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RESEARCH ARTICLE

Association of COVID-19 with impaired endothelial glycocalyx, vascular function and myocardial deformation 4 months after infection

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Aims SARS-CoV-2 infection may lead to endothelial and vascular dysfunction. We investigated alterations of arterial stiffness, endothelial coronary and myocardial function markers 4 months after COVID-19 infection.

Methods and results In a case-control prospective study, we included 70 patients 4 months after COVID-19 infection, 70 age- and sex-matched untreated hypertensive patients (positive control) and 70 healthy individuals. We measured (i) perfused boundary region (PBR) of the sublingual arterial microvessels (increased PBR indicates reduced endothelial glycocalyx thickness), (ii) flow-mediated dilatation (FMD), (iii) coronary flow reserve (CFR) by Doppler echocardiography, (iv) pulse wave velocity (PWV), (v) global left and right ventricular longitudinal strain (GLS), and (vi) malondialdehyde (MDA), an oxidative stress marker, thrombomodulin and von Willebrand factor as endothelial biomarkers. COVID-19 patients had similar CFR and FMD as hypertensives (2.48 ± 0.41 vs. 2.58 ± 0.88, $P = 0.562$, and $5.86 \pm 2.82\%$ vs. $5.80 \pm 2.07\%$, $P = 0.872$, respectively) but lower values than controls (3.42 ± 0.65, $P = 0.0135$, and $9.06 \pm 2.11\%$, $P = 0.002$, respectively). Compared to controls, both COVID-19 and hypertensives had greater PBR5–25 (2.07 ± 0.15 μm and 2.07 ± 0.26 μm, $P = 0.8$ vs. 1.89 ± 0.17 μm, $P = 0.001$), higher PWV (carotid–femoral PWV 12.09 ± 2.50 vs. 11.92 ± 2.94, $P = 0.7$ vs. 10.04 ± 1.80 m/s, $P = 0.036$) and impaired left and right ventricular GLS ($-19.50 \pm 2.56\%$ vs. $-19.23 \pm 2.67\%$, $P = 0.864$ vs. $-21.98 \pm 1.51\%$, $P = 0.020$ and $-16.99 \pm 3.17\%$ vs. $-18.63 \pm 3.20\%$, $P = 0.002$ vs. $-20.51 \pm 2.28\%$, $P < 0.001$). MDA and thrombomodulin were higher in COVID-19

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patients than both hypertensives and controls (10.67 ± 0.32 vs 1.76 ± 0.03, $P = 0.003$ vs. 1.01 ± 0.05 nmol/L, $P = 0.001$ and 3716.63 ± 188.36 vs. 3114.46 ± 179.18 pg/mL, $P = 0.017$ vs. 2590.02 ± 156.51 pg/mL, $P < 0.001$). Residual cardiovascular symptoms at 4 months were associated with oxidative stress and endothelial dysfunction markers.

Conclusions SARS-CoV-2 may cause endothelial and vascular dysfunction linked to impaired cardiac performance 4 months after infection.

Keywords COVID-19 infection • Endothelial glycocalyx • Arterial stiffness • Oxidative stress • Myocardial deformation • Heart failure

Introduction

Coronavirus disease 19 (COVID-19), a newly recognized infectious disease with a rapid spread worldwide, is caused by a novel enveloped RNA beta-coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1,2] Despite the growing number of publications regarding the epidemiological and clinical characteristics of SARS-CoV-2,[3] the underlying pathophysiological mechanisms of the disease remain unclear.[4] Although COVID-19 infection primarily affects the respiratory system, numerous patients display manifestations from the cardiovascular system including acute myocardial injury, arrythmias and myocarditis.[5]

According to research evidence, SARS-CoV-2 affects the cardiovascular system through multiple mechanisms. Firstly, *in vitro* studies showed that angiotensin-converting enzyme 2 (ACE2) receptors, which are expressed in various human tissues, are the main targets for SARS-CoV-2.[6] Therefore, this raises the possibility that the virus may directly damage endothelial and cardiac cells. Secondly, the overproduction of proinflammatory cytokines during disease progression is possibly associated with endothelial derangement and myocardial injury.[7–9]

Recent guidelines suggest that the evaluation of endothelial biomarkers may contribute to the risk stratification of COVID-19 patients.[10]

Carotid–femoral pulse wave velocity (cfPWV) and central blood pressure are reliable markers of aortic elastic properties and have been suggested as valuable prognostic markers for cardiovascular events.[11] Glycocalyx damage, impaired artery flow-mediated dilatation (FMD) and coronary flow reserve (CFR) as well as measurement of circulating thrombomodulin and von Willebrand factor (vWF) may represent early manifestations of endothelial dysfunction.[12–14] Global left (LV) and right ventricular (RV) global longitudinal strain (GLS) permit early detection of subclinical myocardial deformation.[15] These markers are impaired in many cardiometabolic diseases, which share chronic inflammation and oxidative stress as common pathophysiological background such as atherosclerosis, hypertension and diabetes mellitus.[16–18]

In the present study, we hypothesized that (i) infection by SARS-CoV-2 increases arterial stiffness and impairs endothelial integrity and coronary and cardiac performance, and (ii) COVID-19 patients may have similarities in endothelial, vascular and cardiac function with hypertensive patients. The main objective of our study was to investigate the early differences in PWV, endothelial glycocalyx thickness, FMD, LV and RV myocardial strain, CFR and in biomarkers of oxidative stress and endothelial function, 4 months after COVID-19 infection. Hypertensive patients and healthy individuals served as control groups.

Methods

Study population

This prospective, observational, case-control study was conducted from September 2020 to January 2021 in the Laboratory of Preventive Cardiology in University General Hospital 'Attikon'. We consecutively recruited 70 patients who were examined in a dedicated post-COVID-19 outpatient clinic during a scheduled follow-up visit 4 months after a confirmed infection by SARS-CoV-2. We have chosen to examine our patients 4 months after COVID-19 infection as in our previous studies we observed significant alterations in cardiovascular and endothelial function after modification or persistence of atherosclerotic risk factors after 4 months of follow-up.[19] The patients were divided into three groups according to the latest National Institutes of Health (NIH) severity criteria: mild, moderate, severe.[20] Furthermore, we recorded the presence of post-infection symptoms related to the cardiovascular system (dyspnoea, fatigue, cough, chest pain) 4 months post-infection. Inclusion criteria for the study were age > 18 years old and a recent diagnosis of SARS-CoV-2 proven by polymerase chain reaction. Exclusion criteria were age < 18 years old, pregnancy, obstructive coronary disease, dyslipidaemia, diabetes mellitus and diagnosed hypertension under treatment. Seventy newly diagnosed and untreated patients with hypertension of similar age and sex to the COVID-19 patients served as positive control. Arterial hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg during at least three measurements at clinic visits.[21] Furthermore, 70 individuals of similar age, sex to the COVID-19 patients that visited the outpatient clinics of our hospital for check-up served as control group. This study was approved by the institutional ethical board of University General Hospital 'Attikon'. All participants signed an informed consent prior to any procedure included in the study protocol. All methods were conducted according to relevant guidelines and regulations (Declaration of Helsinki).

Study design

In all patients we recorded age, sex, comorbidities, and concomitant medications. All subjects were studied in the morning, having abstained from alcohol, caffeine and food for 8 h prior to the study; all vasoactive

medications were withheld for 48 h before the study. The operators who performed the vascular and cardiac measurements were blinded to patient history.

Blood pressure measurement

Prior to the study procedures, each patient rested in a supine position for 10 min in a quiet room. For the evaluation of the brachial blood pressure and the heart rate we used an automated digital oscillometric sphygmomanometer (TensioMed, Budapest, Hungary). In each patient, blood pressure was measured three times with an interval of 2 min. The operators who performed the examination were blinded to patient history. We used the mean value for statistical analysis.

Endothelial function

Endothelial glycocalyx. The perfused boundary region (PBR) of the sublingual arterial microvessels with diameter ranging 5–25 μm was assessed using Sidestream Darkfield (SDF) imaging (Microscan, Glycocheck, Microvascular Health Solutions Inc., Salt Lake City, UT, USA). The PBR represents the depth of penetration of red blood cells into endothelial glycocalyx. Higher PBR values are associated with increased penetration of red blood cells into the endothelium indicating an impaired endothelial glycocalyx.[22] The evaluation of endothelial glycocalyx using SDF imaging is a non-invasive, reproducible technique which lasts 3 min and provides recording and automated analysis of >3000 sublingual microvessel segments.[22] Therefore, the European Society of Cardiology Working Group on Peripheral Circulation suggests that the aforementioned technique is valid for the assessment of endothelial function.[12] The operators who performed the examination were blinded to patient history.

Brachial artery flow-mediated vasodilatation. In all patients we assessed the endothelium-dependent FMD in the right brachial artery. We obtained images of the brachial artery and we recorded a resting scan with the use of a Doppler ultrasound system (Vivid E95, GE Medical Systems, Horten, Norway). Afterwards, we inflated a blood pressure cuff on the forearm at a pressure of 200 mmHg for 5 min and subsequently we deflated it, causing reactive hyperaemia. Images form the brachial artery were obtained continuously 30 s before and 90 s after cuff deflation. Artery diameter measurements were performed using electronic calipers from the anterior to the posterior m-line. FMD was calculated as the percentage increase in arterial diameter during hyperaemia as compared with the resting scan.[23] The operators who performed the assessment of FMD were blinded to patient history.

Central haemodynamics-arterial stiffness

Carotid–femoral PWV, central aortic pressures (systolic and diastolic) and central pulse pressure (PP) were measured using tonometry by Complior (Alam Medical, Vincennes, France). Carotid–femoral PWV is calculated as the quotient of the pulse transit time and the distance travelled between the two recording sites [PWV (m/s) = travel distance (m)/transit time (s)]. Normal values were PWV <10 m/s.[21] Using pulse wave analysis, the central SBP, DBP and PP were calculated. The operators who performed the examination were blinded to patient history.

Echocardiography

Studies were performed using a Vivid E95 (GE Medical Systems, Horten, Norway) ultrasound system. All studies were digitally stored in a computerized station (EchoPac GE 203, Horten, Norway) and analysed by two observers, who had no access to clinical and laboratory data. The operators who performed the echocardiography were blinded to patient history.

Coronary flow reserve measurement. Coronary flow velocities in the left anterior descending coronary artery were obtained with colour-guided pulse-wave Doppler from long-axis apical projections with a 7 MHz transducer. The maximal velocity of the diastolic component of the coronary flow wave was measured at baseline and after adenosine infusion (140 mg/kg/min) for 3 min. CFR was calculated as the ratio of hyperaemic to resting maximal flow velocity. Measurements from three cardiac cycles were averaged. Inter- and intra-observer variability were calculated as the standard deviation (SD) of the differences between the first and second measurements and expressed as a percentage of the average value.

Assessment of the left ventricle. In all patients we measured LV GLS from standard two-dimensional echocardiography images (frame rate of 70–80/s), from the apical four-, two-, and three-chamber views using a 17 LV segment model. The normal value for GLS is $-22.5 \pm 2.7\%$.[24]

Assessment of the right ventricle. The RV GLS and RV free wall strain (RV FWS) was calculated using an RV focused apical four-chamber view (>50 frames/s).[25] RV GLS and FWS values less than −17% and −19%, respectively, are considered abnormal. Tricuspid annular plane systolic excursion (TAPSE) is measured using two-dimensional M-mode recordings from the apical four-chamber view (normal values are considered greater than 16 mm).[25] The systolic (S′) wave velocity of the tricuspid annulus (RV S') was obtained by tissue Doppler imaging (normal reference range of values is 13.3 ± 2.5 cm/s).

Biomarkers of endothelial function

We quantified in all participants the circulating levels of vWF and thrombomodulin to assess endothelial function. vWF was measured by ELISA using a commercial kit [Abcam, Human von Willebrand Factor ELISA Kit (ab223864); range: 469–30 000 ng/mL]. Thrombomodulin was quantified by ELISA using a commercial kit [Abcam, Human Thrombomodulin ELISA Kit (ab214029); range: 20.3–1300 pg/mL].[14]

Oxidative stress assessment

For the assessment of oxidative stress, we measured malondialdehyde (MDA) spectrophotometrically with a commercial kit (Oxford Biomedical Research, Rochester Hills, MI, USA) of colorimetric assay for lipid peroxidation (measurement range: 1–20 nmol/L).

Statistical analysis

Data are presented as mean ± SD/binary variables were compared using the χ^2 test. The normal distribution of the examined variable was assessed using Kolmogorov–Smirnov test. Variables with non-normal distributions were log transformed for analysis. Continuous variables were compared with full factorial ANOVA and the respective F- and *P*-values of the model are presented. Post hoc analysis using Bonferroni correction was used for comparisons between COVID-19, hypertensive and normal control groups or COVID-19 patients with mild, moderate and severe disease at admission. The interaction terms between the variable of the three study groups and the other

clinical co-variables (smoking, brachial blood pressure, heart rate and disease severity and symptoms) were also examined. The associations between measured markers were assessed using Pearson correlation and the respective correlation coefficient (r) and *P*-values are reported. A *P*-value <0.05 was considered statistically significant.

Results

Study population

The general characteristics of the study population are summarized in *Table 1*. Twenty-four patients (34.28%) were diagnosed with mild disease and were not subsequently hospitalized at any time of the course of the disease. Furthermore, 23 (32.85%) patients were diagnosed to have moderate and 23 (32.85%) severe disease at initial clinical assessment and thus were admitted to hospital. None of the examined patients required mechanical ventilation and none required hospitalization for a period of more than 15 days. Twenty-six patients (37.87%) presented with post-infection symptoms 4 months after COVID-19 disease. Among the symptoms, fatigue was the most common and it was present in 11 patients (15.71%), followed by dyspnoea in 9 (12.8%) cough in 3 (4.3%) and chest pain in 3 (4.3%). There was no significant difference among patients with or without post-infection symptoms regarding clinical characteristics (age, body mass index; data not shown). The mean age of participants was 54.59 ± 8.85 years and there was no significant difference among the three groups regarding age ($P = 0.991$) and sex (44 males per group, $P = 1$).

Blood pressure

By ANOVA, brachial SBP and DBP were different among the three study groups ($F = 9.51$, $P < 0.001$ and $F = 8.11$, $P = 0.001$, respectively). More specifically, hypertensives had higher brachial SBP compared with both COVID-19 patients and control group (145.27 ± 20.61 mmHg vs. 129.70 ± 12.78 mmHg, $P = 0.001$ vs. 126.60 ± 18.93 mmHg, $P < 0.001$, respectively). However, COVID-19 patients had similar brachial SBP as the control group ($P = 0.501$). Hypertensives showed increased DBP compared with COVID-19 patients and the control group ($P < 0.05$) (*Table 1*). However, COVID-19 patients and the control group had similar DBP ($P = 0.386$). Heart rate was also similar among the three groups ($P = 0.685$). No significant interaction was found between disease severity (mild, moderate, severe) and examined blood pressure markers or heart rate in the three study subgroups ($P > 0.05$).

Endothelial and coronary function

By ANOVA, FMD values were found different among the three study groups ($F = 8.71$, $P = 0.001$). More specifically, COVID-19 patients and hypertensives had similar FMD (5.86 ± 2.82% vs. 5.80 ± 2.07%, $P = 0.872$) while both groups had lower FMD values than the control group (9.06 ± 2.11%, $P = 0.002$ and $P = 0.002$, respectively) (*Figure 1A*).

Likewise, a similar trend to that of FMD was observed for PBR5–25 among the three study groups (PBR5–25: $F = 7.70$, $P = 0.001$; PBR5–9: $F = 1.44$, $P = 0.241$; PBR10–19: $F = 5.54$, $P = 0.005$; PBR20–25: $F = 8.78$, $P < 0.001$). COVID-19 patients and hypertensives had similar PBR values but both groups had greater PBR values than the control group (PBR5–25: 2.07 ± 0.15 μm vs. 2.07 ± 0.26 μm, $P = 0.8$ vs. 1.89 ± 0.17 μm, $P = 0.001$ for all comparisons) (*Table 2* and *Figure 1B*).

Finally, we observed that CFR values were also different among the three study groups ($F = 7.82$, $P = 0.001$). More precisely, hypertensives and COVID-19 patients had similar CFR (2.48 ± 0.41 vs. 2.58 ± 0.58, $P = 0.562$) whereas both groups had lower CFR values compared with the control group (3.42 ± 0.65, $P = 0.013$ and $P = 0.032$, respectively). No significant interaction was found between disease severity (mild, moderate, severe) and examined markers (FMD, PBR and CFR) in the three study subgroups ($P > 0.05$) (*Figure 1C*).

Arterial stiffness

By ANOVA, central PP, cfPWV and central SBP (*Table 2*) were found different among the three groups ($F = 3.10$, $P = 0.050$; $F = 4.23$, $P = 0.040$ and $F = 6.20$, $P = 0.003$, respectively).

Specifically, COVID-19 patients and hypertensives had similar arterial stiffness markers (cfPWV 12.09 ± 2.50 m/s vs. 11.92 ± 2.94, $P = 0.7$, $P > 0.05$ for all comparisons for central SBP and PP).

Table 1 General characteristics of the study population

	All participants ($n = 210$)	COVID-19 patients ($n = 70$)	Hypertensives ($n = 70$)	Control group ($n = 70$)	*P*-value
Age (years)	54.59 ± 8.85	54.53 ± 9.07	54.47 ± 8.83	54.77 ± 8.95	0.991
Male sex	132 (62.85)	44 (62.85)	44 (62.85)	44 (62.85)	1.000
Current smoking	55 (26.19)	16 (22.85)	18 (25.71)	21(30)	0.738
SBP (mmHg)	133.86 ± 19.39	129.70 ± 12.78*	145.27 ± 20.61**	126.60 ± 18.93	<0.001
DBP (mmHg)	82.48 ± 11.91	78.17 ± 7.99*	89.88 ± 10.29**	80.70 ± 14.01	0.001
HR (bpm)	76.29 ± 10.18	73.38 ± 10.89	78.45 ± 9.06	75.14 ± 10.24	0.685

Data are presented as mean ± standard deviation, or *n* (%). Continuous variables were compared with factorial ANOVA. Binary variables were compared with χ^2 test.
DBP, diastolic blood pressure; HR, heart rate; SBP, systolic blood pressure.
*$P < 0.05$, obtained by post hoc analysis between COVID-19 patients and hypertensives.
**$P < 0.05$, obtained by post hoc analysis between hypertensives and control group. Significant differences at $P < 0.05$ level were not observed for comparisons of COVID-19 patients and control group using post hoc analysis.

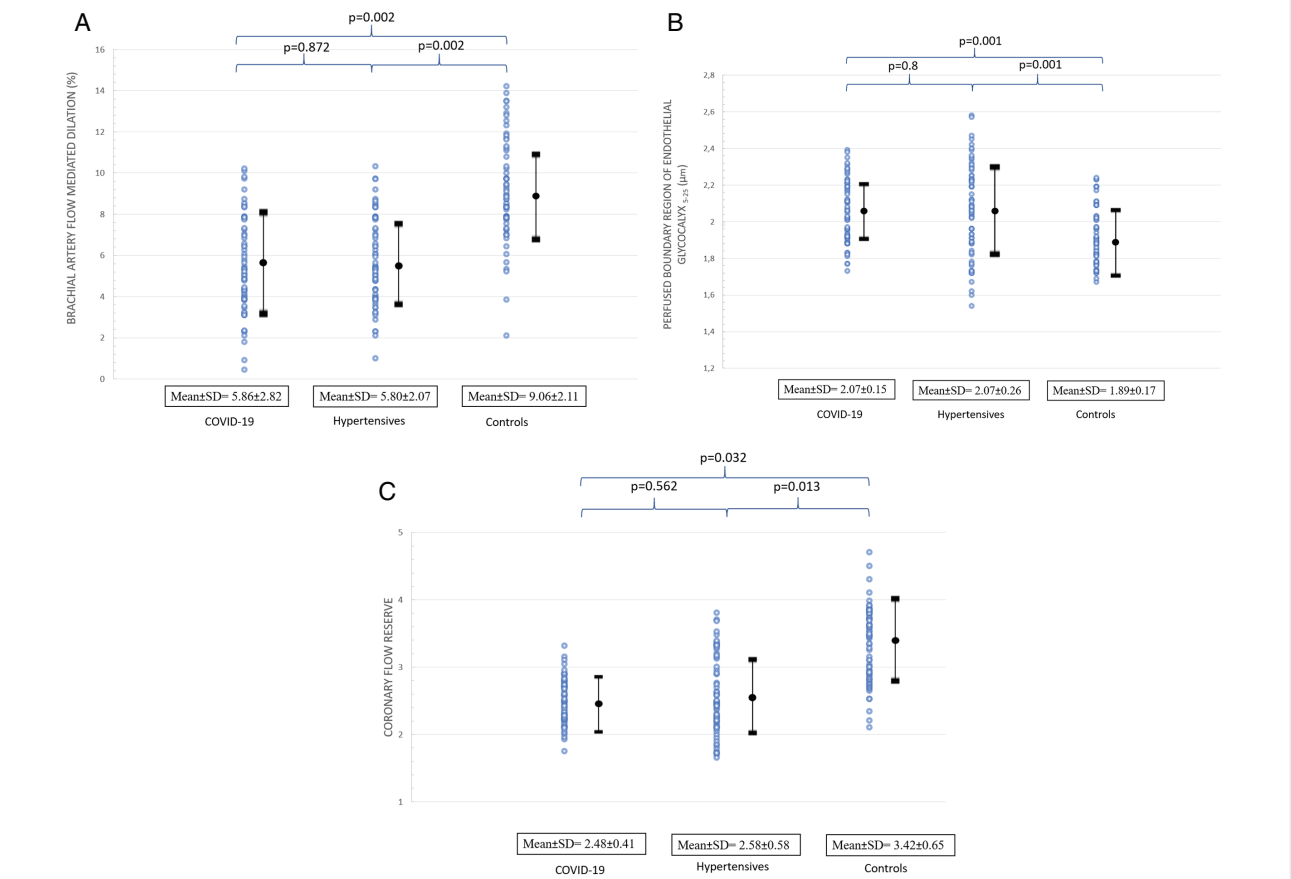

Figure 1 Scatterplot representing values of (*A*) brachial artery flow-mediated vasodilatation, (*B*) perfused boundary region endothelial glycocalyx$_{5-25}$, and (*C*) coronary flow reserve in COVID-19 patients compared with with hypertensives and the control group. Black mark and black lines represent mean ± standard deviation (SD).

COVID-19 patients and hypertensives had higher values of cfPWV and central SBP compared with the control group (cfPWV 12.09 ± 2.50 m/s and 11.92 ± 2.94 m/s, respectively, vs. 10.04 ± 1.80 m/s, $P = 0.036$ and $P = 0.045$, respectively; *Table 2*). No significant interaction was found between disease severity (mild, moderate, severe) and examined markers (central PP, cfPWV and central SBP) in the three study subgroups ($P > 0.05$).

Cardiac function

By ANOVA, there were differences of LV GLS values among the three study groups (F = 5.14, $P = 0.006$). COVID-19 patients had similar LV GLS values with hypertensives but significantly different (less negative) from the control group (−19.55 ± 2.56% vs. −19.23 ± 2.67%, $P = 0.864$ vs. −21.98 ± 1.51%, $P = 0.020$). Interestingly, we observed that 51/70 (72.50%) individuals in the COVID-19 group had LV GLS value above the cut-off value of −20% which is considered as normal (*Figure 2A*).

By ANOVA, there were differences of RV GLS and RV FWS values among the three study groups (F = 27.35, $P < 0.001$ and F = 25.44, $P < 0.001$, respectively). COVID-19 patients presented more deteriorated RV GLS than hypertensives and controls (−16.99 ± 3.17% vs. −18.63 ± 3.20%, $P = 0.002$ vs. −20.51 ± 2.28%, $P < 0.001$) (*Figure 2B*). Also, RV FWS was more impaired in COVID-19 patients compared with hypertensives and controls (−19.34 ± 4.41% vs. −21.70 ± 4.71%, $P = 0.002$ vs. −24.44 ± 2.90%, $P < 0.001$) (*Figure 2C*). Finally, TAPSE and RV S′ were lower in COVID-19 patients compared to both hypertensives and controls ($P < 0.05$) (*Table 2*).

No significant interaction was found between disease severity (mild, moderate, severe) and LV GLS, RV GLS and RV FWS in the three study subgroups ($P > 0.05$).

Oxidative stress

By ANOVA, there were significant differences between the three study groups in blood levels of MDA (F = 9.6, $P = 0.001$). Specifically, COVID-19 patients displayed much higher MDA levels than both hypertensives and healthy individuals (10.67 ± 0.32 vs. 1.76 ± 0.03 vs. 1.01 ± 0.05 nmol/L, $P = 0.003$ and $P = 0.001$, respectively). No significant interaction was found between disease severity (mild, moderate, severe) and MDA in the three study subgroups ($P > 0.05$) (*Figure 3A*).

Table 2 Markers of cardiac and vascular function

	All participants ($n = 210$)	COVID-19 patients ($n = 70$)	Hypertensives ($n = 70$)	Control group ($n = 70$)	F-value	*P*-value
CFR	2.82 ± 0.64	2.48 ± 0.41*	2.58 ± 0.58**	3.42 ± 0.65	7.82	0.001
FMD (%)	6.90 ± 2.54	5.86 ± 2.82*	5.80 ± 2.07**	9.06 ± 2.11	8.71	0.000
LV GLS (%)	−20.42 ± 2.24	−19.55 ± 2.56*	−19.23 ± 2.67**	−21.98 ± 1.51	5.14	0.006
RV GLS (%)	−18.68 ± 3.22	−16.99 ± 3.17	−18.63 ± 3.20	−20.51 ± 2.28	27,35	<0.001
RV FWS	−21.79 ± 4.54	−19.34 ± 4.41	−21.70 ± 4.71	−24.44 ± 2.90	25,44	<0.001
TAPSE	19.93 ± 3.98	17.43 ± 4.14	20.60 ± 3.20	21.90 ± 3.22	8.59	0.001
RV S'	12.85 ± 2.06	11.86 ± 1.82	13.70 ± 2.31	13.05 ± 1.63	4.74	0.012
PBR5−25 (μm)	2.01 ± 0.21	2.07 ± 0.15*	2.07 ± 0.26**	1.89 ± 0.17	7.70	0.001
PBR5-9 (μm)	1.12 ± 0.09	1.11 ± 0.08	1.15 ± 0.10	1.12 ± 0.10	1.44	0.241
PBR10−19 (μm)	2.17 ± 0.26	2.25 ± 0.21*	2.22 ± 0.30**	2.04 ± 0.19	5.54	0.005
PBR20−25 (μm)	2.48 ± 0.31	2.58 ± 0.25*	2.60 ± 0.36**	2.26 ± 0.33	8.78	<0.001
Central PP (mmHg)	45.32 ± 14.59	50.26 ± 14.37*	43.83 ± 12.85	41.14 ± 15.17	3.10	0.050
cfPWV (m/s)	11.35 ± 2.52	12.09 ± 2.50*	11.92 ± 2.94**	10.04 ± 1.80	4.23	0.040
Central SBP (mmHg)	126.91 ± 18.85	128.43 ± 17.39*	135.17 ± 16.83**	117.89 ± 18.85	6.20	0.003
MDA (nmol/L)	4.48 ± 0.075	10.67 ± 0.32*,***	1.76 ± 0.03**	1.01 ± 0.05	9.60	0.001
Thrombomodulin (pg/mL)	3237.73 ± 116.70	3716.63 ± 188.36*,***	3114.46 ± 179.18**	2590.02 ± 156.51	9.34	<0.001
vWF (ng/mL)	3445.14 ± 319.81	4018.03 ± 474.31*	3756.65 ± 293.28**	2079.33 ± 855.10	3.28	0.043

Data are expressed as mean ± standard deviation. MDA, thrombomodulin, and vWF are expressed as mean ± standard error. The variables were compared with factorial ANOVA and the respective F and *P*-values are presented.

cfPWV, carotid–femoral pulse wave velocity; CFR, coronary flow reserve; FMD, flow-mediated dilatation; FWS, free wall strain; GLS, global longitudinal strain; LV, left ventricular; MDA, malondialdehyde; PBR5−25, PBR10−19, PBR20−25, perfused boundary region of the sublingual vessels with diameter 5−25 μm,10−19 μm, 20−25 μm, respectively; PP, pulse pressure; RV, right ventricular; S', systolic wave velocity of the tricuspid annulus; SBP, systolic blood pressure; TAPSE, tricuspid annular plane systolic excursion; vWF, von Willebrand factor.

*$P < 0.05$, obtained by post hoc analysis between COVID-19 patients and control group.

**$P < 0.05$, obtained by post hoc analysis between hypertensives and control group.

***$P < 0.05$, obtained by post hoc analysis between COVID-19 patients and hypertensives.

Biomarkers of endothelial dysfunction

By ANOVA, there were significant differences between the three study groups in plasma levels of vWF and soluble thrombomodulin ($F = 9.34$, $P < 0.001$ and $F = 3.28$, $P = 0.043$, respectively). More notably, COVID-19 patients presented higher thrombomodulin levels compared to both hypertensives and normal controls (3716.63 ± 188.36 vs. 3114.46 ± 179.18 pg/mL, $P = 0.017$ vs. 2590.02 ± 156.51 pg/mL, $P < 0.001$). No significant interaction was found between disease severity (mild, moderate, severe) and thrombomodulin in the three study subgroups ($P > 0.05$) (*Figure 3B*).

Also, COVID-19 patients displayed similar vWF values as hypertensives but higher compared with healthy controls (4018.03 ± 474.31 vs. 3756.65 ± 293.28 vs. 2079.33 ± 855.10 ng/mL, $P = 0.718$ and $P = 0.016$, respectively). No significant interaction was found between disease severity and vWF in the three study subgroups ($P > 0.05$) (*Figure 3C*).

Association between post-infection symptoms and vascular and endothelial function

COVID-19 patients who presented with any cardiovascular post-infection symptoms (dyspnoea, fatigue, cough, or chest pain) had higher values of PBR5−25, thrombomodulin and MDA and lower FMD than patients without symptoms (PBR5−25:2.06 ± 0.24 μm vs. 1.90 ± 0.31 μm, $P = 0.045$; FMD: 5.99 ± 2.43% vs. 4.99 ± 5.14%, $P = 0.036$; thrombomodulin: 4083.36 ± 397.88 vs. 3195.36 ± 402.64 pg/mL, $P = 0.035$; MDA: 12.02 ± 0.51 vs. 10.85 ± 0.31 nmol/L, $P = 0.045$). Additionally, patients that presented with fatigue 4 months after COVID-19 infection had more impaired values of LV GLS, PWV and MDA compared to those without this symptom (LV GLS: −19.20 ± 1.93% vs. −20.24 ± 3.15%, $P = 0.036$; cfPWV: 12.27 ± 2.95 vs. 11.28 ± 3.11 m/s, $P = 0.032$; MDA: 12.06 ± 0.53 vs. 11.23 ± 0.03 nmol/L, $P = 0.028$). Finally, patients suffering of dyspnoea 4 months post-infection had more impaired RV GLS values than those without this symptom (−15.72 ± 2.08% vs. −16.41 ± 2.52%, $P = 0.042$).

Associations between vascular and cardiac function in the COVID-19 group

In the COVID-19 group, increased PBR5−25 was corelated positively with increased central SBP and PP ($r = 0.480$, $P = 0.007$ and $r = 0.410$, $P = 0.024$, respectively). In turn, elevated central SBP and PP were related with more impaired LV GLS values ($r = 0.554$, $P = 0.003$ and $r = 0.566$, $P = 0.002$, respectively). Additionally, in the COVID-19 group, MDA was associated with both PBR5−25

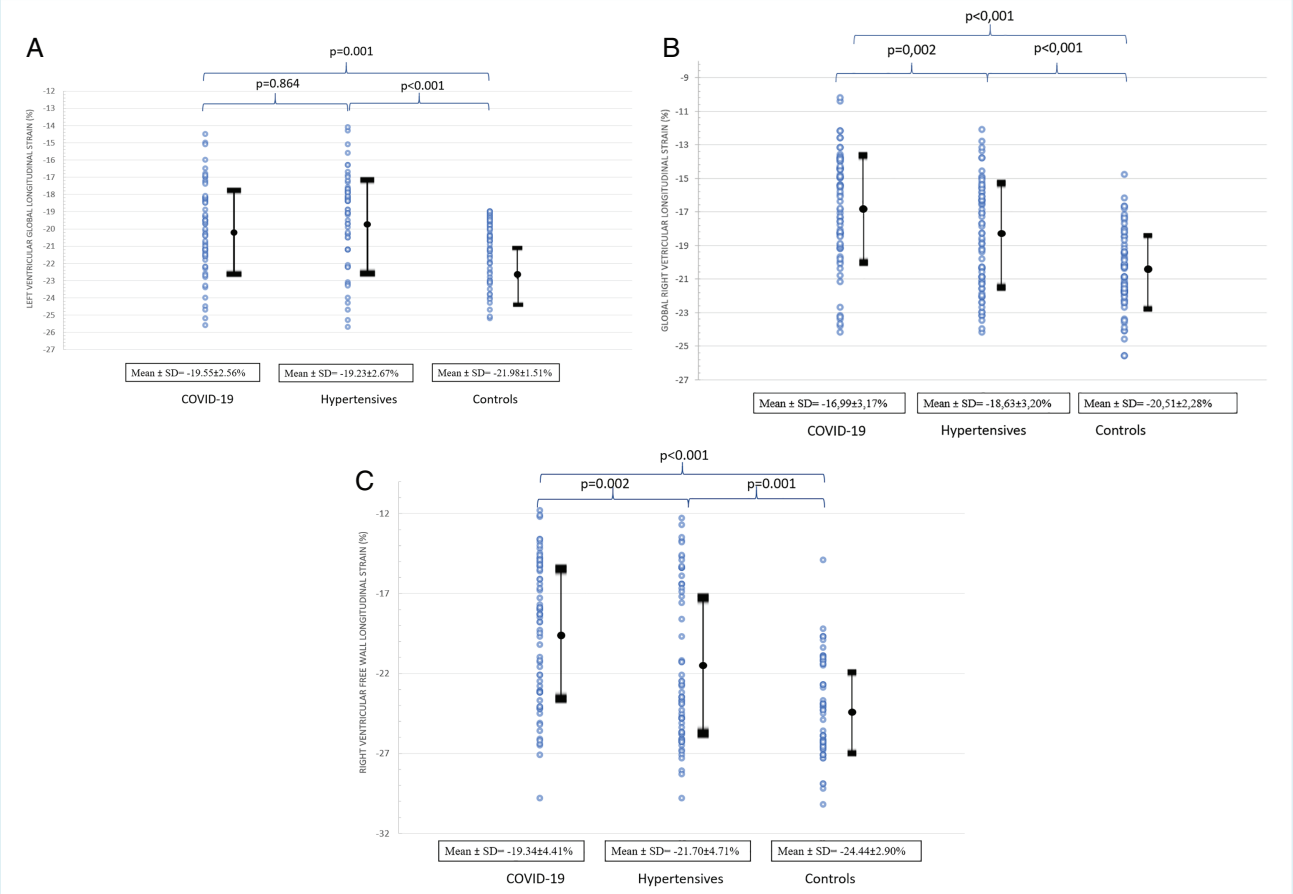

Figure 2 Scatterplot representing values of (*A*) left ventricular global longitudinal strain, (*B*) right ventricular global longitudinal strain, and (*C*) right ventricular free wall strain in COVID-19 patients compared with with hypertensives and the control group. Black mark and black lines represent mean ± standard deviation (SD).

and CFR (r = 0.584, *P* = 0.022 and r = 0.568, *P* = 0.027, respectively). Moreover, in the COVID-19 group thrombomodulin was associated with cfPWV and FMD (r = 0.524, *P* = 0.029 and 0.44, *P* = 0.040, respectively). while vWF was correlated with PBR5–25 (r = 0.43, *P* = 0.045).

In the COVID-19 group, we did not observe significant difference in PBR5–25, cfPWV, central SBP and central PP among the three subgroups of disease severity (mild, moderate, severe) (F = 0.107, *P* = 0.899; F = 1.173, *P* = 0.317; F = 1.039, *P* = 0.360; F = 1.032, *P* = 0.362; respectively) Additionally, LV GLS, RV GLS CFR, FMD, MDA, thrombomodulin and vWF were similar among the three subgroups of disease severity (F = 0.781, *P* = 0.469; F = 0.885, *P* = 0.569; F = 2.513, *P* = 0.112; F = 0.006, *P* = 0.994; F = 0.485, *P* = 0.627; F = 0.489, *P* = 0.629; F = 0.585, *P* = 0.527, respectively; data not shown).

Discussion

Our study supports that SARS-CoV-2 causes endothelial and vascular dysfunction that remains 4 months after initial infection and is, therefore, linked to reduced cardiac performance. In accordance with our hypothesis, we observed that COVID-19 patients displayed greater PBR5–25, lower FMD, and CFR values as well as thrombomodulin and vWF factor compared to healthy individuals indicating endothelial dysfunction and impaired coronary microcirculatory function. Most interestingly, MDA, a marker of lipid peroxidation, was nearly 10-fold higher than that of healthy controls, 4 months after COVID-19 infection. Likewise, we observed greater cfPWV, central PP and central SBP among COVID-19 patients, suggesting increased arterial stiffness. In addition, COVID-19 patients presented impaired LV and RV GLS values compared to the control group implying reduced cardiac performance. The above markers of endothelial vascular and LV function were similar between COVID-19 and hypertensive patients, suggesting a similar vascular damage to that observed in hypertension. Moreover, RV function, as assessed by echocardiography, was more impaired in COVID-19 patients than both hypertensives and normal controls, suggesting the prolonged effects of chest infection on RV function. According to our data, oxidative stress appears the main factor contributing to vascular dysfunction in our COVID-19 patients as it was 10-fold higher compared to hypertensives and normal controls, in contrast to hypertensives where additional mechanisms

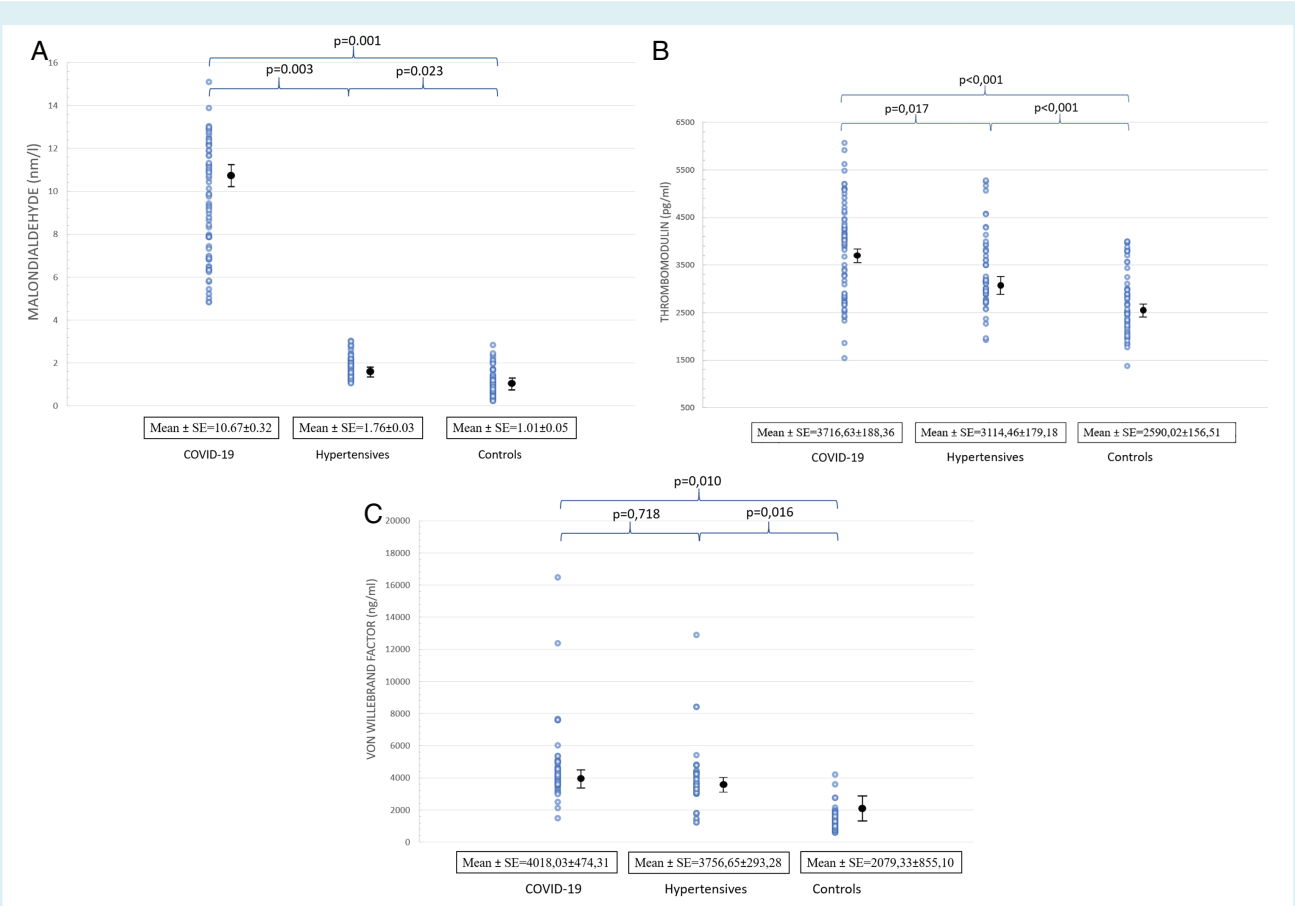

Figure 3 Scatterplot representing values of (*A*) malondialdeyde, (*B*) thrombomodulin, and (*C*) von Willebrand factor in COVID-19 patients compared with with hypertensives and the control group. Black mark and black lines represent mean ± standard error (SE).

such as the activation of the renin–angiotensin–aldosterone system are likely more important for the similar vascular and cardiac dysfunction observed in COVID-19 patients. Our findings support that COVID-19 infection *per se* contributes to increased arterial stiffness and vascular dysfunction in parallel with an impairment of myocardial deformation.

In our study, no significant interaction was found between disease severity based on simple clinical criteria (mild, moderate, severe) and markers of endothelial, vascular, and cardiac function and oxidative stress. This finding suggests that either clinical criteria cannot adequately predict future cardiovascular dysfunction or that oxidative stress and endothelial dysfunction persist at follow-up independently of disease severity at initial presentation. Indeed, in our study, 65.7% of patients were diagnosed to have mild to moderate disease. None of the patient required mechanical ventilation. Additionally, the mean duration of hospitalization was short. Not only the initial clinical presentation at hospital admission but also the disease course during hospitalization may be equally important for post-infection endothelial and cardiovascular function.

Indeed, in our study, there was a 10-fold increase of MDA levels in nearly all COVID-19 patients compared to controls, and this increase was associated with both PBR5–25 and CFR. Furthermore, increased PBR5–25 was associated with increased central SBP and PP and both aortic markers with more impaired LV GLS values. Thus, an excess oxidative stress is strongly associated with cardiovascular and endothelial dysfunction 4 months post-COVID-19 infection. To our knowledge, this is the first study to evaluate subclinical markers of endothelial, vascular and cardiac function as well as oxidative stress at 4 months after COVID-19 infection, suggesting that SARS-CoV-2 exerts prolonged effects on cardiovascular health. In line with our findings, a recent prospective observational cohort study pointed out that 78 and 60 patients out of 100 displayed cardiac involvement and myocardial inflammation after recovery from COVID-19 infection, respectively.[26] Furthermore, recent guidelines suggest follow-up evaluation of endothelial function in patients infected by SARS-CoV-2 for early detection of long-term adverse cardiovascular outcomes.[10]

The endothelium has a binary role in the progression of COVID-19 disease; it is both a target organ for the virus and a mediator in the activation of systemic inflammation. The ACE2 cellular receptor which permits the entrance of SARS-CoV-2 in host cells and TMPRSS2, a serine protein, which mediates the cleavage of the viral spike (S) protein, are both expressed in endothelial cells.[6] The infected cells produce increased levels of

proinflammatory cytokines which induce immune-mediated effects resulting in acute respiratory distress syndrome and multi-organ failure.[27] Indeed, histological and clinical evidence demonstrates that damaging of endothelial cells by SARS-CoV-2 induces vasculitis in multiple organs.[28,29] Finally, a recent study showed that thrombomodulin and vWF, markers of endothelial dysfunction, were elevated in hospitalized COVID-19 patients and were associated with adverse in-hospital outcome,[14] suggesting the presence of severe endotheliopathy. Extending the findings of this study, we found that thrombomodulin levels were higher in COVID-19 patients compared to both hypertensives and normal controls and vWF levels similar to those in hypertensives but higher than those in normal controls. Furthermore we found that increased thrombomodulin was associated with cfPWV and FMD as well as vWF with impaired endothelial glycocalyx confirming the presence of residual endothelial damage 4 months post-COVID-19 infection.

SARS-CoV-2 infection and systemic autoinflammatory diseases such as psoriasis and rheumatoid arthritis share overproduction of proinflammatory cytokines and oxidative stress as common pathophysiological mechanisms. Chen *et al.*[30] showed increased levels of pro-inflammatory cytokines including soluble interleukin-2 receptor (IL-2R), interleukin-6 (IL-6), and tumour necrosis factor-α (TNF- α) in patients with severe COVID-19 disease, suggesting that cytokine storm might be associated with disease severity. Glycocalyx damage by proinflammatory cytokines (e.g. IL-6, TNF-α) and oxidative stress increase vascular permeability inducing interstitial fluid shift and generalized oedema. Patients with psoriasis display vascular and coronary microcirculatory dysfunction which leads to impaired LV myocardial function, via an inflammation-driven cascade.[31] Similarly, Ciftci *et al.*[32] found that patients with rheumatoid arthritis displayed reduced CFR values compared to controls. Tocilizumab, a recombinant humanized monoclonal antibody against IL-6, which is used for the treatment of rheumatoid arthritis, reduces inflammation and oxidative stress and subsequently enhances endothelial and myocardial function. The investigators suggest that this mechanism may explain the efficacy of tocilizumab on COVID-19 disease.[33] Indeed, in the present study, MDA, a marker of lipid peroxidation, was approximately 10-fold higher in patients compared to controls and was associated with impaired glycocalyx and CFR.

The evidence regarding endothelial and vascular function in COVID-19 patients is scarce. At present, three studies have investigated the effect of SARS-CoV-2 on arterial stiffness.[34–36] A recent observational retrospective study, including 12 170 patients, showed that brachial artery PP is an independent risk factor for all-cause mortality in hospitalized COVID-19 patients.[34] In a cross-sectional study, Ratchford *et al.*[35] assessed FMD, single passive limb movement (sPLM) and cfPWV as markers of vascular function and arterial stiffness in 11 young adults compared to the control group, 4 weeks after confirmed SARS-CoV-2 infection. Like our results, a lower FMD and a higher cfPWV were observed in the SARS-CoV-2 group compared to controls. The prospective, observational COSEVAST study examined cfPWV as a marker of arterial stiffness in 64 patients with confirmed SARS-CoV-2 infection divided into three groups (mild, moderate, severe) according to the NIH severity criteria. An increased cfPWV was observed in the moderate and severe COVID-19 groups, whereas cfPWV values were significantly lower in the mild group.[36]

Two recent studies showed alterations in sublingual microcirculation in ventilated COVID-19 patients.[37,38] Rovas *et al.*[39] assessed glycocalyx dimensions (PBR) in sublingual microvessels in 23 hospitalized patients with moderate-to-severe SARS-CoV-2 infection. They reported higher PBR values in COVID-19 patients on mechanical ventilation compared to non-ventilated patients and controls Additionally, in accordance with our data, this evidence suggests significant alterations of the microcirculation and the endothelial glycocalyx in COVID-19 patients which are linked with the elevated thrombomodulin and vWF found in our COVID-19 patients in the present study.

Finally, the presence of vascular and cardiac dysfunction in COVID-19 patients was associated with presence of symptoms (dyspnoea, cough, chest pain, or fatigue) in our study. In particular, impaired endothelial glycocalyx, FMD, and increased thrombomodulin were associated with presence of cardiovascular symptoms suggesting the contribution of residual endothelial dysfunction to lack of symptom resolution 4 months post-infection. Furthermore, the presence of fatigue was linked with increased oxidative stress, arterial stiffness and impaired LV myocardial deformation. In our study, markers of vascular dysfunction were associated with impaired LV myocardial dysfunction linking endothelial dysfunction with lack of symptom resolution likely related to subclinical cardiac dysfunction. COVID-19 patients also displayed more deteriorated RV myocardial function than hypertensives which may be attributed to the effects of the acute respiratory infection. In support of this mechanism, we found that COVID-19 patients showing residual dyspnoea 4 months after infection presented more impaired RV GLS values compared to patients without this symptom.

In our study, the degree of oxidative stress was 10-fold higher in COVID-19 patients compared to hypertensives and normal controls and was associated with both impaired endothelial glycocalyx and CFR. Moreover, in the COVID-19 group, increased thrombomodulin was associated with increased cfPWV a well validated prognostic marker. Finally, elevated central aortic pressures suggesting arterial stiffening were related with more impaired LV GLS values. Thus, the changes in oxidative stress and endothelial function during COVID-19 infection resulted in impairment of arterial stiffness and coronary flow linked to subtle but important changes in myocardial function implying a potential worsening of future cardiovascular prognosis. In support of this study implication, we observed that patients presenting with fatigue 4 months after COVID-19 infection had more impaired values of LV GLS, PWV and MDA compared to those without this symptom.

Conclusions

Our data suggest a significant association between SARS-CoV-2 infection and oxidative stress, endothelial and vascular dysfunction, which is linked to impaired longitudinal myocardial deformation 4 months after COVID-19 infection and a concomitant persistence COVID-related symptoms despite recovery from infection. The above associations appear independent of disease severity.

The assessment of these endothelial function and arterial stiffness markers in SARS-CoV-2 patients may contribute to the risk stratification of cardiovascular complications and future development of heart failure, along with the prediction of COVID-19 adverse clinical outcomes. Our results demonstrate the importance of strict surveillance in post-COVID-19 outpatient clinics of 'well-recovered patients', who may present subclinical cardiovascular complications which could increase long-term morbidity. Additionally, the assessment of these markers may contribute to the detection of patients with coexisting atherosclerotic risk factors in need of more intensive treatment of comorbidities leading to the improvement of prognosis. Targeted therapies against endothelial impairment may improve clinical outcomes of COVID-19 infection. Larger prospective clinical studies with defined cardiovascular endpoints are required to determine the principal effects of SARS-CoV-2 on vascular and endothelial function and the underlying pathophysiological mechanisms. An evaluation of endothelial, vascular and myocardial function markers at a longer follow-up period post-COVID-19 infection would be important to clarify whether the changes observed at 4 months post-infection are reversible and at which time period after hospital discharge.

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