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Original Article

Endothelial Biomarkers in Patients Recovered from COVID-19 One Year after Hospital Discharge: A Cross-Sectional Study

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Abstract. ***Background:*** **COVID-19 is characterized by endothelial dysfunction and is presumed to have long-term cardiovascular sequelae. In this cross-sectional study, we aimed to explore the serum levels of endothelial biomarkers in patients who recovered from COVID-19 one year after hospital discharge.**

Methods**: In this clinical follow-up study, 345 COVID-19 survivors from Huanggang, Hubei, and 119 age and gender-matched medical staff as healthy controls were enrolled. A standardized symptom questionnaire was performed, while electrocardiogram and Doppler ultrasound of lower extremities, routine blood tests, biochemical and immunological tests, serum soluble vascular cell adhesion molecule-1(VCAM-1), intercellular cell adhesion molecule-1(ICAM-1), P-selectin, and fractalkine were measured by enzyme-linked immunosorbent assays (ELISA).**

Results**: At one year after discharge, 39% of recovers possessed post-COVID syndromes, while a few had abnormal electrocardiogram manifestations, and no deep vein thrombosis was detected in all screened survivors. There were no significant differences in circulatory inflammatory markers (leukocytes, neutrophils, lymphocytes, C-reactive protein and interleukin-6), alanine aminotransferase, estimated glomerular filtration rate, glucose, triglycerides, total cholesterol and D-dimer observed among healthy controls with previously mild or severe infected. Furthermore, serum levels of VCAM-1, ICAM-1, P-selectin, and fractalkine do not significantly differ between survivors and healthy controls.**

Conclusions**: SARS-CoV-2 infection may not impose a higher risk of developing long-term cardiovascular events, even for those recovering from severe illness.**

Keywords: COVID-19; Endothelial biomarkers; VCAM-1; ICAM-1; P-Selectin; Fractalkine; 1-year follow-up;

Cardiovascular sequaele.

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Introduction. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the pathogen of coronavirus disease 2019 (COVID-19), is highly contagious and pathogenic and is responsible for more than 351 million infected and nearly 5.6 million deaths worldwide as of Jan 24, 2022.[1] In addition, persistent and diverse post-COVID symptoms have been described in survivors of COVID-19, including those with a mild initial disease course.[2] Therefore, more than 340 million survivors are at high risk for post-COVID syndrome worldwide.[3]

The available clinical evidence suggests that COVID-19, although damaging the respiratory system initially, is a systemic disease with extrapulmonary complications.[4] The cardiovascular system is one of the most involved systems,[5] while endotheliitis is a prominent feature of COVID-19,[6] thus is suggested to be responsible for life-threatening thrombogenesis and coagulopathy in those with severe illness.[7] During the acute phase of SARS-CoV-2 infection, cytokine storm and subsequent endothelial injury and thrombosis are involved in the pathogenesis of cardiovascular complications.[8] However, few studies have focused on the endothelial dysfunction in patients who recovered from COVID-19.

Endothelial cells play an essential role in maintaining vascular homeostasis, such as controlling inflammation, regulating platelet aggregation, and preventing thrombosis.[9] Dysfunction of endothelial cells has been identified as a central feature of COVID-19.[10] The abnormal elevation of soluble endothelial biomarkers, such as vascular cell adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1 (ICAM-1), P-selectin, and fractalkine, is closely related to the development of arteriosclerosis,[11] which is the underlying pathology of coronary artery disease,[12] peripheral artery disease,[13] and cerebrovascular disease[14] in most cases. Therefore, the severity of endothelial dysfunction is associated with increased cardiovascular risks, and it is of great significance to monitor endothelial biomarkers in patients recovering from COVID-19.

In this study, we investigated demographics, laboratory findings, symptoms, electrocardiogram manifestations, screened lower extremity thrombosis and measured serum endothelial biomarkers of participants one year after discharge, thus evaluating long-term cardiovascular risk in patients recovered from COVID-19.

Methods.

Study design and participants. From Mar 16 to Mar 28, 2021, 473 survivors of COVID-19, who had been previously hospitalized from Jan 24 to Mar 18, 2020, in Huanggang, Hubei, China, were recruited to this cross-sectional cohort study. The inclusion criteria were adults previously diagnosed with COVID-19 (positive in a reverse-transcription polymerase chain reaction for SARS-CoV-2), and the stratification of disease severity has been described in our published report.[15] Of these patients, 114 cases were excluded for diabetes, suffering from chronic systemic infection, malignant tumors or hematological and autoimmune diseases, pregnancy, chronic smoking (defined as 20 pack-years), long-term use of medications (angiotensin-converting enzyme inhibitors, angiotensin II type-1 receptor antagonists, corticosteroids or statins), and fourteen recovers did not show up for the follow-up appointment (**Figure 1**). As a result, 345 survivors were recruited into the study from Mar 1 to 30, 2021 from Mar 1 to May 30, 2021. During this time, 119 age and sex-matched healthy controls, medical personnel at Hunan Provincial People's Hospital, were recruited during the annual routine physical examination. All medical staff has repeatedly undergone throat swab screening to exclude SARS-CoV-2 infection every 1 to 2 weeks since the pandemic. Moreover, those who were pregnant, long-term using medications or chronic smoking, or suffering from diabetes, chronic systemic infection, malignant tumors or hematological and autoimmune diseases were excluded.

Collection of clinical data. According to the guidelines of the National Health Commission of China, in the cohort, survivors were divided into the mild group and the severe group according to the severity of the disease during previously acute infection, as described in our

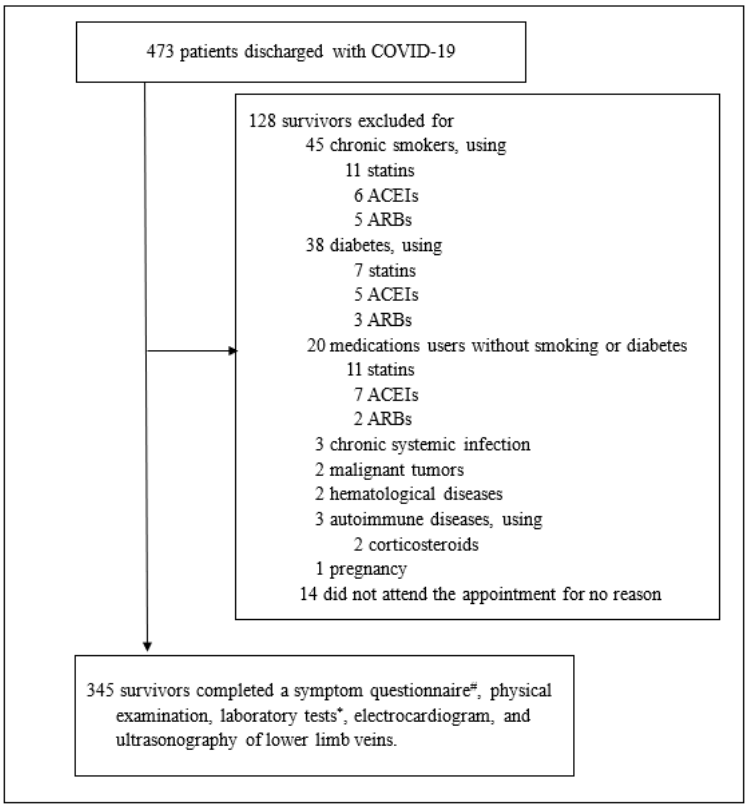

Figure 1. Flow chart of patients with COVID-19 discharged from Huanggang Hospitals between January 24 to March 18, 2020.
#A self-reported symptom questionnaire included Low-grade fever, Fatigue or muscle weakness, Palpitations, Chest tightness or shortness of breath, Dizziness. *Laboratory tests included routine blood tests, biochemical and immunologic tests, the plasma levels of CRP, IL-6, and D-dimer, and the serum levels of VCAM-1, ICAM-1, P-Selectin and fractalkine.
Abbreviations: ACEIs: angiotensin-converting enzyme inhibitors; ARBs: angiotensin II type-1 receptor antagonists.

previous report.[15] In addition, all the survivors were subjected to a standardized symptom questionnaire and received a physical examination, electrocardiogram, and ultrasonography of the lower extremities for detecting deep venous thrombosis. All data were collected and triple-checked by three physicians.

Sample collection and processing. Blood samples were taken from each participant by standard venipuncture in a fasting state on the day of appointments. Routine blood tests, biochemical and immunologic tests, and the plasma levels of C-reactive protein (CRP), interleukin (IL)-6, and D-dimer were measured by conventional laboratory methods. The serum for endothelial biomarker detection was isolated by centrifugation for 15 minutes at 1500×g and frozen at -80°C until thawed and analyzed.

This study has strictly followed the recommendations of the Helsinki Declaration. Therefore, the institutional review boards of the Medical Ethics Committee of the Hunan Provincial People's Hospital approved this study (NO.2021-92), and all participants signed informed consent.

Enzyme-linked immunosorbent assays for serum endothelial biomarkers measurement. Quantitative measurement of serum soluble VCAM-1, ICAM-1, P-Selectin and fractalkine was tested for survivors and healthy participants using 96-well enzyme-linked immunosorbent assay kits (Boster Biological Technology Co. Ltd, Wuhan, China). Quality control was carried out strictly following the manufacturer's instructions for each batch of tests.

Statistic analysis. Categorical variables were compared using $\chi 2$ analysis and expressed in numbers (proportions). Continuous variables with normal distribution were compared using independent group *t*-tests and expressed as mean ± standard deviation (SD), while those not normally distributed were compared using the Mann-Whitney *U* test and expressed as median and interquartile range (IQR) values. All statistical analyses were performed using the SPSS programme, V.19.0 (SPSS Inc., Chicago, IL, USA), and plots were generated using GraphPad Prism, version 8 (GraphPad Software, San Diego, CA). A two-sided *P*-value of < 0.05 was defined as statistically significant.

Results.

Clinical characteristics of the study participants. A total of 345 COVID-19 survivors (291 recovered from the mild situation and 54 from the severe) and 119 age and sex-matched healthy medical volunteers participated in this study. Demographic information and laboratory findings are shown in **Table 1**. In our cohort, 46 male

Table 1. Demographics and laboratory findings of participants.

	Overall(n=464)	Control(n=119)	Mild(n=291)	Severe(n=54)
Gender(n)				
Male	183(39)	46(39)	118(41)	19(35)
Female	281(61)	73(61)	173(59)	35(65)
Age(years)	53(43-62)	52(42-61)	53(46-62)	54(41-61)
Brachial artery pressure(mmHg)				
Systolic	125(113-136)	124(113-135)	125(113-138)	125(116-131)
Diastolic	77(71-85)	77(70-84)	77(71-85)	75(70-83)
Body mass index (Kg/m^2)[a]	24.0±2.5	24.0±3.7	24.1±1.9	23.6±2.2
Leukocytes ($\times10^9/L$)	5.70(4.80-6.60)	5.98(5.19-6.74)	5.60(4.70-6.50)	5.60(4.50-6.48)
Neutrophils($\times10^9/L$)	3.20(2.50-3.85)	3.38(2.71-4.12)	3.10(2.50-3.80)	3.00(2.48-3.83)
Lymphocytes($\times10^9/L$)	1.82(1.54-2.12)	2.01(1.67-2.27)	1.79(1.50-2.08)	1.79(1.44-2.07)
Hemoglobin($\times10^{12}/L$)	144(135-155)	139(131-152)	146(136-157)	145(137-156)
Alanine aminotransferase (U/L)	17.8(13.1-24.9)	20.1(13.7-25.1)	17.1(13.0-24.5)	18.9(12.8-25.0)
eGFR (mL/min)	88.6(73.1-105.0)	92.7(76.2-111.5)	87.9(71.3-102.4)	85.2(76.0-110.0)
D-dimer(mg/L)	0.20(0.12-0.30)	0.25(0.18-0.34)	0.20(0.10-0.30)	0.20(0.10-0.25)
Glucose (mmol/L)	5.26(4.75-5.62)	4.90(4.66-5.20)	5.40(4.87-5.74)	5.39(4.97-5.69)
Triglycerides (mmol/L)	0.98(0.79-1.27)	1.05(0.79-1.34)	0.96(0.80-1.23)	0.92(0.77-1.22)
Total cholesterol(mmol/L)	4.51(4.05-4.94)	4.56(3.95-5.03)	4.51(4.08-4.92)	4.50(4.12-4.86)
C-reactive protein(mg/L)	0.30(0.11-0.46)	0.32(0.16-0.48)	0.30(0.10-0.45)	0.37(0.10-0.45)
Interleukin-6(pg/mL)	2.90(1.00-5.10)	3.20(1.00-6.60)	2.60(1.00-4.60)	2.75(1.00-6.08)
Time from discharge to follow-up(days)[a]	375.0±11.0	NA	375.3±10.7	373.3±12.3

Values are expressed as Median (25, 75 percentile) or number (%) if not otherwise stated. [a]Mean±standard deviation (SD). Abbreviation: eGFR: Estimated glomerular filtration rate.

and 73 female medical volunteers were enrolled as healthy controls, while 118 male and 173 female survivors and 19 adult males and 35 females who recovered from mild and severe situations were recruited. The median ages in the control, mild, and severe groups were 52, 53, and 54 years, respectively, and the average visiting interval after discharge for the recovers was 375.0 days (SD, 11.0 days). No significant differences were found among the mild, severe and control participants in sex, age, systolic or diastolic brachial artery pressure, BMI, circulatory level of leukocytes, neutrophils, lymphocytes, hemoglobin, D-dimer, glucose, triglycerides (TG), total cholesterol (TC), alanine aminotransferase (ALT), C-reactive protein (CRP) and estimated glomerular filtration rate (eGFR). Furthermore, there were no significant differences in plasma interleukin-6 (IL-6) levels between the mild and severe groups. For all survivors, no venous thrombosis was observed by lower extremities ultrasound.

Post-COVID symptoms of participants. Regarding the post-COVID symptoms, a standardized symptom questionnaire was performed and presented in **Table 2**. In general, 135 (39%) recovered patients had persistent symptoms during the 1-year follow-up and no significant differences were found between the severe and mild groups. Among them, 4 (1%) subjects complained of persistent low-grade fever, and 128 (37%) recovers were troubled by fatigue or muscle weakness. For cardiovascular disease-related symptoms, 21 (6%) participants possessed persistent palpitations, 10 (3%) subjects had chest tightness or shortness of breath, and 8 (2%) complained of dizziness. In addition, there was no significant difference observed in those post-COVID symptoms between the mild and severe groups.

Electrocardiogram abnormalities in patients recovering from COVID-19. An electrocardiogram examination was performed for each patient who recovered from COVID-19 at a one-year follow-up. As presented in **Table 2**, the most frequent abnormalities are ST-T changes (16%) and sinus bradycardia (11%), the frequency of left ventricular high voltage (6%), sinus tachycardia (1%), prolonged PR interval (1%) and other abnormalities (such as ventricular premature contraction, atrial fibrillation, prolonged Q-T interval) is relatively small. Of those abnormalities, the frequency of prolonged PR interval seemed to be positively related to the previously infected disease severity (P=0.015), while there was no significant difference in the frequency of other abnormalities between the mild and severe groups.

Serum levels of endothelial biomarkers. Compared with the control group, the serum VCAM-1 levels showed no

Table 2. Symptoms, electrocardiogram manifestations and lower extremities Doppler ultrasound of recovers.

	Overall(n=345)	Mild(n=291)	Severe(n=54)
Symptons[n(%)]			
Anyone of the following symptoms	135(39)	117(40)	18(33)
Low-grade fever	4(1)	2(0.7)	2(4)
Fatigue or muscle weakness	128(37)	112(38)	16(30)
Palpitations	21(6)	20(7)	1(2)
Chest tightness or shortness of breath	10(3)	9(3)	1(2)
Dizziness	8(2)	6(2)	2(4)
Electrocardiogram abnormalities[n(%)]			
Sinus tachycardia	4(1)	4(1)	0(0)
Sinus bradycardia	39(11)	33(11)	6(11)
ST-T changes	56(16)	48((16)	8(15)
Left ventricular high voltage	19(6)	17(6)	2(4)
Prolonged PR interval	3(1)	1(0)	2(4)*
Other abnormalities	10(3)	9(3)	1(2)
Venous thrombosis of lower extremities	0(0)	0(0)	0(0)

Values are expressed as numbers (%). *P=0.015, compared with the mild group.

significant differences in patients who recovered from mild (median, 1.69 *vs* 1.67 ng/mL, P=0.363) or severe (median, 1.69 *vs* 1.67 ng/mL, P=0.962) situation, in line with that between mild and severe recovers (P=0.553) (**Figure 2A**). Although not significant, serum ICAM-1 levels were lower in the mild group than in controls (median, 427.3 *vs* 469.7 pg / mL, P = 0.139) and the severe group than in the control (median, 424.6 *vs* 469.7 pg/mL, P=0.789) or in the mild groups (median, 424.6 *vs* 427.3 pg / mL, P = 0.444) (**Figure 2B**). Similarly, serum P-selectin levels were lower in the mild group than in controls (median, 965.6 vs 1076.6 pg / ml, P = 0.296) and in the severe group than in the control (median, 960.4 *vs* 1076.6 pg / ml, P = 0.104) or mild groups (median, 960.4 *vs* 965.6 pg / ml, P = 0.260) (**Figure 2C**). Regarding fractalkine, the mild group showed lower serum levels than the control (mean values, 204.5±24.0 *vs* 206.8±25.0 pg/mL, P=0.378) and the severe groups (mean values, 204.5±24.0 *vs* 206.8±19.0 pg/mL, P=0.493), and there were no significant differences between the control and the severe groups (P=0.992) (**Figure 2D**).

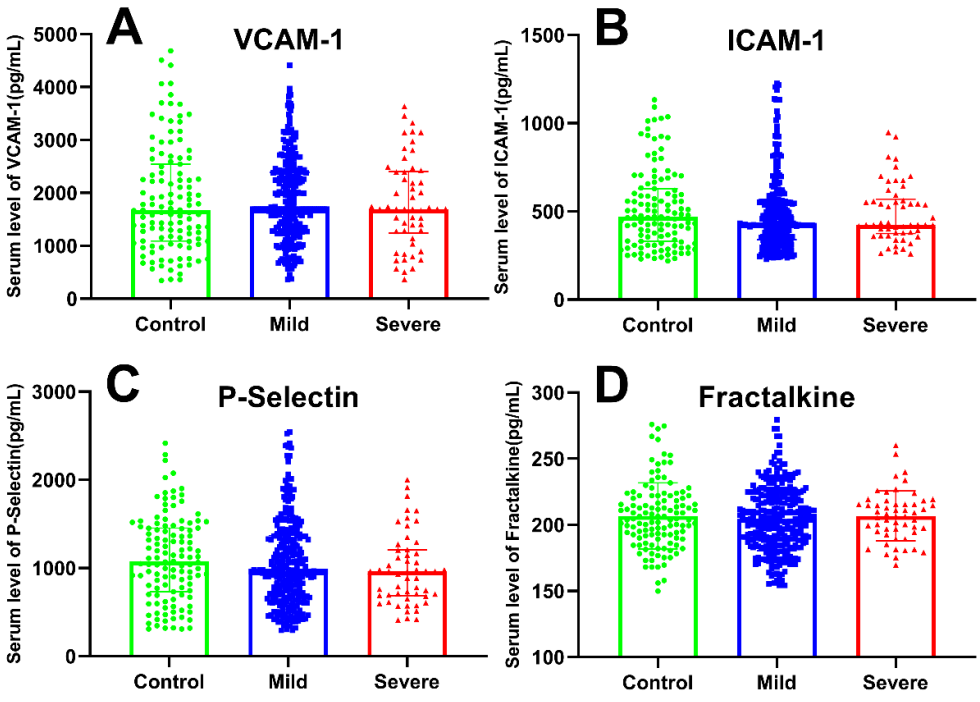

Figure 2. Serum levels of vascular cell adhesion molecule-1(VCAM-1), intercellular cell adhesion molecule-1(ICAM-1), P-selectin, and fractalkine in controls and COVID-19 recovers of previously mild or severe infected.

Discussion.

Principal Findings of Our Study. This study is the first to report endothelial biomarkers of patients who recovered from COVID-19 one year after discharge. In our cohort, a considerable number of survivors are still bothered by post-COVID symptoms. Secondly, inflammatory markers of the normal range, including neutrophils, CRP, and IL-6, indicate the remission of inflammatory reactions in those survivors. In addition, significant elevated D-dimer levels and deep vein thrombosis were absent in all screened survivors, suggesting a relatively low risk of coagulopathy in the long term. Furthermore, the levels of circulating endothelial biomarkers, including VCAM-1, ICAM-1, P-selectin and fractalkine, do not show significant differences in those survivors and healthy controls, implying that SARS-CoV-2 infection may not impose a higher risk of the development of long-term cardiovascular events, even for those recovering from severe illness.

Comparison with related studies. As previously observed in the SARS epidemic,[16] recovered patients have persistent symptoms and unexpected higher rates of diabetes, respiratory and cardiovascular disease, named the post-COVID syndrome after SARS-CoV-2 infection.[3,17] Until now, few clinical studies have focused on cardiovascular sequelae in the aftermath of COVID-19. In a 3-month follow-up study, myocardium injury was detectable in 30% of recovered COVID-19 patients by cardiac magnetic resonance (CMR),[18] while in a cohort of twenty-six patients who recovered from COVID-19 who reported cardiac symptoms and underwent CMR examinations, fifteen (58%) of them had abnormal CMR findings, including myocardial edema, fibrosis, and impaired right ventricle function.[19] In another study of a cohort of 100 German patients who recently recovered from COVID-19 infection, CMR imaging revealed cardiac involvement in 78 patients (78%) and ongoing myocardial inflammation in 60 patients (60%).[20] The prevalence of cardiovascular complications is alarmingly in those studies, indicating the existence of the short to medium-term cardiovascular consequences of COVID-19. However, a reasonable explanation is that the appearance of new or persistent symptoms in the cohorts could increase the positive CMR detection rate, implying that some of these patients are not genuine 'convalescent patients'. However, inconsistent with the studies above, in a single-center longitudinal study, 13% of COVID-19 survivors experienced significant cardiovascular symptoms three months after discharge, including an increase in resting heart rate, occasional palpitations, and newly diagnosed hypertension requiring blood pressure-lowering medications.[21] At the same time, in an observational prospective multicentre trial 60 and 100 days after confirmed diagnosis, cardiac impairment, including reduced left ventricular function or signs of pulmonary hypertension, was present only in a minority of subjects.[22] Moreover, in another preliminary 6-month follow-up study, no survivor reported any obvious cardiopulmonary symptoms, although 29.6% (8/27) of them were detected cardiac injury by CMR.[23] These findings provide the contradictory prevalence of SARS-CoV-2 infection in short- or medium-term cardiovascular sequelae, and most of the conclusions are descriptive and imaging-based, lacking objective biomarkers and long-term follow-up data.

A large number of studies have suggested that endothelial function reflects the comprehensive influence of various risk factors on the vascular system,[24] and endothelial dysfunction is an early predictor of subclinical atherosclerosis[25] and subsequent long-term cardiovascular events.[26] Therefore, early detection of soluble endothelial biomarkers contributes to early detection of disease, quantification of risk, and early intervention to reduce the incidence of cardiovascular adverse events in patients.[27] Both indirect induction by hypercytokinemia (e.g., IL-1, IL -6 and tumor necrosis factor-alpha), hyperchemokinemia and coagulopathy (named after a high-inflammatory response),[28] and direct damage to endothelial cells by SARS-CoV-2 infection contributed to endothelial injuries in patients with COVID-19,[6,29] thus improving the expression of endothelial biomarkers, including ICAM-1, VCAM-1, P-selectin, and fractalkine.[15,30,36] However, few studies have focused on the alterations of endothelial biomarkers and cytokines in patients recovering from COVID-19. In a prospective longitudinal multicenter cohort study, regulators of endothelial activation such as vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF), and macrophage inflammatory protein-1β (MIP-1β) were persistently elevated in convalescence patients with COVID-19, potentially promoting the development of atherosclerosis and cardiovascular sequelae.[31] In another 3-month follow-up study, persistent abnormal levels of endothelial biomarkers, pro-inflammatory cytokines and chemokines (VCAM-1, ICAM-1, TNF-α, MIP-1α, and MIP-1β) were observed in those recovered from COVID-19, especially in severe/critical patients.[32] Therefore, by describing the post-COVID symptoms and abnormal ECG changes, detection of both lower extremities thrombosis, and measuring the circulatory levels of inflammatory factors and endothelial biomarkers, our study may provide a more comprehensive cardiovascular perspective for COVID-19 recovers one year after discharge.

In our cohort, the influence of various confounders, such as older age, pregnancy, chronic smoking, preexisting conditions (malignancy, diabetes mellitus, hyperlipidemia, obesity), and current medications was strictly excluded, thus may not reflect the overall

situation of cardiovascular sequelae in COVID-19 recovers with a preexisting higher risk of endothelial dysfunction. In the study, relatively low levels of circulating endothelial biomarkers at one-year follow-up for those survivors without preexisting endothelial dysfunction risks were observed, although injuries to endothelium cells are believed to have long-lasting effects.[33] Several mechanisms were speculated to be majorly ascribed to the remission of endothelial biomarkers. First, the clearance of SARS-CoV-2 infection in all recruited patients, confirmed by repeated screening after discharge, is conducive to the remission of endothelial biomarkers. Second, with the clearance of viral infection, the levels of inflammatory markers (such as neutrophils, CRP, and IL-6) returned to normal, and a coordinated and dynamic immune response, characterized by reduced inflammation, was developed.[34] The indirect mechanism of endothelial dysfunction induced primarily by high inflammatory responses could be interrupted. Third, the activation of endothelial cells leads to a procoagulant phenotype, which in turn continuously activates endothelial injuries during the acute infection phase.[35] In addition to the normal ranged D-dimer, deep venous thrombosis of lower extremities was excluded by ultrasonography in our cohort, consistently with the previous study,[17] indicating the termination of this vicious cycle that sustained activating endothelial injuries. Therefore, our study could help to explain why COVID-19 survivors no longer need to endure the risk of long-term thrombosis at the level of endothelial phenotype.

Conclusions. Our findings are encouraging, in light of the endothelial dysfunction is involved in the pathogenesis of venous thromboembolism and vasculitis in patients with COVID-19 during the acute phase, thus arousing widespread concern for cardiovascular sequelae in long-term. In our cohort of COVID-19 survivors one year after discharge, significantly higher levels of endothelial biomarkers and higher risk of deep vein thromboembolism in the lower extremities were absent, although the longer-term risk of cardiovascular disease development remains to be elucidated.

Limitations. Limitations should be noted before interpreting the results of this study. First, due to the inaccessibility of the samples from COVID-19 patients during the acute phase, the lack of comparative longitudinal data makes it impossible to dynamically observe the changes of endothelial biomarkers and electrocardiogram, which may affect the causal inference of the SARS-CoV-2 infection and the incidence of cardiovascular events, as well as the accuracy of the conclusions in this cross-sectional study. Second, parameters that more comprehensively reflect vascular function (such as the vascular stiffness index and the intima/media thickness ratio) may better predict future cardiovascular events. However, due to the convenience of the equipment, the failure to combine these parameters with endothelial biomarkers is one major limitation in our study. Third, additional mechanistic work is required to understand better the potential role of the adaptive immune response in the recovery process of endothelial biomarkers and inflammatory markers in patients with COVID-19.

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Data availability. The datasets used and/or analyzed are provided in this paper. Any other raw data supporting the findings of this study are available from the corresponding authors upon reasonable request.

Authors' Contributions. YMZ, FC, and YZ conceptualized and designed the studies. ZXJ, SJZ, LHZ, GQC, RFG, ZLZ, XTH, JMH, SQY, and CCM collected the clinical data. MT, YJ, and YJL performed an ELISA. YMZ put forward the outline of the article with XQY and WX. MT, QZ, and XQY performed data analysis and drew pictures. MT and YJ drafted the manuscript, YMZ, XQY, and FC revised the article, and all authors read and approved the final version.

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References:

1. Johns Hopkins University. Coronavirus Resource Center. https://coronavirus.jhu.edu/map.html Accessed Oct 30 2021.
2. Song WJ, Hui CKM, Hull JH, et al. Confronting COVID-19-associated cough and the post-COVID syndrome: role of viral neurotropism, neuroinflammation, and neuroimmune responses. The Lancet Respiratory medicine 2021; 9:533-44. https://doi.org/10.1016/S2213-2600(21)00125-9
3. Ayoubkhani D, Khunti K, Nafilyan V, et al. Post-covid syndrome in individuals admitted to hospital with covid-19: retrospective cohort study. BMJ (Clinical research ed) 2021; 372:n693. https://doi.org/10.1136/bmj.n693 PMid:33789877 PMCid:PMC8010267
4. Gupta A, Madhavan MV, Sehgal K, et al. Extrapulmonary manifestations of COVID-19. Nature medicine 2020; 26:1017-32. https://doi.org/10.1038/s41591-020-0968-3

PMid:32651579
5. Zheng YY, Ma YT, Zhang JY, Xie X. COVID-19 and the cardiovascular system. Nature reviews Cardiology 2020; 17:259-60. https://doi.org/10.1038/s41569-020-0360-5 PMid:32139904 PMCid:PMC7095524
6. Ackermann M, Verleden SE, Kuehnel M, et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. The New England journal of medicine 2020; 383:120-8. https://doi.org/10.1056/NEJMoa2015432 PMid:32437596 PMCid:PMC7412750
7. Gu SX, Tyagi T, Jain K, et al. Thrombocytopathy and endotheliopathy: crucial contributors to COVID-19 thromboinflammation. Nature reviews Cardiology 2021; 18:194-209. https://doi.org/10.1038/s41569-020-00469-1 PMid:33214651 PMCid:PMC7675396
8. Dou Q, Wei X, Zhou K, Yang S, Jia P. Cardiovascular Manifestations and Mechanisms in Patients with COVID-19. Trends in endocrinology and metabolism: TEM 2020; 31:893-904. https://doi.org/10.1016/j.tem.2020.10.001 PMid:33172748 PMCid:PMC7566786
9. Li X, Sun X, Carmeliet P. Hallmarks of Endothelial Cell Metabolism in Health and Disease. Cell Metab 2019; 30:414-33. https://doi.org/10.1016/j.cmet.2019.08.011 PMid:31484054
10. Libby P, Lüscher T. COVID-19 is, in the end, an endothelial disease. European heart journal 2020; 41:3038-44. https://doi.org/10.1093/eurheartj/ehaa623 PMid:32882706 PMCid:PMC7470753
11. Caligiuri G. CD31 as a Therapeutic Target in Atherosclerosis. Circ Res 2020; 126:1178-89. https://doi.org/10.1161/CIRCRESAHA.120.315935 PMid:32324506
12. Ford TJ, Ong P, Sechtem U, et al. Assessment of Vascular Dysfunction in Patients Without Obstructive Coronary Artery Disease: Why, How, and When. JACC Cardiovascular interventions 2020; 13:1847-64. https://doi.org/10.1016/j.jcin.2020.05.052 PMid:32819476 PMCid:PMC7447977
13. Polonsky TS, McDermott MM. Lower Extremity Peripheral Artery Disease Without Chronic Limb-Threatening Ischemia: A Review. Jama 2021; 325:2188-98. https://doi.org/10.1001/jama.2021.2126 PMid:34061140
14. Blevins BL, Vinters HV, Love S, et al. Brain arteriolosclerosis. Acta neuropathologica 2021; 141:1-24. https://doi.org/10.1007/s00401-020-02235-6 PMid:33098484 PMCid:PMC8503820
15. Tong M, Jiang Y, Xia D, et al. Elevated Expression of Serum Endothelial Cell Adhesion Molecules in COVID-19 Patients. The Journal of infectious diseases 2020; 222:894-8. https://doi.org/10.1093/infdis/jiaa349 PMid:32582936 PMCid:PMC7337874
16. Zhang P, Li J, Liu H, et al. Long-term bone and lung consequences associated with hospital-acquired severe acute respiratory syndrome: a 15-year follow-up from a prospective cohort study. Bone research 2020; 8:8. https://doi.org/10.1038/s41413-020-0084-5 PMid:32128276 PMCid:PMC7018717
17. Huang C, Huang L, Wang Y, et al. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. Lancet 2021; 397:220-32. https://doi.org/10.1016/S0140-6736(20)32656-8
18. Wang H, Li R, Zhou Z, et al. Cardiac involvement in COVID-19 patients: mid-term follow up by cardiovascular magnetic resonance. Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance 2021; 23:14. https://doi.org/10.1186/s12968-021-00710-x PMid:33627143 PMCid:PMC7904320
19. Huang L, Zhao P, Tang D, et al. Cardiac Involvement in Patients Recovered From COVID-2019 Identified Using Magnetic Resonance Imaging. JACC Cardiovascular imaging 2020; 13:2330-9. https://doi.org/10.1016/j.jcmg.2020.05.004 PMid:32763118 PMCid:PMC7214335
20. Puntmann VO, Carerj ML, Wieters I, et al. Outcomes of Cardiovascular Magnetic Resonance Imaging in Patients Recently Recovered From Coronavirus Disease 2019 (COVID-19). JAMA cardiology 2020; 5:1265-73. https://doi.org/10.1001/jamacardio.2020.3557 PMid:32730619 PMCid:PMC7385689
21. Xiong Q, Xu M, Li J, et al. Clinical sequelae of COVID-19 survivors in Wuhan, China: a single-centre longitudinal study. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 2021; 27:89-95. https://doi.org/10.1016/j.cmi.2020.09.023 PMid:32979574 PMCid:PMC7510771
22. Sonnweber T, Sahanic S, Pizzini A, et al. Cardiopulmonary recovery after COVID-19: an observational prospective multicentre trial. The European respiratory journal 2021; 57.
23. Wu X, Deng KQ, Li C, et al. Cardiac Involvement in Recovered Patients From COVID-19: A Preliminary 6-Month Follow-Up Study. Frontiers in cardiovascular medicine 2021; 8:654405. https://doi.org/10.3389/fcvm.2021.654405 PMid:34055936 PMCid:PMC8155269
24. Münzel T, Sinning C, Post F, Warnholtz A, Schulz E. Pathophysiology, diagnosis and prognostic implications of endothelial dysfunction. Annals of medicine 2008; 40:180-96. https://doi.org/10.1080/07853890701854702 PMid:18382884
25. Celermajer DS, Sorensen KE, Gooch VM, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 1992; 340:1111-5. https://doi.org/10.1016/0140-6736(92)93147-F
26. Kitta Y, Obata JE, Nakamura T, et al. Persistent impairment of endothelial vasomotor function has a negative impact on outcome in patients with coronary artery disease. J Am Coll Cardiol 2009; 53:323-30. https://doi.org/10.1016/j.jacc.2008.08.074 PMid:19161880
27. Koga H, Sugiyama S, Kugiyama K, et al. Elevated levels of VE-cadherin-positive endothelial microparticles in patients with type 2 diabetes mellitus and coronary artery disease. J Am Coll Cardiol 2005; 45:1622-30. https://doi.org/10.1016/j.jacc.2005.02.047 PMid:15893178
28. Perico L, Benigni A, Casiraghi F, Ng LFP, Renia L, Remuzzi G. Immunity, endothelial injury and complement-induced coagulopathy in COVID-19. Nature reviews Nephrology 2021; 17:46-64. https://doi.org/10.1038/s41581-020-00357-4 PMid:33077917 PMCid:PMC7570423
29. Varga Z, Flammer AJ, Steiger P, et al. Endothelial cell infection and endotheliitis in COVID-19. Lancet 2020; 395:1417-8. https://doi.org/10.1016/S0140-6736(20)30937-5
30. Goshua G, Pine AB, Meizlish ML, et al. Endotheliopathy in COVID-19-associated coagulopathy: evidence from a single-centre, cross-sectional study. The Lancet Haematology 2020; 7:e575-e82. https://doi.org/10.1016/S2352-3026(20)30216-7
31. Ong SWX, Fong SW, Young BE, et al. Persistent Symptoms and Association With Inflammatory Cytokine Signatures in Recovered Coronavirus Disease 2019 Patients. Open forum infectious diseases 2021; 8:ofab156. https://doi.org/10.1093/ofid/ofab156 PMid:34095336 PMCid:PMC8083585
32. Zhou M, Yin Z, Xu J, et al. Inflammatory profiles and clinical features of COVID-19 survivors three months after discharge in Wuhan, China. The Journal of infectious diseases 2021.
33. Daiber A, Steven S, Weber A, et al. Targeting vascular (endothelial) dysfunction. British journal of pharmacology 2017; 174:1591-619. https://doi.org/10.1111/bph.13517 PMid:27187006 PMCid:PMC5446575
34. Kared H, Redd AD, Bloch EM, et al. SARS-CoV-2-specific CD8+ T cell responses in convalescent COVID-19 individuals. The Journal of clinical investigation 2021; 131. https://doi.org/10.1172/JCI145476 PMid:33427749 PMCid:PMC7919723
35. Jin Y, Ji W, Yang H, Chen S, Zhang W, Duan G. Endothelial activation and dysfunction in COVID-19: from basic mechanisms to potential therapeutic approaches. Signal transduction and targeted therapy 2020; 5:293. https://doi.org/10.1038/s41392-020-00454-7 PMid:33361764 PMCid:PMC7758411
36. Agrati C., Bordoni V., Sacchi A., Petrosillo N., Nicastri E., Del Nonno F., D'Offizi G., Palmieri F., Marchioni L., Capobianchi M.R., Antinori A., Ippolito G., Bibas M.Elevated P-Selectinin severe Covid-19: considerations for therapeutic options.Mediterr J Hematol Infect Dis 2021, 13(1): e2021016 https://doi.org/10.4084/mjhid.2021.016 PMid:33747397 PMCid:PMC7938922