In studies of patients with COVID-19, it has been pointed out that abnormalities in coagulation function of these patients are often related to poor prognosis, especially fibrinogen degradation products and D-dimer [17]. In fact, fibrinogen deposition is a fairly typical feature of tissue damage caused by infection, and activation of the coagulation system and fibrinogen deposition have some commonalities in inflammatory diseases and tissue damage models. Current research has shown that fibrinogen can change the function of white blood cells and affect the local inflammatory response. In various disease models, fibrinogen deposition can aggravate inflammation and promote the disease, but the cause and mechanism of the effects of novel coronavirus on the coagulation system and fibrinogen are still unknown. Our study showed that 13.1% of patients had an increase in D-dimer, while 42.9% had an increase in fibrinogen. After grouping by oxygenation index, the percentage of patients with increased fibrinogen and D-dimer in Group I was higher than that in Group II, but the difference was not statistically significant. However, the increase of fibrinogen and D-dimer in Group I was more significant than that in Group II, and the difference was statistically significant. Based on data in Table 3 and Table 4, we consider that first, there was no significant correlation between the patient numbers of increased fibrinogen and of lung injury, but there was a significant correlation between the degree of increased fibrinogen and the severity of lung injury. The more severe the lung injury, the more obvious the increase of fibrinogen, suggesting that an obvious increase of fibrinogen may be an early indicator of the severity of lung injury; second, it may be related to the small sample size of Group I. This study indicates that high fibrinogen may be able to predict the degree of lung injury and determine disease condition at an early stage. Patients with obvious lung injury also usually have higher fibrinogen, leading to high risks for thrombosis. Therefore, we recommend that for patients with the oxygena-

tion index < 300 mmHg and with no obvious contraindications, treatment of preventive anticoagulation may be considered.

Our study has some limitations. First, we only included a single-center research in Suzhou, and did not include multi-center research in Wuhan. Second, the collected cases were generally relatively mild patients, and further studies are needed to determine whether the findings are applicable to all types of COVID-19 patients.

CONCLUSION

In summary, age, WBC count, LDH, and SCr are related to the duration of COVID-19. Fever, WBC count, LYM count, CRP, ALT, AST, albumin, LDH, SCr, D-dimer, and fibrinogen are related to the severity of the disease. In particular, the decline level of LYM count and albumin, CRP, LDH, and fibrinogen can be considered as factors predicting the degree of lung injury and prognosis. We also recommend that for patients with the oxygenation index < 300 mmHg and with no contraindications, early treatment of preventive anticoagulation may be considered.

Acknowledgment:
We thank Professor Yuchao Zhang from the Chinese Academy of Sciences for her help in the statistical analysis.

Authors' Contributions:
Yukun Zhang, Jun Chen, and Xin Yu designed the study and co-authored the manuscript. Yukun Zhang, Qing Chen and Lin Yao collected the data, Qing Chen and Lin Yao entered data into the database. Yukun Zhang and Qing Chen did the statistical analysis and wrote the original article. All authors reviewed and approved the final version.

Declaration of Interest:
The authors declare that they have no conflicts of interest.

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