Early View

Original article

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Diffusion Capacity Abnormalities for Carbon Monoxide in Patients with COVID-19 At Three-Month Follow-up

Running title: DLCO in COVID-19

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Abstract

Objective: To evaluate pulmonary function and clinical symptoms in coronavirus disease 2019 (COVID-19) survivors within three months after hospital discharge, and to identify risk factors associated with impaired lung function.

Methods and material: COVID-19 patients were prospectively followed up with pulmonary function tests and clinical characteristics for three months following discharge from a hospital in Wuhan, China between January and February 2020.

Results: 647 patients were included. 87 (13%) patients presented with weakness, 63 (10%) with palpitation and 56 (9%) with dyspnea. Prevalences of the three symptoms were markedly higher in severe patients than non-severe patients (19% vs. 10% for weakness, P=0.003; 14% vs. 7% for palpitation, P=0.007; 12% vs. 7% for dyspnea, P=0.014). Results of multivariable regression showed an increased odd in the ongoing symptoms among severe patients (OR: 1.7, 95%CI: 1.1-2.6, P=0.026) or patients with longer hospital stay (OR: 1.03, 95%CI: 1.00-1.05, P=0.041). Pulmonary function test results were available for 81 patients, including 41 non-severe and 40 severe patients. In this subgroup, 44 (54%) patients manifested abnormal diffusion capacity for carbon monoxide (DLCO) (68% severe vs 42% non-severe patients, P=0.019). Chest CT total severity score (TSS) > 10.5 (OR: 10.4; 95%CI: 2.5-44.1; P=0.001) on admission and ARDS (OR: 4.6; 95%CI: 1.4-15.5; P=0.014) were significantly associated with impaired DLCO. Pulmonary interstitial damage may be associated with abnormal DLCO.

Conclusion: Pulmonary function, particularly DLCO, declined in COVID-19 survivors. This decrease was associated with TSS of chest CT >10.5 and ARDS occurrence. Pulmonary interstitial damage might contribute to the imparied DLCO.

Key words: COVID-19, sequela, lung function, diffusion function

Take home message: COVID-19 patients present with impaired DLCO at 90 days after discharge, particularly severe patients. Chest CT TSS>10.5 and ARDS occurrence are associated with impaired DLCO. Pulmonary interstitial damage may contribute to the impaired DLCO.

Introduction

The epidemic of coronavirus disease 2019 (COVID-19) has had devastating effects. Patients surviving hospitalization are frequently reported to have pulmonary sequelae. It is challenging to evaluate lung function throughout COVID-19 progression because of the difficulty related to infection control risks in obtaining lung function testing during this contagious pandemic disease [1].

There are short reports of lung function of COVID-19 patients at discharge and at 30 days post-discharge [2, 3]. Reduced lung function was demonstrated in survivors of Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) up to 6 months following hospital discharge [4, 5]. SARS patients were reported to have a mild decrease in carbon monoxide diffusing capacity (DLCO) 6-8 weeks after discharge with improving lung function over time [6]. We performed a prospective cohort study to identify main sequelae and lung function changes in hospitalized SARS-CoV-2 patients during three-month follow-up.

Methods

Study design

This was a prospective cohort study performed at the Affiliated Hospital of Jianghan University, Wuhan, China. COVID-19 was confirmed and diagnosed according to Chinese management guideline for COVID-19 (Trial Version 5 Revised) [7]. Nucleic acid tests were provided for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by real-time reverse transcriptase-polymerase chain reaction (PCR)

assays. The severity was defined by World Health Organization (WHO) guideline for COVID-19 [8]. Severe pneumonia refers to fever or suspected respiratory infection, plus one of: respiratory rate > 30 breaths/min; severe respiratory distress; or SpO2 ≤ 93% on room air. A total of 749 COVID-19 patients with full data available were admitted from January to February, 2020. Of those, 81 patients died within three months and 21 patients were lost during follow-up. 647 patients were followed-up for three months after discharge. Patients' clinical baseline details, major clinical characteristics and lung function within three months' follow-up were recorded. The study was approved by the institutional ethical committee and patients gave standard written consent to the use of their data.

Pulmonary function test

81 (13%) patients underwent pulmonary function test (PFT) at three months after discharge. PFT was performed by a professional doctor with 20 years of experience using the MasterScreen PFT system (Jaeger, German) at the three-month follow-up visit. The recorded parameters are as follows: total lung volume (TLC), residual volume (RV), forced vital capacity (FVC), forced expiratory volume in the first second (FEV_1), FEV_1/FVC ratio, maximum mid-expiratory flow (MMEF), and diffusing capacity of the lung for carbon monoxide (DLCO).

Chest CT visual quantitative evaluation

Chest CT scan were performed on 16 or 64-multidector CT scanners (GE LightSpeed 16, GE Healthcare; Somatom Sensation 64, Siemens Healthcare). All patients

underwent chest CT scan at admission. In addition, 45 in 81 patients who had PFT had chest CT scan at three months after discharge. Two experienced radiologists (Wan and Li) reviewed CT images without knowledge of mild or severe, normal or reduced DLCO. Pulmonary interstitial changes on follow-up chest CT graph was defined as a combination of findings including fibrous stripe, ground-glass opacity, consolidation, subpleural curvilinear shadow, coarse reticular pattern, and traction bronchiectasis [9]. Meanwhile, main pulmonary artery (MPA), ascending aorta (AAo) diameters and the ratio MPA/AAo were measured to evaluate the relationship between pulmonary vascular disease and impaired DLCO[10]. To explore the relationship between impaired DLCO and radiographic changes, we conducted chest CT total severity score (TSS) which was evaluated by percentage of involvement in each lobe and overall lung. The percentage of the lobar involvement in each of the five lung lobes were classified in 5 levels' score, ie, 0 (0%), 1 (1–25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%). The TSS was obtained by adding the five lobar scores [11].

Statistical Analysis

Data were expressed as number (percentage) for categorical variables, mean (standard deviation, SD) when normally distributed, and median (interquartile range, IQR) when they had skewed distributions. T-test, Mann Whitney U test, Chi-square test or Fisher exact test were used to compare differences in characteristics and pulmonary function between groups of different characteristics. We used univariable and

multivariable logistic regression models to explore the risk factors associated with the occurrence of sequelae and impaired lung function. To avoid overfitting in the multivariable logistic regression model, we just chose four variables for analysis considering the total number of impaired DLCO (n=41) in our study. The receiver operating characteristic (ROC) curve and the area under the curve were utilized to assess the predicted value of TSS for impaired DLCO. All statistical analyses were performed using SPSS (version 24) and Prism (version 8.0.1) with two-tailed p<0.05.

Results

Patient characteristics

647 COVID-19 patients attended the three-month follow-up visit after hospital discharge, including 399 non-severe patients and 248 severe patients (Supplementary Figure 1). The mean age was 58 (SD, 15) years old, with 44% being male. The sequential organ failure assessment (SOFA) score on admission was 1 (inter quartile range, 0-2). Top three comorbidities were hypertension (30%), diabetes (11%), and chronic respiratory disease (CRD) (6%), respectively. Moreover, severe patients were significantly older and had higher SOFA score at admission (Supplementary Table 1).

The residual symptoms at three-month follow-up

Ongoing symptoms for COVID-19 at the three-month follow-up visit were weakness, palpitation, dyspnea, cough, lower limb edema, chest pain and hemoptysis, respectively. 87 (13%) patients presented with fatigue in their daily lives, 63 (10%) with palpitation and 56 (9%) with dyspnea. Prevalences of the three symptoms in

patients with severe COVID-19 were markedly higher than those in the non-severe

patients (19% vs 10% for weakness, P=0.003; 14% vs 7% for palpitation, P=0.007; 12%

vs 7% for dyspnea, P=0.014) (Supplementary Table 1). In supplementary table 2, we

compared the clinical characteristics between COVID-19 patients who had sequelae

or not. Main differences between these two groups were disease severity, inpatient

days, inflammation and coagulant disorder on admission. In univariable analysis, odds

of sequelae were significantly higher in severe patients with long hospital stay. Higher

white blood cell count, higher hypersensitive c-reactive protein and abnormal

D-dimer level were also associated with the occurrence of sequelae. In the

multivariable model, severity and inpatient days were significantly associated with the

occurrence of sequelae. (Supplementary Table 3)

Lung function at three-month follow-up

81 COVID-19 patients were assessed for pulmonary function test at three months

after discharge, including 41 non-severe and 40 severe patients. In this subgroup of

the cohort, the mean age was 59 (SD, 14) years old, 34 (42%) patients were male and

the mean body mass index (BMI) was 23.87 (3.18) kg/m^2. As shown in Table 1,

pulmonary function was impaired in 61 (75%) of 81 survivors. 8 (10%) patients had

reduced TLC, 17 (21%) patients had decreased FVC, 5 (6%) patients' FEV_1/FVC

were less than 70%, 44 (54%) patients' DLCO were less than 80%. To determine

whether abnormal lung function was associated with disease severity, we compared

characteristics and pulmonary function parameters between non-severe and severe

cases. There were no significant differences in TLC, FVC, FEV_1, FEV_1/FVC,

MMEF75/25, DLCO/VA according to the spirometry between non-severe and severe patients. However, significant difference was found for DLCO, which was less than 80% of predicted for 68% of severe patients compared to 42% of non-severe patients (P<0.05) (Figure 1).

44 in 81 COVID-19 patients had impaired DLCO. To figure out the differences between normal and impaired DLCO patients, we compared clinical characteristics between two groups in table 2. We found that parameters including severity, the TSS of chest CT, lymphocyte count, MPA diameter on admission and ARDS were higher in impaired DLCO, and the difference between two groups was statistically significant.

TSS of chest CT, pulmonary interstitial damage, vascular disease and impaired DLCO

All patients underwent chest CT scan at admission, 90% patients' lesions could be seen in bilateral lung on admission, there was no statistical difference between normal and impaired DLCO group (P=0.459). To evaluate the effect of CT assessment on DLCO decline in patients with COVID-19, we performed CT TSS for all patients. The median TSS was 9 (inter quartile range, 5-13) at admission. We conducted a ROC curve to explore cutoff of TSS to predict abnormal DLCO. We found that the cutoff of TSS was 10.5 on admission, the area under the curve was 0.765 (95% confidence interval, 0.663 to 0.867; p <0.001) with the sensitivity of 64% (95% CI 49-76%) and the specificity of 84% (95% CI 69-92%) (Figure 2).

Besides, 45 out of 81 patients with PFT performed had a chest CT scan at three-month follow-up after discharge. To determine whether pulmonary interstitial damage contributed to impaired DLCO or not, we analyzed pulmonary CT changes at three months after discharge. It was found that abnormal DLCO patients were more likely to have interstitial damage, especially manifesting the sign of traction bronchiectasis, subpleural curvilinear shadow and coarse reticular pattern, indicating pulmonary interstitial damage may contribute to impaired DLCO in COVID-19 patients (Table 3). When exploring the relationship between vascular disease and impaired DLCO, no significant differences were found at three months after discharge.

Predictors for lung function decline

Univariable logistic analysis showed that severity, TSS>10.5, MPA diameter at admission, and ARDS were significantly associated with impaired DLCO. Other variables were not associated with DLCO decline. Finally, we put age, MPA diameter, TSS over 10.5 based on the ROC curve and ARDS into the multivariable logistic regression model. Details of univariable and multivariable analyses were shown in Supplementary Table 4 and Figure 3. We found that TSS > 10.5 (odds ratio: 10.5; 95%CI: 2.5-44.1; P=0.001) and ARDS (odds ratio: 4.6; 95%CI: 1.4-15.5; P=0.014) were significantly associated with impaired DLCO.

Discussion

In the present study, we focused on investigating the residual symptoms and pulmonary functions in COVID-19 patients after hospital discharge. Our study revealed that the most common residual symptoms were weakness (13%), palpitation (10%), and dyspnea (9%). Prevalences of these three symptoms were significantly higher in severe COVID-19 patients than that in non-severe patients. In addition, COVID-19 patients presented with abnormal pulmonary function, especially impaired DLCO during recovery. Furthermore, severe COVID-19 patients were found to have a higher prevalence of impaired DLCO. Finally, multivariable analysis in our study demonstrated that TSS > 10.5 and meeting ARDS were significantly associated with impaired DLCO. Pulmonary interstitial damage may contribute to impaired DLCO at three months after discharge.

During the initial epidemic of COVID-19, the most common symptoms at the onset of illness were fever, cough, fatigue and shortness of breath [12, 13]. Some symptoms may continue since hospital discharge. It has been reported that weakness is common after acute lung injury and is associated with substantial impairments in physical function and quality of life [14]. The potential cause of these sequelae was multiple organ injury following infection of SARS-CoV-2. A previous study has shown that COVID-19 patients can have an impaired physical functioning when they were discharged home, even after early physiotherapy [15]. For patients with SARS and MERS, the six-minute walk distance is also reduced at 3 months after hospital discharge, but could be slowly improved by 12 months [16, 17]. Fatigue was reported

for at least one-third of the patients when followed-up for 18 months [18] and 40 months [19]. In this study, 10% patients felt palpitation. It was well reported of myocardial injury in COVID-19 patients. According to autopsy findings, the viremia in 6 of 10 and 5 of 12 patients demonstrated high viral RNA titers in the liver, kidney, or heart [20]. Myocardial injury was also highly associated with fatal outcomes in this infectious disease [21, 22]. This might be a reason for palpitation due to different degrees of myocardial injury.

Reports by Mo [3] and Huang [2] showed lung carbon monoxide diffusion dysfunction in COVID-19 patients at discharge and one month from discharge, respectively. According to their study, anomalies were noted in DLCO % predicted in 47.2% and 52.6%, respectively. They all reported the significant difference in impaired diffusing capacity among the different groups of severity. In our study, 44 (54%) patients had impaired diffusing capacity and there was a significant difference between non-severe and severe COVID-19 patients at three-month follow-up, which is in agreement with previous studies. Lung function disorder is also one of the common issues with patients suffering SARS and MERS. Pulmonary function defects were detected in half of the recovered severe acute respiratory syndrome patients 3 months after hospital discharge [23]. Interstitial or pulmonary vascular abnormalities are associated with reduced DLCO [24], but it is unclear if impaired DLCO in COVID-19 is due to pulmonary interstitial or pulmonary vascular disease or both. In order to illuminate the reason for impaired DLCO in COVID-19, we analyzed the TSS score on admission, pulmonary interstitial abnormalities at three-month after

discharge, and markers of vasculopathy (including D-dimer, Padua score, MPA, MPA/AAo and low molecular weight heparin use) and explored their associations with DLCO decline. As a method to score the severity of inflammation on CT images [11], TSS > 10.5 was found significantly associated with impaired DLCO, indicating that the severity of pulmonary inflammation may be the reason for impaired DLCO. The results implied that we should follow up the COVID-19 patients for the pulmonary function, especially the individuals with high TSS of chest CT. We also found patients with impaired DLCO had a higher percentage of interstitial lesions, indicating pulmonary interstitial damage may contribute to impaired DLCO at three months after discharge. There was no significant difference in vascular diseases between impaired DLCO and normal ones at three-month follow-up. However, due to small sample size and lack of computer tomography pulmonary angiography (CTPA), the results could not accurately reflect the relationship between vascular abnormalities and impaired DLCO. The pathogenesis of impaired DLCO in COVID-19 merits further study in the future.

We also analyzed inherent relationships of corticosteroids treatment, inflammatory on admission and meeting ARDS with impaired DLCO. As a result, meeting ARDS contributed to impaired DLCO, which was consistent with previous reports that ARDS survivors had striking decline in DLCO, the most common abnormality in pulmonary function [25, 26].The exact pathologic causes of lung dysfunction in recovered COVID-19 patients remain unknown. Structural pulmonary damage caused by the ARDS and subsequent chronic changes may damage gas

exchange [27]. Furthermore, neuromuscular weakness may also contribute to the impaired pulmonary function [28].

Although large number of patients were followed up in our cohort, there were several limitations in our study. Firstly, pulmonary function test was not carried out for all patients and not all patients undergoing lung function test received chest CT scan at three-month follow-up, which is mainly attributed to our limited knowledge of this novel virus and poor awareness about its' impact on patients' lung function in the early epidemic era. This is the inherent limitation of this real-world study. Secondly, there were lack in CTPA and other instruments to evaluate cardiovascular conditions. Furthermore, there were no direct evidence to explain the etiology of sequelae and impaired DLCO of COVID-19 survivors. Although psychiatric and traumatic stress disorder were reported for patients with SARS and MERS [29, 30], a larger study with long-term follow-up needs to be carried out.

Conclusion

Weakness, palpitation and dyspnea were the most common sequelae of COVID-19. Lung carbon monoxide diffusion dysfunction was the major damage in pulmonary function of COVID-19 survivors at three months after discharge. Chest CT TSS>10.5 and ARDS occurrence in COVID-19 were associated with impaired DLCO. Pulmonary interstitial damage may contribute to impaired DLCO at three months after discharge. This indicates that there is a necessity to adopt pulmonary rehabilitation strategy to improve outcomes in COVID-19 patients.

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Table 1. Lung function in severe and non-severe COVID-19 patients.

Variables*	All (N=81)	Non-severe (N=41)	Severe (N=40)	P
Age, year	59±14	55±15	63±12	0.022
Gender, male	34 (42)	17 (42)	17 (43)	0.925
BMI, kg/m^2	23.87±3.18	23.72±3.41	24.04±2.95	0.677
Comorbidities, N (%)				
Hypertension	23 (28)	12 (29)	11(28)	0.860
CHD	5 (6)	2 (5)	3 (8)	0.675
Diabetes	7 (9)	2 (5)	5 (13)	0.264
CRD	6 (7)	1 (2)	5 (13)	0.201
Tumor	2 (3)	2 (5)	0 (0)	0.494
Arrhythmia	2 (3)	2 (5)	0 (0)	0.494
Lung function				
TLC % pred	99.25±24.44	102.15±26.25	96.28±22.38	0.288
TLC <80% pred	8 (10)	1(2)	7 (18)	0.029
RV % pred	144.85±66.75	154.78±76.75	134.68±53.73	0.176
FVC % pred	89.73±13.25	90.79±14.75	88.65±11.59	0.469
FVC <80 % pred	17 (21)	9 (22)	8 (20)	0.829
FEV$_1$ % pred	93.95±11.32	94.66±11.33	93.23±11.41	0.573
FEV$_1$<80% pred	5 (6)	2 (5)	3 (8)	0.675

FEV1/FVC	87.23±10.06	87.63±9.62	86.82±10.59	0.719
FEV_1/FVC<70%	3 (4)	0 (0)	3(8)	0.116
MMEF75/25	85.90±24.25	87.73±24.34	84.03±24.32	0.496
MMEF75/25<65%	16 (20)	8 (20)	8 (20)	0.956
DLCO % pred	82.60±23.80	88.13±28.11	76.93±16.91	0.033
DLCO < 80% pred	44 (54)	17 (42)	27 (68)	0.019
DLCO/VA % pred	86.10±15.72	88.72±17.21	83.41±13.74	0.128
DLCO/VA < 80% pred	31(38)	14 (34)	17 (43)	0.439

Abbreviations: pred, predict; TLC, total lung volume; FVC, forced vital capacity; RV, residual volume; FEV1, forced expiratory volume in the first second; MMEF, maximum mid-expiratory flow; DLCO, diffusing capacity of the lung for carbon monoxide.

* Continuous variables were summarized as mean (SD) and categorical variables were number (percentage).

Table 2. The characteristics in COVID-19 patients with normal DLCO and impaired DLCO at 3-month follow-up

Variables*	Total (N=81)	Normal DLCO (N=37)	Impaired DLCO (N=44)	P
Demographics and clinical characteristics				
Age, year	59 (14)	58±14	60±14	0.458
Gender, male, n (%)	34 (42)	12 (32)	22 (50)	0.111
SOFA score	1 (0-2)	1 (0-2)	1 (0-3)	0.217
BMI, kg/m^2	23.87±3.18	23.86±3.36	23.88±3.09	0.977
Severity				0.019
Severe	40 (49)	13(35)	27(61)	
Non-severe	41(51)	24(65)	17(39)	
Padua score	4 (1-5)	2 (1-5)	5 (1-6)	0.080
Comorbidities, N (%)				
Hypertension	23 (28)	9 (24)	14 (32)	0.456
Diabetes	7 (9)	5 (14)	2 (5)	0.237
CRD	5 (6)	2 (5)	3 (7)	1.000
Tumor	2 (3)	1 (3)	1 (2)	1.000
Laboratory findings on admission				
WBC, 10^9/L	4.98 (3.80-6.28)	5.54 (4.46-6.94)	5.39(4.35-6.94)	0.894

Lymphocyte, 10^9/L	0.90 (0.65-1.22)	1 (0.82-1.35)	0.79(0.60-1.05)	0.014
HCRP, mg/L	33.31 (8.71-83.79)	30.08 (8.33-79.84)	51.90(17.49-136.20)	0.157
NT-proBNP, pg/mL	286.4 (95.8-566.55)	194.5 (92.15-537.10)	445.85(85.7-882.85)	0.286
CK-MB, U/L	10.45 (7.7-13.6)	9.9 (7.285-12.64)	11.55(8.47-15.31)	0.105
D-dimer, mg/L	0.4 (0.31-0.62)	0.41 (0.32-0.60)	0.47(0.38-0.82)	0.471
Fibrinogen, g/L	3.91 (2.96-4.69)	4 (3.45-5.12)	4.05(2.88-4.83)	0.560
Platelet, 10^9/L	187 (141-235)	202 (174-258)	163(133-223)	0.032

Chest CT scan on admission

Unilateral	8 (10)	5 (14) [†]	3 (7)	0.459
Bilateral	73 (90)	32 (87) [†]	41 (93)	
Unilobar	5 (6)	3 (8)	2 (5) [†]	0.656
Multilobar [§]	76 (94)	34 (92)	42 (96) [†]	
TSS	9 (5-13)	7 (2-10)	12 (8.25-15)	<0.001
MPA	26.05±3.26	25.16±3.16	26.80±3.19	0.023
AAo	30.33±3.71	29.97±3.83	30.62±3.63	0.433
MPA/AAo	0.86±0.09	0.84±0.07	0.88±0.10	0.053

Treatments during hospitalization, N (%)

Corticosteroids	17 (21)	7 (19)	10 (23)	0.675
LMWH [#]	33 (41)	13 (35)	20 (46)	0.346
HFNC	13 (16)	5 (14)	8 (18)	0.569

Noninvasive MV	32 (40)	12 (32)	20 (46)	0.232
ARDS, N (%)	24 (30)	6 (16)	18 (41)	0.015

Abbreviations: SOFA, Sequential Organ Failure Assessment; CRD, Chronic Respiratory Disease; CT, Computerized Tomography; TSS, Total Severity Score; MPA, Main pulmonary artery; AAo, ascending aorta; LMWH, Low Molecular Weight Heparin; HFNC, Transnasal Hyperflow Oxygen Therapy; MV, Mechanical Ventilation; ARDS, Acute Respiratory Distress Syndrome.

*Continuous variables were summarized as mean (SD) and categorical variables were number (percentage).

§ Multilobar involvement refers to greater than or equal to 2 lobar in lung.

† The summed percentage may exceed 100% due to rounding.

LMWH was used for prevent venous thrombus thrombosis.

Table 3. Pulmonary CT scan and impaired DLCO*at three month

Variables	Total (N=45)	Normal DLCO (N=16)	Impaired DLCO (N=29)	P
Pulmonary interstitial damage	32 (71)	8 (50)	24 (83)	0.037[**]
Fibrous stripe	23 (51)	7 (44)	16 (55)	0.463
GGO	9 (20)	2 (13)	7 (24)	0.465
Consolidation	5 (11)	1 (6)	4 (14)	0.636
Traction bronchiectasis	14 (31)	1 (6)	13 (45)	0.008[**]
Subpleural curvilinear shadow	22 (49)	2 (13)	20 (69)	<0.001 [**]
Coarse reticular pattern	7 (16)	0 (0)	7 (24)	0.04[**]
Pulmonary vascular parameters				
MPA	26.09±2.79	25.52±2.94	26.40±2.70	0.327
AAo	30.21±3.29	29.67±3.54	30.50±3.17	0.442
MPA/AAo	0.87±0.08	0.86±0.09	0.87±0.08	0.855

[*] Impaired DLCO defined as < 80% predicted.   [**] $P<0.05$.

Abbreviation: GGO, ground-glass opacity; MPA, Main pulmonary artery; AAo, ascending aorta.
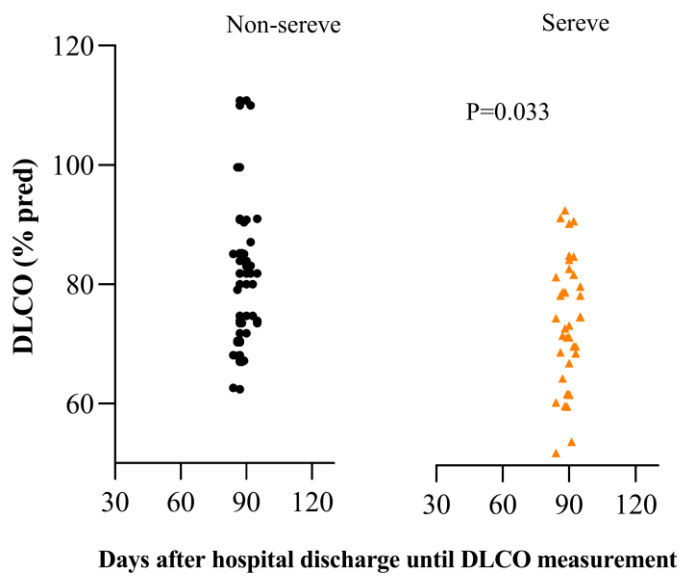
Figure 1. DLCO (diffusion capacity for carbon monoxide) % of predicted at 3 months after discharge in non-severe and severe COVID-19 patients.
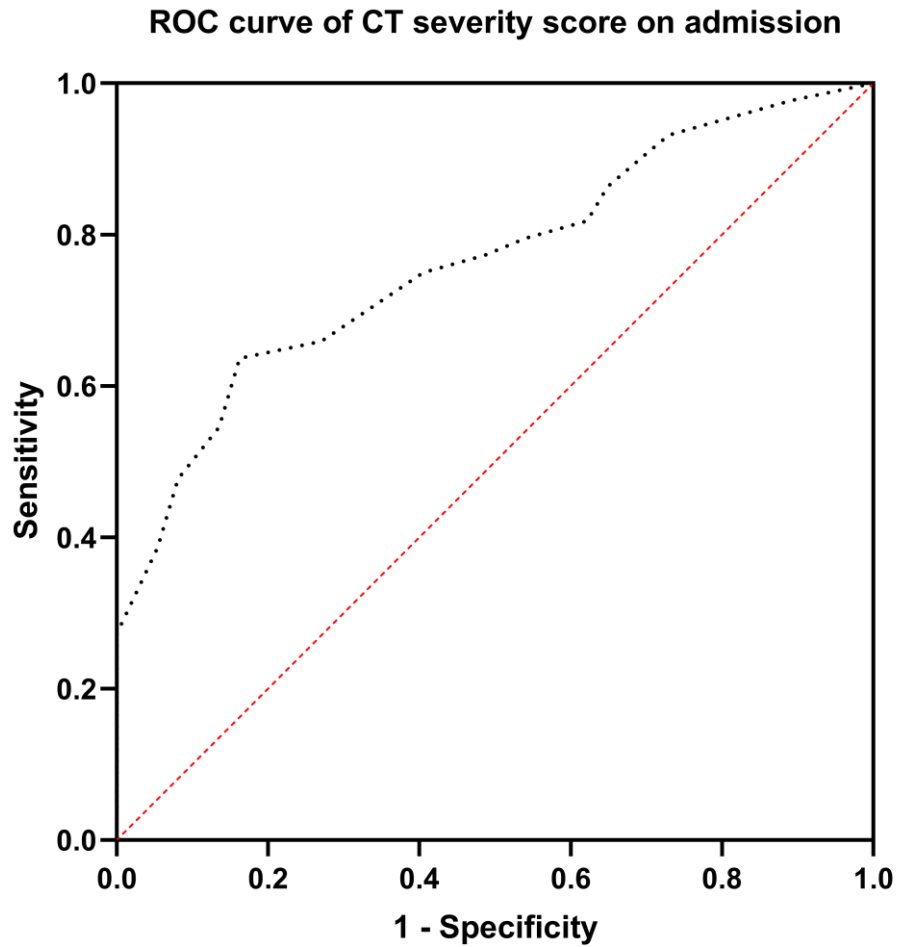
Figure 2. Receiver operating characteristic curve (ROC) analysis of total CT severity score (TSS) on admission for prediction of impaired DLCO during three-month follow-up.

With the cut off value of 10.5 for the TSS, the area under the curve was 0.765 (95% confidence interval, 0.663 to 0.867; p <0.001) with the sensitivity of 64% and specificity of 84%.

Figure 3. Factors associated with impaired DLCO during three-month follow-up in multivariable regression analysis.

Abbreviation: TSS, total severity score; MPA, main pulmonary diameter; ARDS, Acute Respiratory Distress Syndrome

Supplementary material

Supplementary Table 1. The main symptoms of COVID-19 patients with 3-month follow-up.

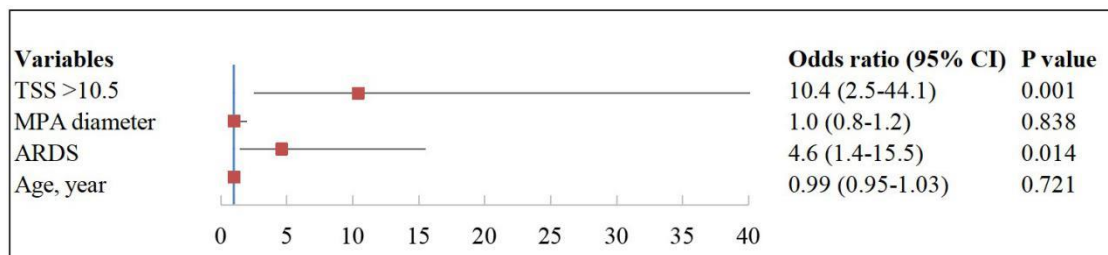| Variables*	Total (N=647)	Non-severe (N=399)	Severe (N=248)	P
Age, year	58 (15)	55 (15)	63 (13)	<0.001
Gender, male, n (%)	287 (44)	165 (41)	122 (49)	0.051
SOFA score	1 (0-2)	0 (0-1)	2 (1-3)	<0.001
Comorbidities, N (%)				
Hypertension	191 (30)	104 (26)	87 (35)	0.015
Diabetes	70 (11)	41 (10)	29 (12)	0.572
CRD	36 (6)	17 (4)	19 (8)	0.067
CHD	33 (5)	19 (5)	14 (6)	0.620
Tumor	19 (3)	14 (4)	5 (2)	0.274
Arrhythmia	11 (2)	8 (2)	3 (1)	0.545
Symptoms at three-month follow-up, N (%)				
Weakness	87 (13)	41 (10)	46 (19)	0.003
Palpitation	63 (10)	29 (7)	34 (14)	0.007
Dyspnea	56 (9)	26 (7)	30 (12)	0.014

Cough	38 (6)	20 (5)	18 (7)	0.238
Lower limb edema	8 (1)	4 (1)	4 (2)	0.490
Chest pain	6 (1)	3 (1)	3 (1)	0.680
Hemoptysis	1 (0.2)	1 (0.3)	0 (0)	1.000

Abbreviations: SOFA, Sequential Organ Failure Assessment; CRD, Chronic Respiratory Disease; CHD, Chronic Heart Disease.

* Continuous variables were summarized as mean (SD) or median (interquartile ranges [IQR]) where appropriate and categorical variables were number (percentage).

Supplementary Table 2. The characteristics in COVID-19 patients with or without sequelae at three-month follow-up.

Variables	Total (N=647)	No sequelae (N=509)	With sequelae (N=138)	P
Demographics and clinical characteristics				
Age, year	58 (15)	58±15	60±15	0.120
Gender, male, n (%)	287 (44)	230 (45)	57 (41)	0.416
SOFA score	1 (0-2)	1(0-2)	1 (0-2)	0.662
BMI, kg/m^2	23.87±3.18	24.07±3.33	23.06±2.47	0.229
Severity				<0.001[*]
Severe	248(38)	177(35)	71(51)	
Non-severe	399(62)	332(65)	67(49)	
Comorbidities, n (%)				
Hypertension	191(30)	150 (30)	41 (30)	0.956
Diabetes	70 (11)	57 (11)	13 (9)	0.551
CRD	36 (6)	27 (5)	9 (7)	0.580
CHD	33 (5)	30 (6)	3 (2)	0.078
Tumor	19 (3)	16 (3)	3 (2)	0.777
Arrhythmia	11(2)	9 (2)	2 (1)	1.000
Laboratory findings on admission				

WBC, 10^9/L	4.98 (3.80-6.28)	4.90 (3.78-6.20)	5.27 (3.87-6.79)	0.045*
Lymphocyte, 10^9/L	0.90 (0.65-1.22)	0.91 (0.66-1.22)	0.88 (0.64-1.22)	0.521
HCRP, mg/L	33.31 (8.71-83.79)	30.09 (7.53-79.85)	48.21 (13.05-100.33)	0.009*
NT-proBNP, pg/mL	286.40 (95.80-566.55)	278.20 (95.90-553.20)	312.90 (90.68-705.53)	0.160
CK-MB, U/L	10.45 (7.70-13.60)	10.45 (7.54-13.46)	10.52 (8.05-14.06)	0.445
D-dimer, mg/L	0.40 (0.31-0.62)	0.40(0.31-0.58)	0.45(0.31-0.92)	0.006*
Fibrinogen, g/L	3.91(2.96-4.69)	3.81(2.96-4.69)	3.91(3.01-4.69)	0.450
Platelet, 10^9/L	187(179-285)	186(140-231)	191(147-247)	0.227
Laboratory findings on discharge				
WBC, 10^9/L	5.23 (4.21-6.35)	5.17 (4.25-6.28)	5.38 (4.05-6.68)	0.576
Lymphocyte, 10^9/L	1.30 (1.00-1.59)	1.31 (1.01-1.61)	1.26 (0.91-1.58)	0.126
HCRP, mg/L	4.03 (1.37-15.53)	3.99 (1.32-15.53)	4.36 (1.61-15.32)	0.742
D-dimer, mg/L	0.41 (0.29-0.73)	0.41 (0.29-0.73)	0.43 (0.30-0.73)	0.411
Fibrinogen, g/L	3.20 (2.56-4.00)	3.20 (2.56-4.00)	3.13 (2.64-3.99)	0.936
Platelet, 10^9/L	223 (179-285)	223 (179-287)	223 (176-275)	0.771
Inpatient days	18±8	17±8	19±10	0.002*

Abbreviations: SOFA, sequential organ failure assessment; BMI, body mass index; CRD, chronic respiratory disease; CHD, chronic heart disease; WBC, White blood cell; HCRP, Hypersensitive c-reactive protein; NT-proBNP, N-terminal pro brain natriuretic peptide; CK-MB, Creatine kinase-MB. * P<0.05

Supplementary Table 3. Risk factors associated with the occurrence of sequelae.

	Univariable OR (95%CI)	P Value	Multivariable OR (95%CI)	P Value
Demographics and clinical characteristics				
Age, year	1.01 (1.00-1.02)	0.114	1.0 (0.99-1.01)	0.909
Gender, male (vs. female)	0.9 (0.6-1.3)	0.416	0.7 (0.5-1.1)	0.718
Sofa score	1.0 (0.9-1.1)	0.905		
BMI, kg/m^2	0.9 (0.7-1.1)	0.303		
Severe pneumonia	2.0 (1.4-2.9)	<0.001[*]	1.7 (1.1-2.6)	0.026[*]
Comorbidities present (vs. not present)				
Hypertension	1.0 (0.7-1.5)	0.956		
Diabetes	0.8 (0.4-1.6)	0.551		
CRD	1.2 (0.6-2.7)	0.581		
CHD	0.4 (0.1-1.2)	0.091		
Tumor	0.7 (0.2-2.4)	0.552		
Arrhythmia	0.8 (0.2-3.8)	0.797		
Laboratory findings on admission				
WBC, 10^9/L	1.1 (1.0-1.2)	0.017[*]	1.08 (0.99-1.17)	0.079
Lymphocyte, 10^9/L	0.9 (0.6-1.3)	0.533		
HCRP, mg/L	1.004 (1.001-1.007)	0.015[*]	1.002 (0.998-1.006)	0.332

|				
CK-MB, U/L	1.00 (0.98-1.03)	0.835		
D-dimer, mg/L	1.07 (1.00-1.14)	0.043[*]	1.03 (0.97-1.10)	0.348
Fibrinogen, g/L	1.1 (0.9-1.2)	0.343		
Platelet, 10^9/L	1.002 (0.999-1.004)	0.181		
Inpatient days	1.03 (1.01-1.06)	0.003[*]	1.03 (1.00-1.05)	0.041[*]

Abbreviations: SOFA, sequential organ failure assessment; BMI, body mass index; CRD, chronic respiratory disease; CHD, chronic heart disease; WBC, White blood cell; HCRP, Hypersensitive c-reactive protein; NT-proBNP, N-terminal pro brain natriuretic peptide; CK-MB, Creatine kinase-MB. [*] $P<0.05$

Supplementary Table 4. Factors associated with impaired DLCO in univariable and multivariable analysis.

	Univariable OR (95%CI)	P Value	Multivariable OR (95%CI)	P Value
Demographics and clinical characteristics				
Age, year	1.01 (0.98-1.05)	0.455	0.99(0.95-1.03)	0.721
Male (vs. female)	2.1 (0.8-5.2)	0.113		
Sofa score	1.3 (0.9-1.8)	0.160		
BMI, kg/m^2	1.0 (0.9-1.2)	0.976		
Severe pneumonia	2.9 (1.2-7.3)	0.020[*]		
Padua score	1.2 (1.0-1.5)	0.055		
Comorbidities present (vs. not present)				
Hypertension	1.5 (0.5-3.9)	0.457		
Diabetes	0.3 (0.1-1.7)	0.171		
Chronic respiratory disease	1.3 (0.2-8.1)	0.793		
Tumor	0.8 (0.1-13.9)	0.901		
Laboratory findings on admission				
WBC, 10^9/L	1.0(0.8-1.2)	0.752		
Lymphocyte, 10^9/L	0.5(0.2-1.3)	0.137		
HCRP, mg/L	1.01(1.00-1.01)	0.157		
CK-MB, U/L	1.1(1.0-1.2)	0.155		

D-dimer, mg/L	1.1(0.9-1.2)	0.467		
Fibrinogen, g/L	0.9(0.6-1.2)	0.388		
Platelet, 10^9/L	0.99(0.99-1.00)	0.055		
Treatments during hospitalization, N (%)				
Corticosteroids	1.3(0.4-3.7)	0.675		
HFNC	1.4(0.4-4.8)	0.570		
Noninvasive MV	1.7(0.7-4.3)	0.234		
ARDS	3.6(1.2-10.3)	0.019[*]	4.6(1.4-15.5)	0.014[*]
TSS > 10.5 at admission	7.7(2.5-23.4)	<0.001[*]	10.4(2.5-44.1)	0.001[*]
MPA diameter at admission	1.2(1.0-1.4)	0.027[*]	1.0(0.8-1.2)	0.838

Abbreviations: SOFA, sequential organ failure assessment; BMI, body mass index; WBC, White blood cell; HCRP, Hypersensitive c-reactive protein; NT-proBNP, N-terminal pro brain natriuretic peptide; CK-MB, Creatine kinase-MB; HFNC, Transnasal Hyperflow Oxygen Therapy; MV, mechanical ventilation; ARDS, Acute Respiratory Distress Syndrome; TSS, total severity score. MPA, main pulmonary artery. [*] $P<0.05$
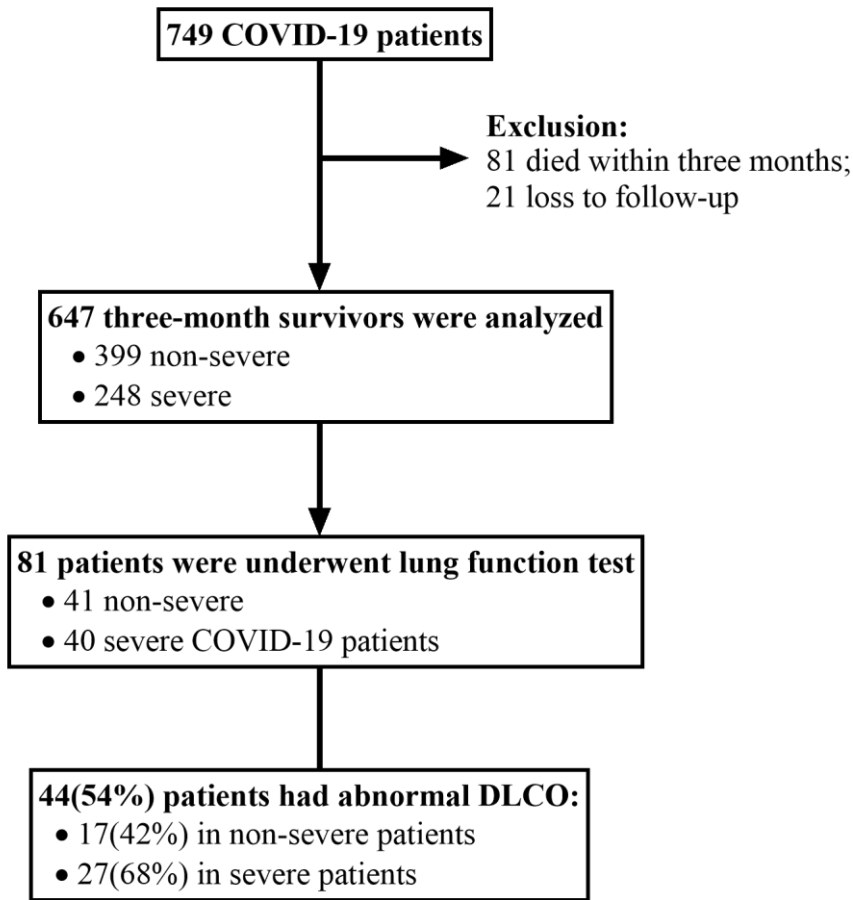
Supplementary Figure 1. The flow chat of studied COVID-19 patient