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

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ABSTRACT

Objectives: The aim of this study was to evaluate the clinical characteristics, pulmonary diffusion function, chest computed 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 protein D (SP-D), the receptor for advanced glycation end products (RAGE), laminin, and von Willebrand factor (vWF) were measured in the 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 with non-severe disease and 21 with severe disease) 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 observation is required.

Author contributions

Hongwei Li, Qian Wu, Xinwei Hou, Linmin Zhang, Jing Guo, Yajie Li, Fangfei Yang, and Yan Zhang performed the 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 Huaiyong 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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1. Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a major global public health emergency. As of August 1, 2021, the COVID-19 pandemic has resulted in the infection of approximately 200 million people and has caused over 4 million deaths.

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 release of cell-specific proteins into the circulation, which can assess cell damage (Bhargava and Wendt, 2012)

Surfactant protein D (SP-D) is the main functional substance produced by alveolar type 2 (AT2) cells. The receptor for advanced glycation end products (RAGE) is mainly expressed on the basal surface of alveolar type 1 (AT1) 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 2 weeks after SARS-CoV-2 infection had been treated (Shao et al., 2020) To date, there have been few reports on the long-term changes in lung cell damage and repair 6 months after the treatment of SARS-CoV-2 infection.

In this study, the serum levels of SP-D, RAGE, laminin, and vWF were measured in COVID-19 survivors 6 months after discharge. By comparing clinical characteristics, pulmonary diffusion function, and chest computed 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.

2. Materials and methods

2.1. Participants

Patients with COVID-19 who were admitted to Tianjin Haihe 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 assigned to the severe group: dyspnea (respiratory rate $\geq$33 breaths/min), finger oxygen saturation $\leq$93% in the resting state, arterial blood partial pressure of oxygen or fraction of inspired oxygen $\leq$300 mmHg (1 mmHg = 0.133 kPa), and lung imaging showing that the lesion had progressed significantly by $>$50% within 24–48 hours. Patients who did not meet the above criteria were assigned to the non-severe group. Patients who died before follow-up, refused to participate in the follow-up, and those who left the local area and could not complete the follow-up were excluded.

Thirty age- and sex-matched health and 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, were negative for SARS-CoV-2 antibodies, and were 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.

2.2. Basic data collection

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

2.3. Pulmonary diffusion function tests

Pulmonary diffusion function tests were performed using a MasterScreen Body plethysmograph (Jaeger MS-PFT Analysis Unit; Jaeger, Würzburg, Germany). In accordance with 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.

2.4. 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 percentage of the lesions in a single lung lobe. A lung lobe without lesions was scored 0, while a lung lobe with a lesion area percentage of $<$25% was scored 1, $\geq$25% to $<$50% was scored 2, $\geq$50% to $<$75% was scored 3, and $\geq$75% was scored 4. 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 $\geq$1 indicated an abnormal chest CT and a score of 0 indicated a normal chest CT.

2.5. ELISA for serum SP-D, RAGE, laminin, and vWF

Serum levels of SP-D, RAGE, laminin, and vWF were measured by ELISA in enrolled healthy controls and COVID-19 survivors. All kits were purchased from Abcam: SP-D (Human Surfactant protein D/SP-D SimpleStep ELISA Kit, ab239431), RAGE (Human SimpleStep ELISA Kit, ab190807), laminin (Human Laminin ELISA Kit, ab119599), and vWF (Human Von Willebrand Factor ELISA Kit, ab108918).

2.6. Statistical analysis

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

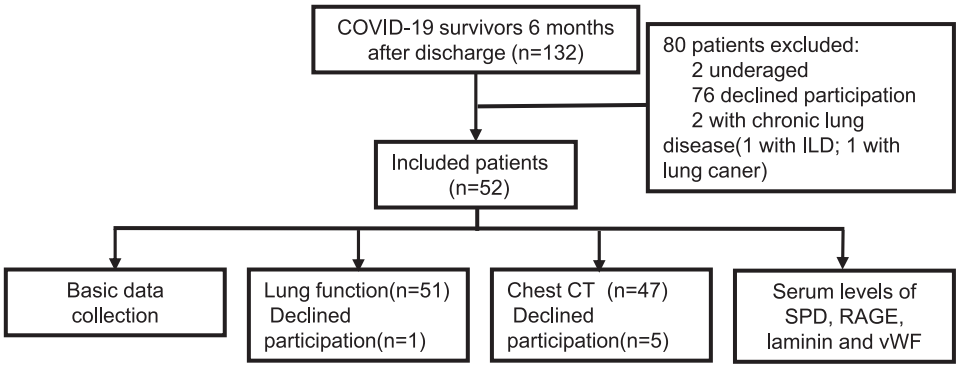

Figure 1. Study flow chart showing the inclusion of COVID-19 survivors 6 months after discharge . COVID-19, coronavirus disease 2019; CT, computed tomography; ILD, interstitial lung disease; RAGE, receptor for advanced glycation end products ; SP-D, surfactant protein D ; vWF, von Willebrand factor.

Table 1
Demographic and clinical characteristics of COVID-19 survivors 6 months after discharge

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

IQR, interquartile range.
[a] Antiviral drugs: arbidol, lopinavir, or ritonavir, and interferon alpha inhalation.

3. Results

3.1. 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 re-examination 6 months after discharge, and two patients with chronic lung disease (one with interstitial lung disease and one after undergoing lung cancer resection), the remaining 52 survivors were finally enrolled (Figure 1): 31 were assigned to the non-severe group and 21 to the severe group. The mean age of severe group COVID-19 survivors (54 ±14 years) was higher than that of non-severe group COVID-19 survivors (43 ± 15 years). Of the 52 COVID-19 patients enrolled, 16 had comorbidities (31%), and the proportion of patients with comorbidities was higher in the severe group (48%) than in 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 that in the patients 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 of the COVID-19 survivors had abnormal pulmonary diffusion function, including six in the severe COVID-19 group (30%) and 11 in the non-severe COVID-19 group (35.5%), although this was not significant (P = 0.685) (**Supplementary Material** Table S1).

Forty-seven patients underwent chest CT (five patients refused). Eleven patients presented with abnormal chest CT findings, including 10 in the severe COVID-19 group (55.6%) and one in the non-severe COVID-19 group (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 the non-severe COVID-19 patients (P = 0.001) (Table 2).

3.2. Serum SP-D, RAGE, laminin, and vWF Levels in COVID-19 survivors 6 months after discharge

In COVID-19 survivors 6 months after discharge, the mean serum laminin level was 4019.71 ± 1413.41 pg/ml, which was significantly higher than the mean level in the healthy controls (948.61 ± 344.19) (P < 0.001). The mean serum level of SP-D was 6896.01 ± 3404.37 pg/ml, which was lower than the mean level in the healthy control group (10 127.47 ± 1764.49) (P < 0.001). The mean serum RAGE level was 797.97 ± 235.85 pg/ml, which did not differ statistically from the mean level in the healthy control group (879.16 ± 323.10) (P = 0.195). The mean serum level of vWF was 1.43 ± 0.38 IU/ml, which was also not statistically different from the mean level in the healthy control group (1.43 ± 0.31) (P = 0.914) (Table 3).

Table 2
Comparison of chest CT findings between non-severe and severe COVID-19 survivors 6 months after discharge

Chest CT findings	Total(n = 47)	Non-severe(n = 29)	Severe(n = 18)	P-value
Abnormal (%)	11 (23.4%)	1 (3.4%)	10 (55.6%)	<0.001
Fibrotic bands (%)	6 (12.8%)	0 (0%)	6 (33.3%)	0.001

CT, computed tomography. P-values of <0.05 indicate statistical significance.

Table 3
Serum SP-D, RAGE, laminin, and vWF levels of COVID-19 survivors 6 months after discharge

Group	Healthy controls (n = 30)	COVID-19 survivors 6 months after discharge (n = 52)	P-value
SP-D (pg/ml)	10 127.47 ± 1764.49	6896.01 ± 3404.37	<0.001
RAGE (pg/ml)	879.16 ± 323.10	797.97 ± 235.85	0.195
Laminin (pg/ml)	948.61 ± 344.19	4019.71 ± 1413.41	<0.001
vWF (IU/ml)	1.43 ± 0.31	1.43 ± 0.38	0.914

SP-D, surfactant protein D; RAGE, receptor for advanced glycation products; vWF, von Willebrand factor. P-values <0.05 indicate statistical significance.

3.3. 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 female patients (P = 0.041) (Figure 2; **Supplementary Material** Tables S2 and S3). The variables disease severity type, presence of symptoms, pulmonary diffusion function, and chest CT findings had no effect on serum laminin levels (**Supplementary Material** Tables S4–S7).

3.4. Serum vWF levels according to different variables in COVID-19 survivors 6 months after discharge

The serum vWF level had decreased to normal in COVID-19 survivors 6 months after discharge. However, the serum vWF level was significantly higher in the severe group than in the non-severe group (P = 0.015) and in patients with abnormal chest CT findings than in those with normal findings (P = 0.002) (Figure 3). The variables age, sex, presence of symptoms, and pulmonary diffusion function had no effect on serum vWF levels (**Supplementary Material** Tables S2, S3, S5, and S6).

3.5. Serum SP-D and RAGE levels according to different variables in COVID-19 survivors 6 months after discharge

There were no differences in serum SP-D or RAGE levels among the groups with different ages, sexes, disease severities, symptoms, pulmonary diffusion function, or chest CT findings (**Supplementary Material** Tables S2–S7).

4. Discussion

COVID-19 is an infectious disease caused by SARS-CoV-2, which can lead to severe acute respiratory distress syndrome (ARDS), a 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

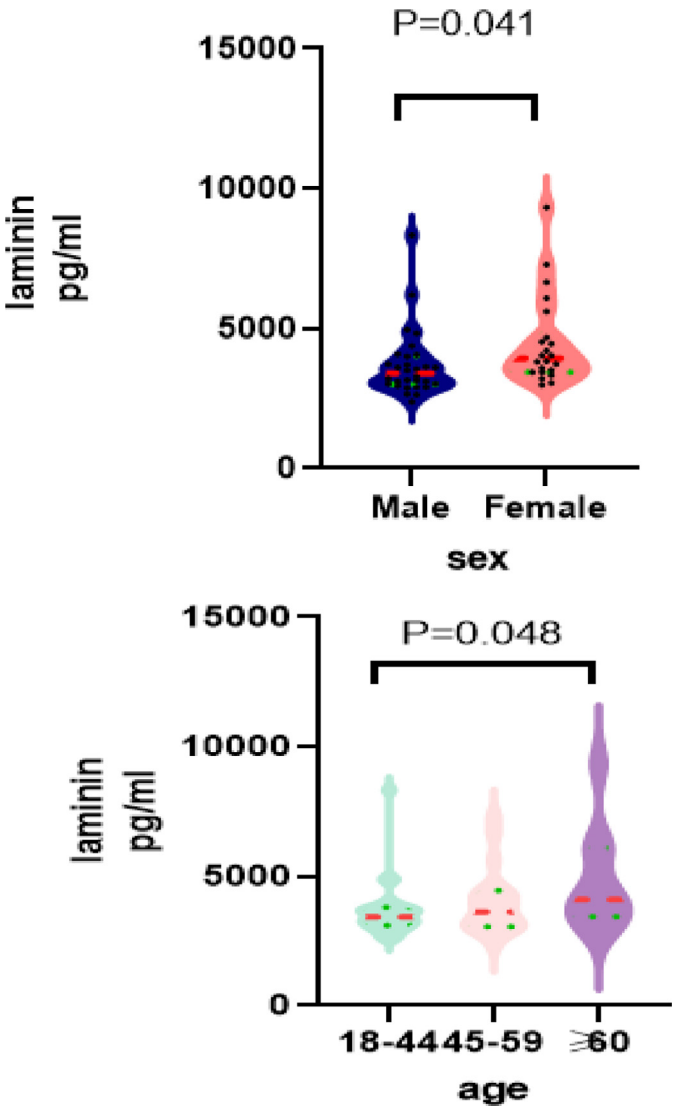

Figure 2. Serum levels of laminin according to different variables in COVID-19 survivors 6 months after discharge. (a) Comparison of serum levels of laminin between male and female COVID-19 survivors 6 months after discharge. (b) Comparison of serum levels of laminin between different age groups of COVID-19 survivors 6 months after discharge. A P-value <0.05 indicates statistical significance.

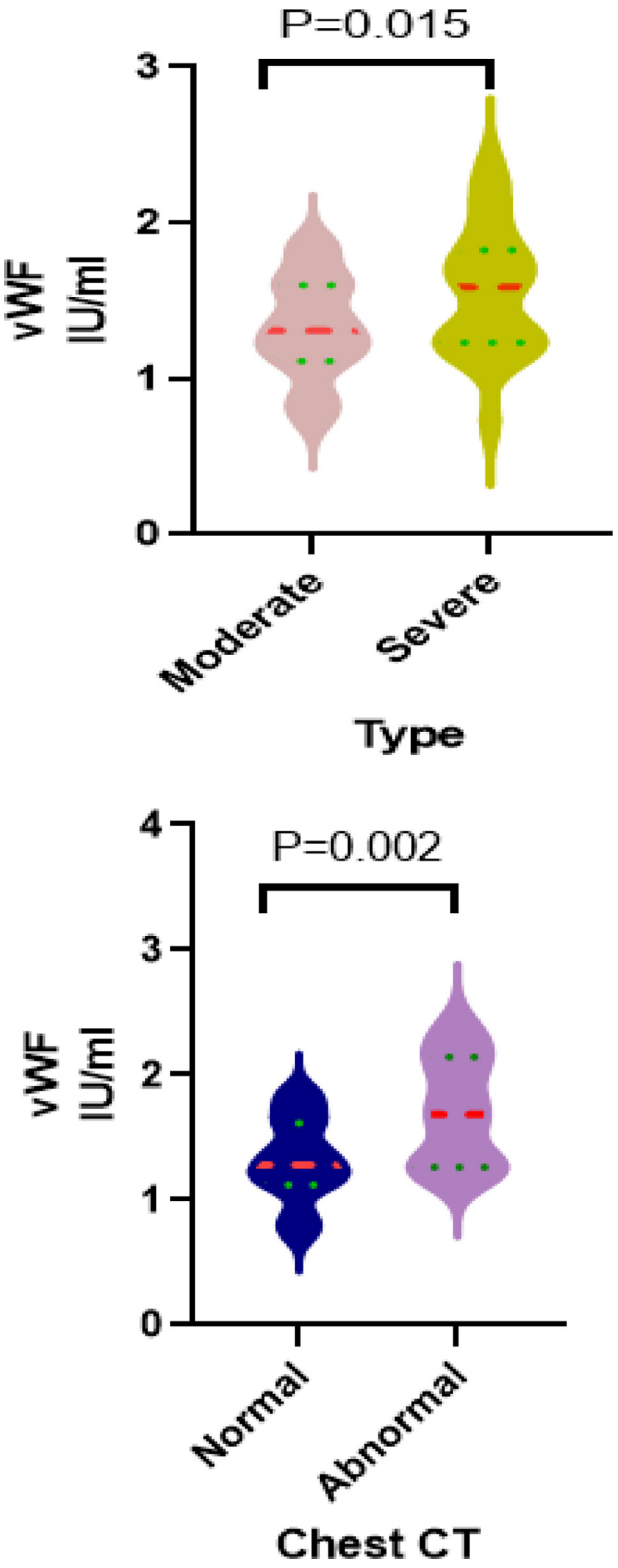

Figure 3. Serum levels of von Willebrand factor (vWF) according to different variables in COVID-19 survivors 6 months after discharge. (a) Comparison of serum levels of vWF between non-severe and severe COVID-19 survivors 6 months after discharge . (b) Comparison of serum levels of vWF between COVID-19 survivors 6 months after discharge with normal and abnormal chest CT findings. A *P*-value <0.05 indicates statistical significance.

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 evolution of COVID-19 lung histopathological lesions over time is similar to that of ARDS (Merdji et al., 2021). The histological characteristics include 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 expresses 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 (surfactant proteins, 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 these, 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 the 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. Due to the current limited data on the levels of lung damage markers in the serum of patients with COVID-19 during the recovery period, it is hypothesized 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). In the present study, an investigation of these four markers was conducted to evaluate the lung damage and recovery of COVID-19 survivors 6 months after discharge from hospital.

This research showed that serum RAGE levels in COVID-19 patients did not increase at 6 months of recovery. The previous literature has reported that during the exudative stage of ARDS, the basal surface of alveolar type I epithelial cells mainly expresses 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 such as nuclear factor kappa B (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 falls by the 14th day of the recovery period (Shao et al., 2020). In the present study it was found that serum RAGE levels had returned to normal after 6 months of recovery from COVID-19.

In the pathogenesis of COVID-19, SARS-CoV-2 binds to ACE2 and is highly expressed in alveolar and endothelial cells of the 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 into the blood, mediating platelet adhesion and aggregation (Roose and Joly, 2020). Meanwhile, vWF is also a biomarker of the intensity of the inflammatory response (Joly et al., 2021).

In a previous study (Shao et al., 2020), serum vWF levels were found to increase significantly in the acute infection stage of the disease, and this increase 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 starts to decrease in the early recovery period (Shao et al., 2020). In the present study, serum vWF levels had 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 had been discharged from the hospital, the plasma vWF level was still slightly elevated, but there was no statistical difference from the control group, consistent with the results of the present study.

However, in a study by Fogarty (Fogarty, 2021) including 50 patients 68 days after SARS-CoV-2 infection to evaluate endothelial cell marker changes, it was 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). In contrast, the present 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 6 months after recovery in our study was higher than the level found in the patients 68 days after discharge in the study by Fogarty.

The plasma vWF value was higher in patients with severe disease and in patients with abnormal chest CT findings in this study. A previous study found that chest CT abnormalities in patients with COVID-19 at 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 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 in those with an abnormal chest CT. This suggests that the influence of the disease on the severity of endothelial injury remained 6 months after discharge, and that endothelial injury in patients with severe COVID-19 was more serious. A larger, multi-center study is needed to further explore the change in vWF levels during the rehabilitation period. In addition, there are reports in the literature that plasma vWF levels in patients with COVID-19 are significantly increased in quantity and quality, and that the multifactorial function of ADAMTS13 is 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-19 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 that 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 development of pulmonary fibrosis (Lee et al., 2018).

Our study found that the serum laminin level of convalescent patients was significantly higher than the level in the normal population and patients with acute COVID-19. This study found that the serum laminin level increased 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 development of pulmonary fibrosis (Spagnolo et al., 2020). In view of these findings, it is suggested that older patients with COVID-19 are more likely to develop pulmonary fibrosis after recovery than the general population.

This 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% confidence interval 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 the diffusive lung dysfunction during rehabilitation is more significant in female patients, but female patients may not have persistent CT abnormalities. This result is consistent with our finding 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 serological markers mentioned above, 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 or 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 recov-

ery period from COVID-19 and found that all indicators of persistent respiratory disease were unrelated to the severity of the initial disease (Fogarty et al., 2021), which is consistent with the present research results.

A significant positive correlation between serum SP-D and the inflammatory response has been reported (Alay et al., 2021). A previous study (Shao et al., 2020) reported that the serum SP-D level increased in the acute phase of the disease course, and that serum SP-D decreased significantly 6 months after discharge, which may be related to the gradual reduction of the body's inflammatory response.

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

In conclusion, the results of this study suggest 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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Declarations

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

Conflict of 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.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2021.11.032.

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