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Serum levels of laminin and von Willebrand factor in COVID-19 survivors 6 months after discharge

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Highlights

- Lung cell damage indicators of COVID-19 survivors 6 months after discharge were explored.

- Levels of laminin were high particularly in elderly and female patients.

- COVID-19 patients had abnormal lung injury indicators 6 months after discharge.

Abstract
Objectives: To evaluate clinical characteristics, pulmonary diffusion function, chest computerized tomography (CT), and serum lung cell damage indicators of coronavirus disease 2019 (COVID-19) survivors 6 months after discharge.

Methods: Data of COVID-19 survivors discharged from hospital between January 21, 2020 and January 11, 2021 and healthy controls were collected. Serum levels of surfactant D (SPD), the receptor for advanced glycation products (RAGE), laminin, and von Willebrand factor (vWF) were measured in healthy controls and COVID-19 survivors 6 months after discharge. The relationships between serum lung cell damage indicator levels and various parameters were explored.

Results: Fifty-two COVID-19 survivors (31 non-severe and 21 severe) and 30 controls were included. Serum levels of laminin in COVID-19 survivors 6 months after discharge were significantly higher than those in the controls. The increase was more significant in elderly and female patients. Serum levels of RAGE and vWF were not statistically different from those of the controls. However, 6 months after discharge, COVID-19 survivors with abnormal chest CT and those in the severe group had higher vWF levels.

Conclusions: COVID-19 patients had abnormal lung injury indicators 6 months after discharge. The recovery time after infection is currently unknown, and long-term observations are required.

Keywords: COVID-19; Follow-up; Chest Computed Tomography; Laminin; von Willebrand factor
Introduction

Coronavirus disease 2019 (COVID-19) is a major global public health emergency caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The COVID-19 pandemic has infected approximately 200 million people and caused over 4 million deaths as of August 1, 2021. SARS-CoV-2 binds to the host's angiotensin-converting enzyme 2 (ACE2) receptor and enters target cells (Hoffmann et al., 2020; Yan et al., 2020). Damage caused by the virus entering the cell usually results in the cell-specific proteins release into the circulation, which can assess cell damage (Bhargava and Wendt, 2012). Surfactant D (SPD) is the main functional substance produced by alveolar type 2 (AT2) cells. The receptor for advanced glycation products (RAGE) is mainly expressed on the basal surface of alveolar type 1 cells. Laminin is a large basement membrane glycoprotein that plays an important role in intercellular adhesion, growth, differentiation, and epithelial cell repair. von Willebrand factor (vWF) is a multimeric glycoprotein that can be used as an endothelial cell marker. In lung tissue, endothelial cells and AT2 cells have abundant ACE2 receptors (Hamming et al., 2004; Mason, 2020). A previous study evaluated the cellular injury associated with SARS-CoV-2 infection and confirmed that the damage to AT2 cells and lung structures remained two weeks after SARS-CoV-2 infection was treated (Shao et al., 2020). To date, there have been few reports on long-term changes of lung cell damage and repair 6 months after SARS-CoV-2 infection was treated.
In this study, the serum levels of SPD, RAGE, laminin, and vWF in COVID-19 survivors were measured 6 months after discharge. By comparing clinical characteristics, pulmonary diffusion function, chest computerized tomography (CT) with the levels of these indicators in COVID-19 survivors 6 months after discharge, an assessment can be made of the lung injury, lung regeneration, and repair ability of patients who have recovered from SARS-CoV-2 infection.

**Materials and Methods**

**Participants**

Patients with COVID-19 who were admitted to our hospital between January 21, 2020 and January 11, 2021 were included. All patients met the diagnostic criteria, clinical classification, and discharge criteria of the Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and Treatment published by the China National Health Commission. The patients were divided into non-severe and severe groups according to the severity of the disease during hospitalization. Patients who met any of the following criteria were classified into the severe group: dyspnea (respiratory rate ≥33 breaths/min), in resting state (finger oxygen saturation ≤93%), arterial blood partial pressure of oxygen or fraction of inspired oxygen ≤300 mmHg (1 mmHg = 0.133 kPa), and lung imaging showing that the lesion had progressed significantly by > 50% within 24–48 h. Patients who did not meet the above criteria were
defined as the non-severe group. We excluded patients who died before the follow-up, refused to participate in the follow-up, and left the local area and could not complete the follow-up.

Thirty age- and sex- matched health or medical staff who completed physical examinations at Tianjin Haihe Hospital between June 17, 2020 and June 24, 2020 were recruited as healthy controls. All had negative SARS-CoV-2 nucleic acid test results and SARS-CoV-2 antibodies, without underlying diseases.

This study was approved by the Institutional Review Board of Tianjin Haihe Hospital (2020HHKT-014). Written informed consent was obtained from all participants.

**Basic Data Collection**

We collected general participant information of COVID-19 survivors who underwent a follow-up examination 6 months after discharge using a standard form. It included age, sex, comorbidity, and clinical treatment. These patients also completed a questionnaire regarding their clinical symptoms.

**Pulmonary Diffusion Function Tests**

Pulmonary diffusion function tests were performed using the Master Screen Body (Jaeger MS-PFT Analysis Unit, Würzburg, Germany). According to the American Thoracic Society standards (1986), the diffusing capacity for carbon
monoxide corrected for hemoglobin (DLCOc-SB) was measured as a percentage of the predicted value. Measured diffusing capacity for carbon monoxide (DLCO) <80% of the predicted value indicated pulmonary diffusion impairment.

**Chest CT Examination**

Chest CT scans were performed using a Canon 64-slice helical CT scanner (Aquilion Prime 128, Canon Medical Systems, Otawara, Japan). A semi-quantitative visual scoring method (Hansell et al., 2008) was used to score the CT images of each single lung lobe according to the area percentages of the lesions in a single lung lobe. A lung lobe without lesions was scored 0, while a lung lobe with lesion area percentages was categorized into <25%, ≥25% to <50%, ≥50% to <75%, and ≥75% scored 1, 2, 3, and 4, respectively. The total score of the five lobe categories ranged from 0 to 20. Each CT image was independently reviewed and scored by three radiologists, and the scores were averaged to obtain the final score of the CT image. A score ≥1 indicated abnormal chest CT, and a score of 0 indicated normal chest CT.

**Enzyme-Linked Immunosorbent Assay (ELISA) for Serum SPD, RAGE, Laminin and vWF**

Serum levels of SPD, RAGE, laminin, and vWF were measured by ELISA in enrolled healthy controls and COVID-19 survivors. All kits were purchased from Abcam: SPD (human surfactant D/SP-D SimpleStep ELISA Kit,
Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows (version 26.0). Numerical data are presented as the number of cases and percentage (n%). Normally distributed data are expressed as mean ± standard deviation; between-group comparisons were performed using an independent samples t-test. Non-normally distributed data are expressed as median (interquartile range); between-group comparisons were performed using the Mann–Whitney U test. P-values were determined by an unpaired bilateral Mann–Whitney U test. Statistical significance was set at $P<0.05$.

Results

Patient Demographic and Clinical Characteristics

A total of 132 local COVID-19 patients were discharged from Tianjin Haihe Hospital, China, between January 21, 2020 and January 11, 2021. After excluding two children, 76 patients who refused to undergo reexamination 6 months after discharge and two patients with chronic lung disease (one with interstitial lung disease and one after undergoing lung cancer resection), 52 survivors were finally enrolled (Figure 1): 31 in the non-severe group and 21 in...
the severe group. The mean age of severe COVID-19 survivors (54 ±14 years) was higher than that of non-severe COVID-19 survivors (43 ±15 years).

Of the COVID-19 patients enrolled, 16 patients had comorbidities (31%), and the proportion of patients with comorbidities in the severe group (48%) was higher than the non-severe group (19%). The main comorbidity was hypertension (17%), followed by type 2 diabetes (10%) and coronary heart disease (8%). Twenty-one patients still had clinical symptoms (40%), nine of them being in the severe group (43%) and 12 in the non-severe group (39%). The main symptoms were myalgia or fatigue (25%) and exertional dyspnea (21%). All patients were treated with antiviral drugs during the acute infection stage. The rate of corticosteroid use in patients severe COVID-19 (43%) was higher than those with non-severe COVID-19 (6%) (Table 1).

Pulmonary diffusion function tests 6 months after discharge were conducted in 51 patients (one patient was non-cooperative due to the presence of a tracheal cannula). Seventeen patients with COVID-19 had abnormal pulmonary diffuse function, including six with severe (30%) and 11 with non-severe COVID-19 (35.5%), although this was not significant (P=0.685) (Table S1).

Forty-seven patients underwent chest CT (five patients refused). Eleven patients presented with abnormal chest CT findings, including 10 with severe (55.6%) and one with non-severe COVID-19 (3.4%) (P<0.001) (Table 2). Among them, six patients in the severe group showed fibrotic bands on chest CT, and the difference was significant compared with non-severe COVID-19 patients (P=0.001) (Table 2).
Serum SPD, RAGE, laminin, and vWF Levels in COVID-19 Survivors 6 Months After Discharge

In COVID-19 survivors 6 months after discharge, serum laminin levels were 4,019.71±1,413.41 pg/mL, which were significantly higher than those of healthy controls (948.61±344.19) (P<0.001). The serum level of SPD was 6896.01±3404.37 pg/mL, which was lower than that of the healthy control group (10127.47±1764.49) (P<0.001). The serum RAGE level was 797.97±235.85 pg/mL, which was not statistically different from that of the healthy control group (879.16±323.10) (P=0.195). The serum level of vWF was 1.43±0.38 IU/mL, which was not statistically different from those of the healthy control group (1.43±0.31) (P=0.914) (Table 3).

Serum Laminin Levels According to Different Variables in COVID-19 Survivors 6 Months After Discharge

The serum level of laminin was high in COVID-19 survivors 6 months after discharge. The increase was most significant in the older patients (P=0.048) and women patients (P=0.041) (Figure 2). Variables, such as disease severity type, symptoms, pulmonary diffusion function, and chest CT findings had no effect on serum laminin levels (Tables S4–S7).

Serum vWF Levels According to Different Variables in COVID-19 Survivors 6 Months After Discharge

The serum vWF level decreased to normal levels 6 months after discharge of COVID-19 survivors. However, this was significantly higher in the severe group (P= 0.015) and in patients with abnormal chest CT findings (P= 0.002)
Variables, such as age, sex, symptoms, and pulmonary diffusion function had no effect on serum vWF levels (Tables S2, S3, S5, S6).

**Serum SPD and RAGE Levels According to Different Variables in COVID-19 Survivors 6 Months After Discharge**

There were no differences in serum SPD and RAGE levels among groups with different ages, sexes, disease severities, symptoms, pulmonary diffusion functions, and chest CT findings (Tables S2-S7).

**Discussion**

COVID-19 is an infectious disease caused by SARS-CoV-2, which can lead to severe acute respiratory distress syndrome (ARDS), characteristic inflammatory response, vascular damage, microvascular lesions, angiogenesis, and extensive thrombosis. The disease process of COVID-19 can be divided into four stages: the first stage is characterized by upper respiratory tract infections, the second presents with dyspnea and pneumonia, the third stage is the deterioration of clinical conditions caused by cytokine storms and subsequent high inflammatory states, and the fourth stage is death or rehabilitation (Stasi et al., 2020). Previous studies have found that the pathological characteristics of COVID-19-induced lung injury are the exudative phase in the first week of the disease, the proliferation or tissue phase in the second and third weeks and the end-stage fibrosis phase in the last third week. The time evolution of COVID-19 lung histopathological lesions is similar to ARDS (Merdji et al., 2021). The histological characteristics are typical diffuse alveolar damage (DAD) (Beasley, 2021). The pathogenesis of
the disease can be summarized as the damage of capillary and alveolar
epithelial cells. Part of the basement membrane of the alveolar wall fuses,
causing fluid and cell breakdown products to leak out of the alveolar cavity,
followed by the proliferation and growth of type II lung cells and a repair period
characterized by fibroblast proliferation (Beasley, 2010; Hughes et al., 2017;
Tomashefski, 1990; Tomashefski, 2000). Lung epithelial tissue includes a
variety of cellular components, such as ciliated, basal, goblet, and Clara cells,
and type I and type II adipocytes, which are widely distributed in lung tissue.
In the exudative stage, the basal surface of alveolar type I epithelial cells
mainly express receptor for RAGE. RAGE plays a central role in inflammation
(Bierhaus et al., 2005; Birts et al., 2021). An important function of alveolar
type II epithelial cells is to produce pulmonary surface-active substances (SP).
These are categorized according to their structure and function as: SP-A, SP-
B, SP-C, and SP-D. These SPs can reduce the surface tension of the alveoli,
open the alveoli, and increase lung compliance. Previous studies have found
that endothelial cell damage is a manifestation of acute lung injury (ALI) or
ARDS. Endothelial cell-specific proteins include soluble intercellular adhesion
molecule-1, angiopoietin-1, angiopoietin-2, and vWF. Among them, the
endothelial cell injury biomarker, vWF, has been shown to be significantly
elevated at 68 days of recovery from COVID-19 (Fogarty, 2021). The main
function of lung extracellular matrix is to maintain the integrity of epithelial
tissue and blood vessel structure, which includes collagen, glycoprotein, and
proteoglycan. Laminin is an extracellular protein deposited on the basement
membrane and plays an important role in cell adhesion, growth,
differentiation, and repair of epithelial cells. A previous study observed that the
expression level of laminin Y2 fragment in the plasma and pulmonary edema fluid of patients with ALI or ARDS increased significantly (Katayama et al., 2010). Therefore, the above four markers can differentiate the stages of lung injury. Because of the current limited data on the level of lung damage markers in the serum of patients with COVID-19 during the recovery period, we hypothesize that this pathological state exists and contributes to the onset of disease during the recovery period. The mechanism may be related to the persistent symptoms that patients experience during the recovery period based on the histological characteristics of ALI or ARDS caused by the typical DAD (Fremont, 2010). We conducted an investigation of these four markers to evaluate the lung damage and recovery of COVID-19 survivors 6 months after discharge from hospital.

Our research showed that serum RAGE levels in COVID-19 patients did not increase at 6 months of recovery. Previous literature reported that during the exudative stage of ARDS, the basal surface of alveolar type I epithelial cells mainly express receptor for RAGE. RAGE plays a central role in the inflammatory response. Combining ligands will stimulate many aspects of inflammation, including the production of key inflammatory mediators NF-κB, and the subsequent production of inflammatory cytokines (Bierhaus, 2005; Birts, 2021). Previous studies have shown that RAGE rises in the acute phase of COVID-19 and drops by the 14th day of the recovery period (Shao et al., 2020). Our research shows that serum RAGE levels return to normal after 6 months of recovery from COVID-19.

In the COVID-19 pathogenesis, SARS-CoV-2 binds to ACE2 and is highly expressed in alveolar and endothelial cells of angiovascular structures (Rovas
et al., 2021). Angiopoietin-2 induces damage to the endothelium (Roose and Joly, 2020). vWF is a biomarker of COVID-19 endothelial lesions (Joly et al., 2021), which release the polysaccharide protein in the blood, mediating platelet adhesion and aggregation (Roose and Joly, 2020). Meanwhile, vWF is also a biomarker of the inflammatory response intensity (Joly et al., 2021). In our previous study (Shao et al., 2020), serum vWF levels significantly increased in the acute infection stage of the disease, which may be strongly related to the inflammatory response during the acute infection stage of COVID-19. The previous study also suggested that the serum vWF level began to decrease in the early recovery period (Shao et al., 2020). In this study, serum vWF levels decreased to normal 6 months after discharge. A study by von Meijenfelt et al. (von Meijenfelt, 2021) found that 4 months after patients with COVID-19 were discharged from the hospital, the plasma vWF level was still slightly increased during follow-up, but there was no statistical difference from the control group, consistent with our research results. However, Fogarty (Fogarty, 2021) included 50 patients 68 days after SARS-CoV-2 infection to evaluate the endothelial cell markers changes. The study results found that compared with the control group, the endothelial cell injury biomarker vWF:Ag was significantly increased during the recovery period (median 1.1 vs. 0.84 IU/mL; \(P=0.004\)), causing persistent endothelial disease (Fogarty, 2021). However, our study revealed no difference in serum vWF levels of SARS-CoV-2 infected patients during the 6-month recovery period compared with healthy controls (1.43 vs. 1.43 IU/mL). Notably, the serum vWF level of patients in our study six months after recovery was higher than the level found in patients in Fogarty's study 68 days after discharge. In addition,
the plasma vWF value was higher in patients with severe disease and
abnormal chest CT findings in our study. A previous study found that chest CT
abnormalities in patients with COVID-19 6 months after discharge were
related to the disease severity and were more obvious in the severe group
(Wu et al., 2021). Given that we found increased vWF levels both in those
with severe disease and those with abnormal CT at follow up, it is likely that
this may represent the same group of patients. Previous studies have shown
that the alveoli of those with severe COVID-19 are damaged by the virus,
resulting in lung injury leading to respiratory failure and ARDS (Wu and
McGoogan, 2020, Yang et al., 2020). Forty percent of COVID-19 patients
develop ARDS (Wu et al., 2020), and that vWF is a biomarker of COVID-19
endothelial injury. Combined with the results of this study, the serum vWF
level increased more significantly in those with severe COVID-19 and with
abnormal chest CT. This suggests that the disease influence on the
endothelial injury severity remained 6 months after discharge, and that
endothelial injury in patients with severe COVID-19 was more serious. To
further explore the change of vWF level during the rehabilitation period, a
larger, multi-center study is needed. In addition, there are reports in the
literature that plasma vWF levels in patients with COVID-19 were significantly
increased in quantity and quality, and that the multifactorial function of
ADAMTS13 was down-regulated (Ward et al. 2021). It would be useful to
conduct further research to determine whether therapeutic interventions to
correct ADAMTS13-VWF polymer dysfunction affect COVID-microvascular
thrombosis and vascular disease.
Pulmonary fibrosis is a recognized sequela of ARDS (Wu and McGoogan, 2020). Although the virus is cleared in COVID-19 patients during the recovery period, eliminating the cause of lung injury cannot prevent progressive fibrosis and the development of irreversible interstitial lung disease (Spagnolo et al., 2020). Pulmonary fibrosis may occur after SARS-CoV-2 infection (Spagnolo et al., 2020). Laminin is a high molecular weight extracellular matrix protein deposited on the basement membrane and participates in cell adhesion, growth, and differentiation (Colognato and Yurchenco, 2000). Lama1 is a protein subunit of laminin, which plays an important role in several lung injury and pulmonary fibrosis processes, including participation in macrophage activation, fibroblast proliferation, myofibroblast transformation, and extracellular matrix production, and affects the pulmonary fibrosis development (Lee et al., 2018). Our study found that the serum laminin level of convalescent patients was significantly higher than that of the normal population and patients with acute COVID-19. This study shows that the serum laminin level rises most significantly in COVID-19 patients after hospital discharge during the 6-month recovery period, particularly among older patients. This may be related to the increased susceptibility of the older population to ARDS during the COVID-19 pandemic (Schuliga et al., 2021) and the incidence of ARDS caused by pneumonia (Spagnolo et al., 2020). In addition, aging is also a risk factor for the pulmonary fibrosis development (Spagnolo et al., 2020). In view of these findings, it is suggested that the older patients with COVID-19 is more likely to develop pulmonary fibrosis after recovery than the general population. Our study also found that the serum laminin level increased during the rehabilitation period, particularly in female
patients. A study of 83 discharged patients who had COVID-19 found that some still had persistent physiological and imaging abnormalities 12 months after discharge, and their DLCO was significantly reduced (Wu et al., 2021).

Regression analysis also showed that the probability of DLCO damage was lower in female patients (odds ratio: 8.61, 95% CI: 2.83–26.2; \( P=0.0002 \)). It is worth noting that there is no significant association between patient sex and persistent CT abnormalities (Wu et al., 2021). It has been suggested that women patients diffuse lung dysfunction during rehabilitation are more significant, but females may not have persistent CT abnormalities. This result is consistent with our conclusion that laminin levels were significantly higher in female patients, and that the serum laminin levels in patients with abnormal CT findings did not differ from those in patients with normal CT findings. This indicates that persistent imaging abnormalities and abnormal blood exchange may have different mechanisms, and their underlying mechanisms are worthy of further study.

In addition to the above-mentioned serological markers, many patients still had persistent symptoms after a COVID-19 recovery period of 6 months. The study results revealed that their main symptoms were fatigue and shortness of breath after exercise. There was no statistically significant difference in the prevalence of symptoms according to disease severity, and there was no significant difference in lung diffusion function and chest CT between patients with severe and non-severe disease after 6 months of recovery. Fogarty et al. followed up 153 patients with a 75-day recovery period from COVID-19 and found that all indicators of persistent respiratory disease was unrelated to the
severity of the initial disease (Fogarty et al., 2021), which is consistent with our research results.

It has been reported that serum SPD has a significant positive correlation with inflammatory response (Alay et al., 2021). Our previous study (Shao et al., 2020) reported that serum SPD level increased in the acute phase of the course of disease, and that serum SPD decreased significantly 6 months after discharge, which may be related to the gradual reduction of the body’s inflammatory response.

Limitations

First, this is a single-centered controlled study with a small sample size, which is due to the small total number of confirmed COVID-19 cases in this region and requires multi-center, larger-scale research support. Second, our results showed that elevated vWF is more common in patients with severe COVID-19 and those with abnormal chest CT during recovery. Previous studies of convalescent COVID-19 patients have shown that abnormal chest CT was more likely to be observed in those with severe acute initial infection (Wu et al., 2021).

In conclusion, our research suggests that older individuals and individuals with more severe COVID-19 have greater increases in lung injury indicators, and that older individuals are more likely to develop pulmonary fibrosis. However, the recovery time after SARS-CoV-2 infection is currently unknown, and longer-term observation is needed.
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Declaration of Competing Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

Hongwei Li, Qian Wu, Xinwei Hou, Linmin Zhang, Jing Guo, Yajie Li, Fangfei Yang, and Yan Zhang performed a database search, screening, quality assessment, and data extraction. Zhonghua Qin performed the experiments. Hongwei Li and Qian Wu conducted the analyses. Qi Wu, Li Li and Haiyong Chen designed the study. Hongwei Li and Qian Wu contributed to the writing of the manuscript. All authors approved the final draft of the manuscript.

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Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

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Katayama M, Ishizaka A, Sakamoto M, Fujishima S, Sekiguchi K, Asano K, et al. Laminin gamma2 fragments are increased in the circulation of patients with...


**Figure 1.** Flow chart of COVID-19 survivors 6 months after discharge.

COVID-19, coronavirus disease 2019; CT, computed tomography; ILD, interstitial lung disease; RAGE, receptor for advanced glycation products; SPD, surfactant D; vWF, von Willebrand factor.

**Figure 2.** Serum levels of laminin according to different variables in COVID-19 survivors 6 months after discharge. (a) Serum levels of laminin between different sex groups of COVID-19 survivors 6 months after discharge. (b) Serum levels of laminin between different age groups of COVID-19 survivors 6 months after discharge. *P*-values < 0.05 indicate statistical significance.

**Figure 3.** Serum levels of vWF according to different variables in COVID-19 survivors 6 months after discharge. (a) Serum levels of vWF between non-severe and severe of COVID-19 survivors 6 months after discharge. (b) Serum levels of vWF between different chest CT findings of COVID-19 survivors 6 months after discharge. *P*-values < 0.05 indicate statistical significance.

vWF: von Willebrand factor.
Table 1. Demographic and clinical characteristics of COVID-19 survivors 6 months after discharge

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total (n=52)</th>
<th>Non-severe (n=31)</th>
<th>Severe (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (IQR), years</td>
<td>47±16</td>
<td>43±15</td>
<td>54±14</td>
</tr>
<tr>
<td>Sex (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>30 (58%)</td>
<td>18 (58%)</td>
<td>12 (57%)</td>
</tr>
<tr>
<td>Female</td>
<td>22 (42%)</td>
<td>13 (42%)</td>
<td>9 (43%)</td>
</tr>
<tr>
<td>Comorbidity (%)</td>
<td>16 (31%)</td>
<td>6 (19%)</td>
<td>10 (48%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>9 (17%)</td>
<td>5 (16%)</td>
<td>4 (19%)</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>5 (10%)</td>
<td>1 (3%)</td>
<td>4 (19%)</td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>4 (8%)</td>
<td>0 (0%)</td>
<td>4 (19%)</td>
</tr>
<tr>
<td>Symptoms (%)</td>
<td>21 (40%)</td>
<td>12 (39%)</td>
<td>9 (43%)</td>
</tr>
<tr>
<td>Myalgia or fatigue</td>
<td>13 (25%)</td>
<td>8 (26%)</td>
<td>5 (24%)</td>
</tr>
<tr>
<td>Exertional dyspnea</td>
<td>11 (21%)</td>
<td>7 (23%)</td>
<td>4 (19%)</td>
</tr>
<tr>
<td>Cough</td>
<td>5 (10%)</td>
<td>4 (13%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Smell and taste dysfunction</td>
<td>5 (10%)</td>
<td>3 (10%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Abdominal pain and diarrhea</td>
<td>3 (6%)</td>
<td>1 (3%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Clinical treatments (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corticosteroid</td>
<td>11 (21%)</td>
<td>2 (6%)</td>
<td>9 (43%)</td>
</tr>
<tr>
<td>Antiviral drug&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52 (100%)</td>
<td>31 (100%)</td>
<td>21 (100%)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Antiviral drug: arbidol, lopinavir or ritonavir, and interferon alpha inhalation
Table 2. Comparison of chest CT findings between non-severe and severe COVID-19 survivors 6 months after discharge

<table>
<thead>
<tr>
<th>Chest CT findings</th>
<th>Total (n=47)</th>
<th>Non-severe (n = 29)</th>
<th>Severe (n = 18)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal (%)</td>
<td>11 (23.4%)</td>
<td>1 (3.4%)</td>
<td>10 (55.6%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fibrotic bands (%)</td>
<td>6 (12.8%)</td>
<td>0 (0%)</td>
<td>6 (33.3%)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

P-values of <0.05 indicate statistical significance.
Table 3. Serum SPD, RAGE, laminin, and vWF levels of COVID-19 survivors 6 months after discharge

<table>
<thead>
<tr>
<th>Group</th>
<th>Healthy (n=30)</th>
<th>COVID-19 survivors 6 months after discharge (n=52)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPD (pg/mL)</td>
<td>10,127.47±1,764.49</td>
<td>6,896.01±3,404.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RAGE (pg/mL)</td>
<td>879.16±323.10</td>
<td>797.97±235.85</td>
<td>0.195</td>
</tr>
<tr>
<td>Laminin (pg/mL)</td>
<td>948.61±344.19</td>
<td>4,019.71±1,413.41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>vWF (IU/mL)</td>
<td>1.43±0.31</td>
<td>1.43±0.38</td>
<td>0.914</td>
</tr>
</tbody>
</table>

P-values <0.05 indicate statistical significance.
Table S1. Comparison of pulmonary diffusion function between non-severe and severe COVID-19 survivors 6 months after discharge

<table>
<thead>
<tr>
<th>Pulmonary diffusion function</th>
<th>Total (n=51)</th>
<th>Non-severe (n = 31)</th>
<th>Severe (n = 20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (%)</td>
<td>34 (66.7%)</td>
<td>20 (64.5%)</td>
<td>14 (70%)</td>
<td>-</td>
</tr>
<tr>
<td>Abnormal (%)</td>
<td>17 (33.3%)</td>
<td>11 (35.5%)</td>
<td>6 (30%)</td>
<td>0.685</td>
</tr>
</tbody>
</table>

P-values <0.05 indicate statistical significance.
**Table S2.** Comparison of SPD, RAGE, laminin, and vWF among different age groups of COVID-19 survivors 6 months after discharge

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>18-44 (n=23)</th>
<th>45-59 (n=19)</th>
<th>≥60 (n=10)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPD</td>
<td>5,973.42±2,554.47</td>
<td>7,812.04±4,440.00</td>
<td>7,277.50±2,487.78</td>
<td>-</td>
</tr>
<tr>
<td>RAGE</td>
<td>824.97±255.68</td>
<td>757.96±215.03</td>
<td>811.88±239.76</td>
<td>-</td>
</tr>
<tr>
<td>laminin</td>
<td>3,703.26±1,161.56</td>
<td>4,010.53±1,287.96</td>
<td>4,765.00±1,957.40</td>
<td>*0.048</td>
</tr>
<tr>
<td>vWF</td>
<td>1.38±0.29</td>
<td>1.40±0.40</td>
<td>1.57±0.53</td>
<td>-</td>
</tr>
</tbody>
</table>

*Comparing the 18–44 year-old group with the ≥60-year-old group.

P-values <0.05 indicate statistical significance.
Table S3. Comparison of SPD, RAGE, laminin and vWF according to sex in COVID-19 survivors 6 months after discharge

<table>
<thead>
<tr>
<th>Sex</th>
<th>Male</th>
<th>Female</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=30)</td>
<td>(n=22)</td>
<td></td>
</tr>
<tr>
<td>SPD</td>
<td>7,035.00±3,678.36</td>
<td>6,706.48±3,065.39</td>
<td>0.735</td>
</tr>
<tr>
<td>RAGE</td>
<td>807.40±256.27</td>
<td>785.11±210.01</td>
<td>0.74</td>
</tr>
<tr>
<td>Laminin</td>
<td>3,679.17±1,183.00</td>
<td>4,484.10±1,589.78</td>
<td>0.041</td>
</tr>
<tr>
<td>vWF</td>
<td>1.45±0.40</td>
<td>1.39±0.37</td>
<td>0.54</td>
</tr>
</tbody>
</table>

P-values <0.05 indicate statistical significance.
**Table S4.** Comparison of SPD, RAGE, laminin and vWF between non-severe and severe COVID-19 survivors 6 months after discharge

<table>
<thead>
<tr>
<th>Type</th>
<th>Non-severe (n=31)</th>
<th>Severe (n=21)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPD</td>
<td>7,062.78±3,528.38</td>
<td>6,649.82±3,282.22</td>
<td>0.672</td>
</tr>
<tr>
<td>RAGE</td>
<td>836.25±258.59</td>
<td>741.46±189.52</td>
<td>0.134</td>
</tr>
<tr>
<td>Laminin</td>
<td>3,927.42±1,335.00</td>
<td>4,155.95±1,545.37</td>
<td>0.572</td>
</tr>
<tr>
<td>vWF</td>
<td>1.32±0.32</td>
<td>1.58±0.42</td>
<td>0.015</td>
</tr>
</tbody>
</table>

*P*-values <0.05 indicate statistical significance.
Table S5. Comparison of SPD, RAGE, laminin and vWF between asymptomatic and symptomatic COVID-19 survivors 6 months after discharge

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Asymptomatic* (n=34)</th>
<th>Symptomatic* (n=18)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPD</td>
<td>6,874.56±3,123.83</td>
<td>6,936.53±3,977.95</td>
<td>0.951</td>
</tr>
<tr>
<td>RAGE</td>
<td>786.07±242.68</td>
<td>820.45±227.46</td>
<td>0.622</td>
</tr>
<tr>
<td>Laminin</td>
<td>3,743.38±1,134.23</td>
<td>4,541.67±1,747.79</td>
<td>0.052</td>
</tr>
<tr>
<td>vWF</td>
<td>1.37±0.33</td>
<td>1.53±0.46</td>
<td>0.169</td>
</tr>
</tbody>
</table>

* Symptomatic patients with COVID-19 were defined as those who had self-reported myalgia, fatigue, or exertional dyspnea at 6 months following discharge, and the remaining patients were defined as asymptomatic.

P-values <0.05 indicate statistical significance.
Table S6. Comparison of serum SPD, RAGE, laminin and vWF between normal and abnormal pulmonary diffusion function of COVID-19 survivors 6 months after discharge

<table>
<thead>
<tr>
<th>Pulmonary diffusion function</th>
<th>Normal (n=34)</th>
<th>Abnormal (n=17)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPD</td>
<td>7,312.43±3,896.99</td>
<td>6,045.51±2,116.01</td>
<td>0.139</td>
</tr>
<tr>
<td>RAGE</td>
<td>826.97±231.45</td>
<td>780.55±255.85</td>
<td>0.517</td>
</tr>
<tr>
<td>Laminin</td>
<td>4,156.62±1,559.31</td>
<td>3,798.53±1,100.48</td>
<td>0.402</td>
</tr>
<tr>
<td>vWF</td>
<td>1.41±0.38</td>
<td>1.42±0.34</td>
<td>0.92</td>
</tr>
</tbody>
</table>

P-values <0.05 indicate statistical significance.
Table S7. Comparison of SPD, RAGE, laminin and vWF between different chest CT findings of COVID-19 survivors 6 months after discharge

<table>
<thead>
<tr>
<th>Chest CT findings</th>
<th>Normal (n=36)</th>
<th>Abnormal (n=11)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPD</td>
<td>6,799.38±3,349.87</td>
<td>6,318.98±1,929.10</td>
<td>0.654</td>
</tr>
<tr>
<td>RAGE</td>
<td>807.78±253.30</td>
<td>727.16±143.11</td>
<td>0.192</td>
</tr>
<tr>
<td>Laminin</td>
<td>3,979.17±1515.65</td>
<td>4,131.82±1,424.30</td>
<td>0.768</td>
</tr>
<tr>
<td>vWF</td>
<td>1.32±0.33</td>
<td>1.71±0.43</td>
<td>0.002</td>
</tr>
</tbody>
</table>

P-values < 0.05 indicates statistical significance.