Circulation Research

ORIGINAL RESEARCH

Vascular Dysfunction of COVID-19 Is Partially Reverted in the Long-Term

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BACKGROUND: COVID-19 is characterized by severe inflammation during the acute phase and increased aortic stiffness in the early postacute phase. In other models, aortic stiffness is improved after the reduction of inflammation. We aimed to evaluate the mid- and long-term effects of COVID-19 on vascular and cardiac autonomic function. The primary outcome was aortic pulse wave velocity (aPWV).

METHODS: The cross-sectional Study-1 included 90 individuals with a history of COVID-19 and 180 matched controls. The longitudinal Study-2 included 41 patients with COVID-19 randomly selected from Study-1 who were followed-up for 27 weeks.

RESULTS: Study-1: Compared with controls, patients with COVID-19 had higher aPWV and brachial PWV 12 to 24 (but not 25–48) weeks after COVID-19 onset, and they had higher carotid Young's elastic modulus and lower distensibility 12 to 48 weeks after COVID-19 onset. In partial least squares structural equation modeling, the higher the hs-CRP (high-sensitivity C-reactive protein) at hospitalization was, the higher the aPWV 12 to 48 weeks from COVID-19 onset (path coefficient: 0.184; P=0.04). Moreover, aPWV (path coefficient: −0.186; P=0.003) decreased with time. Study-2: mean blood pressure and carotid intima-media thickness were comparable at the end of follow-up, whereas aPWV (−9%; P=0.01), incremental Young's elastic modulus (−17%; P=0.03), baroreflex sensitivity (+28%; P=0.049), heart rate variability triangular index (+15%; P=0.01), and subendocardial viability ratio (+12%; P=0.01×10^{-4}) were significantly improved. There was a trend toward improvement in brachial PWV (−6%; P=0.14) and carotid distensibility (+18%; P=0.05). Finally, at the end of follow-up (48 weeks after the onset of COVID-19) aPWV (+6%; P=0.04) remained significantly higher in patients with COVID-19 than in control subjects.

CONCLUSIONS: COVID-19-related arterial stiffening involves several arterial tree portions and is partially resolved in the long-term.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: baroreflex ■ coronavirus ■ elastic modulus ■ heart rate ■ inflammation

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Several cardiovascular complications have been reported during COVID-19, including acute heart disease, cardiac autonomic dysfunction and diffuse endothelial damage, leading to microvascular thrombosis and thromboembolic events. A few small cross-sectional studies have reported increased elastic artery stiffness in the early postacute phase of COVID-19, up to 4 months after disease.[1–4] The mid- and long-term effects of COVID-19 on vascular function and autonomic control of blood pressure (BP) and heart rate remain unknown.

Despite the observation in other models of inflammation that arterial stiffness is increased in both elastic and muscular arteries,[5] no studies have evaluated whether COVID-19 also affects muscular arteries. Moreover, inflammation

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†A list of all Methuselah Study Group participants is given in the Appendix.
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Novelty and Significance

What Is Known?

- Cardiovascular risk is increased during COVID-19. Aortic stiffness, a known cardiovascular risk factor, is increased in the early postacute phase of COVID-19 (up to 4 months after infection).
- In other models of inflammation, arterial stiffness is increased in both elastic and muscular arteries and potentially reversible. The more effective the decrease in inflammation is the greater the reduction in arterial stiffness.

What New Information Does This Article Contribute?

- Higher levels of sensitivity C-reactive protein level at hospitalization are associated with higher aortic stiffness at 12-48 weeks following COVID-19.
- At the end of follow-up (48 weeks after the onset of COVID-19), several measures of cardiovascular function were improved. Despite the improvement, aortic stiffness was not fully normalized at the end of follow-up.

Several portions of the arterial tree appear to be affected by COVID-19. Vascular dysfunction induced by COVID-19 can persist in the mid-term and be partially restored in the long-term, specifically,the more time since COVID-19 onset, the greater the restoration of vascular function. The COVID-19 related cardiac disease appears to improve in parallel with vascular disease after the acute phase of COVID-19. The residual increase in aortic stiffness that remains long-term is of interest and may suggest an increase in global cardiovascular risk and premature vascular aging.

Nonstandard Abbreviations and Acronyms

ACE2	angiotensin-converting enzyme 2
aPWV	aortic pulse wave velocity
BP	blood pressure
bPWV	brachial pulse wave velocity
BRS	baroreflex sensitivity
eGFR	estimated glomerular filtration rate
Einc	incremental Young's elastic modulus
GFR	glomerular filtration rate
IBD	inflammatory bowel disease
MBP	mean blood pressure
PLS-SEM	partial least squares structural equation modeling

may lead to potentially reversible arterial stiffening.[5] In patients with inflammatory bowel disease (IBD), which is an emerging model of chronic severe inflammation, the more effective the decrease in inflammation is, the higher the reduction in aortic stiffness.[6,7] Translating this concept to patients with COVID-19, the present study tested the hypothesis that COVID-19-related vascular dysfunction may be partly reversible in the long-term.

METHODS

Data Availability

The data that support the findings of this study are available on request from the corresponding author (L. Zanoli). The data is not publicly available as it contains information that could compromise research participant privacy/consent.

Please see the Supplemental Major Resources Table.

Study Population

Study-1

Study-1 was a multicenter observational cross-sectional study. The primary outcome was aortic pulse wave velocity (aPWV). Patient selection is reported in Figure 1 and described in the Supplemental Methods. Briefly, we enrolled a random sample of 90 patients (age 55±12 years, male sex 58%, weight 80±14 kg) of Italian ethnicity with a previous hospitalization for COVID-19 from March 2020 to February 2021 in 3 COVID-19 units of 3 hospitals in Italy (Infectious Diseases, Policlinico di Catania, Catania; Internal Medicine, Cannizzaro Hospital, Catania; and Infective Diseases, Giovanni Paolo II Hospital, Ragusa). The patient group was paired with a control group of 180 subjects (2 controls/1 patient) of the same ethnicity matched for age, sex, and body mass index, recruited from a community database and with an available noninvasive vascular study performed before the SARS-CoV-2 pandemic.

Patients with COVID-19 and controls with conditions associated with arterial stiffening (diabetes, chronic kidney disease, dyslipidemia, stroke, ischemic heart disease, and current or former smoking [ie, smoking cessation >1 year from hospitalization]) and those who took drugs that could potentially modify vascular function (antihypertensive drugs) were excluded from this study.

COVID-19 status was confirmed using polymerase chain reaction. All patients in Study-1 were hospitalized for COVID-19. The severity of COVID-19 during hospitalization was defined according to the World Health Organization clinical progression score.[8] Written informed consent was obtained from each participant before enrollment. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was

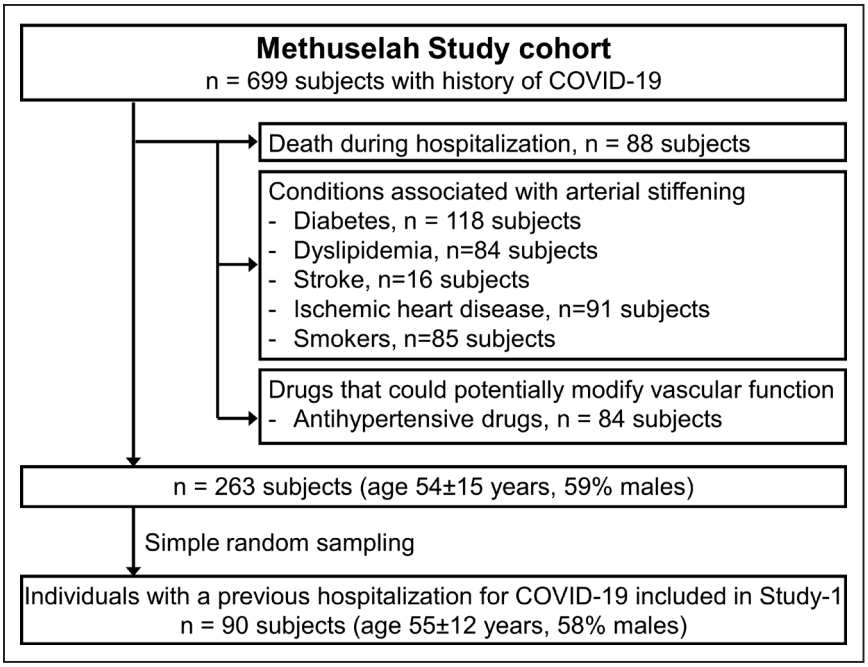

Figure 1. Flowchart describing the selection of subjects for the present analysis.

approved by the Ethics Committee on Research on Humans of the University of Catania.

Study-2

Study-2 was a multicenter longitudinal study. The primary outcome was aPWV. Follow-up was planned in half of the patients with COVID-19 enrolled in Study-1. Therefore, a random number from the uniform distribution was generated for each of the 90 patients with COVID-19 enrolled in Study-1 and a second hemodynamic visit was proposed to 45 patients with the lowest random numbers. A total of 4 patients refused the follow-up visit. Therefore, 41 patients underwent a second hemodynamic visit after 27 weeks of follow-up and were included in Study-2. A group of 82 control subjects, matched for age, sex, and body mass index (2 controls/1 patient) were again paired with patients as in Study-1.

Vascular Study Protocol

All participants were studied between 09:00 and 11:00 while fasting, in a centralized vascular laboratory by an expert operator (L. Zanoli) blinded to clinical data and in a quiet room with a controlled temperature of 22±1 °C after 15 minutes of rest in a supine position. Brachial BP was measured 3 times, 2 minutes apart, using a validated oscillometric device (Spacelabs 90217 ambulatory BP monitor; Issaquah, WA).[9] The mean value of the last 2 measurements was used in this study. Consecutively, a complete noninvasive vascular examination was performed.

Pulse Wave Analysis

The aPWV and brachial pulse wave velocity (bPWV) were measured with a SphygmoCor device (SphygmoCor system, AtCorMedical, Sydney, Australia) as previously reported,[10] using the foot-to-foot velocity method, the intersecting tangent algorithm and the direct distance between the measurement sites[11]: aPWV (m/s)=0.8×(carotid-femoral direct distance [m]/Δt); bPWV (m/s)=0.8×(carotid-radial direct distance [m]/Δt). The mean value of 2 consecutive recordings was used for this analysis. When the difference between the 2 measurements was ≥0.5 m/s, a third recording was performed, and the median value was used.

The right arm radial pulse wave profile was recorded by applanation tonometry (SphygmoCor system, AtCor Medical, Sydney, Australia) after recalibration with brachial mean BP (MBP) and diastolic BP in the contralateral arm and was used to assess the central pulse wave profile, as suggested in the Association for Research into Arterial Structure and Physiology Society task force consensus statement on protocol standardization,[12] and to calculate the subendocardial viability ratio, an index of myocardial oxygen supply and demand. The right common carotid artery pulse wave profile was recorded by applanation tonometry (SphygmoCor system, AtCor Medical, Sydney, Australia) and used to calculate carotid pulse pressure after recalibration with brachial MBP and diastolic BP. Brachial MBP was calculated as brachial diastolic BP+1/3×brachial pulse pressure.[12]

Carotid Study

A carotid study was performed immediately after pulse wave analysis, as previously described,[13] using a high-precision echotracking device (MyLab One; Esaote, Maastricht, The Netherlands) equipped with a high-resolution (13 MHz) linear-array transducer. The diastolic internal diameter and intima-media thickness (B-mode), as well as the stroke change in diameter (fast B-mode) were measured online for the right common carotid artery. The distensibility coefficient, defined as the relative change in luminal area during systole for a given pressure change (pulse pressure), and the incremental Young's elastic modulus (Einc), which represents the elastic properties of the material of the arterial wall, were calculated as previously described.[13]

Cardiovascular Autonomic Function

R-R intervals and beat-to-beat finger BP were recorded with a Finometer Midi device (Finapres Medical Systems, Amsterdam,

The Netherlands) for 5 minutes, during which patients were instructed to breathe spontaneously (range, 10–18 breaths/min) and to refrain from sleeping or speaking. The beat-to-beat R-R interval, baroreflex sensitivity (BRS) and total peripheral resistance were calculated by the Finometer Midi device from 256 consecutive beats.[14] R-R intervals were also analyzed by Kubios HRV v.2.2 software (Biosignal Analysis Medical Imaging Group, Kuopio, Finland) in the frequency domain through power spectral analysis for the assessment of the sympathetic/parasympathetic balance, calculated as the low frequency (0.04–0.15 Hz) to high frequency (0.15–0.40 Hz) power ratio,[15] and in the time domain for the calculation of the heart rate variability triangular index.[16]

Clinical Variables

Enzyme immunoassays were used for the quantitative determination of hs-CRP (high-sensitivity C-reactive protein). The estimated glomerular filtration rate (eGFR) was estimated using the Chronic Kidney Disease Epidemiology Collaboration formula.[17] Acute pericarditis during hospitalization for COVID-19 was defined as the presence of 2 of the following criteria: (1) pericardial chest pain during hospitalization, (2) pericardial rubs upon auscultation, (3) new widespread ST elevation or PR depression on ECG, and (4) pericardial effusion at echocardiography. Impairment in liver enzymes was defined as alanine aminotransferase and aspartate aminotransferase above the upper limit of the normal range (alanine aminotransferase >55 U/L, aspartate aminotransferase >48 U/L).

In patients with COVID-19 and control subjects, clinical data were collected within 1 week of the hemodynamic study. Molecular tests for SARS-CoV-2 RNA were performed using a reverse-transcription polymerase chain reaction assay.

The time from COVID-19 onset was defined as the time that had passed from the onset of the first symptom of COVID-19 to the day of the hemodynamic study. According to the median time from COVID-19 onset (24 weeks from the onset of the first symptom of COVID-19), we reported data of patients with recent onset (12–24 weeks) and those studied after a more prolonged interval (25–48 weeks).

Statistical Analysis

The sample size calculation of Study-1 is reported in the Supplemental Methods.

Continuous variables are presented as the means (±SD); categorical variables are presented as percentages. The D'Agostino-Pearson normality test was used to confirm that variables were well modeled with a normal distribution (Table S1). The Bonferroni correction was used to counteract multiple comparisons. A $P>1.00$ after Bonferroni correction was reported as $P=1.00$. Univariate analyses (ANOVA, Kruskal-Wallis test, χ^2 test and Fisher exact test) were used to compare the clinical and hemodynamic variables of patients with COVID-19 and control subjects. Multiple-group logistic regression analysis adjusted for the matching variables (age, sex, and body mass index) and major confounders (MBP, GFR, and total cholesterol) was used to compare the aPWV of patients with COVID-19 and control subjects. Univariate linear regression analyses were used to identify factors associated with aPWV in patients with COVID-19; we then selected variables that were associated ($P<0.10$) with aPWV (Table S2). These variables were then included in a multivariate linear regression model (enter method, Table S2). The coefficient of determination was used to estimate the proportion of the variation (R^2) in the dependent variable (aPWV) predictable from the variables included in the univariate and multivariate linear regression models reported in Table S2. Normality distribution of the residuals of the multivariate linear regression analysis reported in Table S2 was confirmed using the D'Agostino-Pearson normality test ($P=0.12$).

All variables that were associated ($P<0.10$) with aPWV in univariate linear regression analyses (Table S2) were also included in a partial least squares structural equation modeling (PLS-SEM) to perform mediation analyses and explore potential mechanisms of arterial dysfunction in patients with COVID-19.[18–20] Based on mediation analysis, we quantified both the direct and indirect (mediated) effects of COVID-19 on aortic stiffness. A mediation effect was confirmed when (1) exposure (ie, Einc) was significantly correlated with the mediator (ie, MBP) and (2) the mediator was significantly correlated with the outcome (ie, aPWV). The ratio of indirect-to-total effect was used to determine the proportion of the variance (R^2) explained by the mediation process.[18] An indirect-to-total effect ratio <20% indicates that nearly zero mediation occurs, 20% to 80% indicates partial mediation and >80% indicates full mediation.[20] Statistical analyses were performed using NCSS 2007 and PASS 11 software (Gerry Hintze, Kaysville, UT), except for PLS-SEM analyses, which were performed using SmartPLS 3 software (SmartPLS GmbH, Boenningstedt, Germany). Assessment of multivariate normality of the PLS-SEM was performed using IBM SPSS AMOS 26 software (Amos Development Corporation, PA). A 2-tailed $P<0.05$ was considered to indicate statistical significance.

RESULTS

Study-1

The main data of 90 patients with COVID-19 and 180 control subjects are reported in Tables 1 and 2.

Matching was successful since patients with COVID-19 and control subjects, paired for age, sex, and body mass index, were also comparable for total cholesterol and eGFR. hs-CRP levels were higher in all patients at hospital admission for COVID-19 than 12 to 48 weeks later, at the time of the vascular study ($P=0.01\times10^{-5}$). Compared with control subjects, all patients with COVID-19 had higher aPWV, bPWV and carotid Einc, as well as lower carotid distensibility. aPWV was confirmed to be higher in patients with recent (12–24 weeks) COVID-19 onset than in controls ($P=0.001$), but not in those with longer (24–48 weeks) COVID-19 times ($P=0.78$) in multiple-group logistic regression analysis adjusted for age, sex, body mass index, eGFR, total cholesterol, and MBP. This result was also confirmed when adjusting for the presence or absence of hypertension rather than MBP. All patients with COVID-19 had a lower BRS. Time from COVID-19 onset and BRS were positively correlated in univariate linear regression analysis (1 week

Table 1. Socioeconomic and Clinical Characteristics of Patients Enrolled in Study-1 During Hospitalization for COVID-19

Variable(s)	Patients with previous COVID-19 disease			*P* value
	All	Time from COVID-19 onset		
		12–24 wk	25–48 wk	
	(n=90)	(n=45)	(n=45)	
	A	B	C	B–C
Monthly salary below €1000,* %	8	10	7	1.00†
Education: primary school,* %	30	28	33	1.00†
Single, divorced, or widowed,* %	11	8	14	1.00†
hs-CRP at hospital admission, mg/L	57 (30–104)	57 (27–104)	56 (31–105)	1.00‡
eGFR at hospital admission, mL/min per 1.73 m^2	96±17	94±15	98±18	1.00§
Impaired liver enzymes at hospital admission, %	16	13	18	1.00†
Acute pericarditis during hospitalization, %	6	4	7	1.00†

Values represent the mean±SD, median (interquartile range), or percentage. eGFR indicates estimated glomerular filtration rate; and hs-CRP, high-sensitivity C-reactive protein.

*Data available only in 40 patients in Group B and 43 patients in Group C.

Statistical analyses:

†Fisher exact test;

‡Kruskal-Wallis test; and

§1-way ANOVA with Bonferroni correction were used to test differences between Group B and Group C for categorical variables, continuous variables without normal distribution and continuous variables with normal distribution, respectively.

increase: β, 0.14 ms/mm Hg [95% CI, 0.01–0.27 ms/mm Hg]; *P*=0.03).

Factors associated with aPWV in patients with COVID-19 are reported in Table S2. In univariate analysis, aPWV was positively related to hs-CRP at hospitalization for COVID-19 and negatively associated with the interval from the onset of COVID-19. These relationships were confirmed in a multivariate linear regression analysis (Table S2). eGFR at hospital admission for COVID-19 was not associated with aPWV measured 12 to 48 weeks from COVID-19 (*P*=0.33). Interestingly, eGFR and aPWV were correlated when the former was estimated at the same time of the hemodynamic study (*P*=0.003, Table S2). However, this association was lost in multivariate analysis (Table S2).

In fully adjusted PLS-SEM analyses, the normalized estimate of multivariate kurtosis was 0.497, suggesting that our data follow a multivariate normal distribution. The higher hs-CRP was at hospital admission for COVID-19, the higher aPWV was 12 to 48 weeks later, at the time of the vascular study (path coefficient, 0.184; *P*=0.04); the more time that had passed since COVID-19 onset, the lower aPWV was (path coefficient, –0.186; *P*=0.003; Figure 2).

Symptoms of COVID-19

The main symptoms of COVID-19 during the acute phase and at the time of the vascular study are reported in Table S3. A total of 63% of patients with COVID-19 had persistent symptoms at the time of testing (69% of patients with recent COVID-19 onset and 58% of those with more past disease). Fatigue was the most common symptom (54%), followed by dyspnea (17%), and muscular or joint pain (11%). Younger patients had a greater number of symptoms during the acute phase of COVID-19 ($P=0.01\times10^{-2}$). The higher the number of persistent symptoms at the time of the vascular study was, the higher the aPWV was (*P*=0.001). Accordingly, patients with loss of smell/taste at the time of the vascular study had higher aPWV (11.1±3.1 versus 8.4±1.8 m/s; *P*=0.002). The severity of COVID-19 during hospitalization is reported in Table S4.

Study-2

A total of 41 COVID-19 patients (mean age 54±12 years, males 51%) were followed-up for 27 weeks (baseline visit was performed 21±5 weeks after the onset of COVID-19; follow-up visit was performed 48±6 weeks after the onset of COVID-19). During follow-up, no antihypertensive drugs were added and no patient developed diabetes, chronic kidney disease, dyslipidemia, stroke, or ischemic heart disease. Table S5 shows the effect of COVID-19 on vascular function in this patient population. MBP and carotid intima-media thickness were not modified during follow-up, whereas aPWV (–9%; *P*=0.01), Einc (–17%; *P*=0.03), BRS (+28%; *P*=0.049), heart rate variability triangular index (+15%; *P*=0.01), and subendocardial viability ratio (+12%; $P=0.01\times10^{-4}$) were significantly improved. There was a trend toward improvement in bPWV (–6%; *P*=0.14) and carotid distensibility (+18%; *P*=0.05). Finally, at the end of follow-up (48 weeks after the onset of COVID-19) aPWV (+6%; *P*=0.04; Figure 3) remained significantly higher in patients with COVID-19 than in control subjects.

Table 2. Clinical Characteristics of Patients With COVID-19 and Age-, Sex-, and Body Mass Index-Matched Controls Enrolled in Study-1

	Patients with previous COVID-19 disease			Control subjects
	All	Time from COVID-19 onset		
		12–24 wk	25–48 wk	
Variable(s)	(n=90)	(n=45)	(n=45)	(n=180)
	A	B	C	D
Time from COVID-19 onset, wk	25±8	18±3	33±5	…
Age, y	55±12	55±11	54±13	55±13
Male sex, %	58	56	60	54
Italian ethnicity	100	100	100	100
Body mass index, kg/m^2	28.2±4.1	28.1±4.0	28.3±4.3	28.1±3.6
hs-CRP, mg/L	2.0 (1.1–3.3)	1.9 (1.0–3.0)	2.0 (1.1–4.0)	1.6 (1.0–2.9)
eGFR, mL/min per 1.73 m^2	98±13	96±13	99±13	99±16
Total cholesterol, mg/dL	207±21	208±20	205±22	201±18
Hypertension, %	29	28	29	17
Impaired liver enzymes, %	8	7	9	…
aPWV >10 m/s, %	17	24	9	11
Carotid IMT >900 µm, %	8	9	7	4
Brachial SBP, mm Hg	129±16	128±18	131±14	124±20
Brachial DBP, mm Hg	78±10	78±10	77±10	74±11
Brachial MBP, mm Hg	95±10	95±11	95±10	90±12
Heart rate, b/min	66±9	67±9	66±9	67±11
aPWV, m/s	8.6±2.0*	9.0±2.4*	8.2±1.3	7.9±1.5
bPWV, m/s	6.9±1.4*	7.3±1.4*	6.5±1.3	6.3±1.1
Subendocardial viability ratio, %	155±24	153±25	157±24	162±33
Carotid IMT, µm	695±149	690±153	700±146	649±135
Carotid distensibility, kPa^{-1}×10^{-3}	23.4±10.0*	23.3±10.6	23.5±9.5	28.4±12.2
Carotid Einc, kPa·10^2	470±208*	487±230	453±184	390±175
TPR, dyn×s/cm^5·10^2	12.47±3.95	13.66±4.33	11.29±3.16	12.05±3.18
BRS, ms/mm Hg	7.3 (5.0–12.1)	6.7 (4.2–12.1)	8.3 (6.1–12.1)	9.8 (5.8–15.2)
LF/HF ratio	1.19 (0.65–2.13)	1.33 (0.66–2.14)	1.10 (0.62–2.16)	1.22 (0.72–2.09)
HRVI	6.74 (4.92–8.83)	6.56 (4.92–7.76)	7.02 (5.03–10.24)	6.92 (5.33–8.83)

Values represent the mean±SD, median (interquartile range), or percentage. Statistical analyses: Fisher exact test (Group A versus Group D) or χ^2 test (Group B versus Group C versus Group D) were used for categorical variables; Kruskal-Wallis test (Group A versus Group D; Group B versus Group C versus Group D) were used for continuous variables without normal distribution; 1-way ANOVA (Group A versus Group D; Group B versus Group C versus Group D) were used for continuous variables with normal distribution. aPWV indicates aortic pulse wave velocity; bPWV, brachial pulse wave velocity; BRS, baroreflex sensitivity; DBP, diastolic blood pressure; Einc, incremental Young elastic modulus; HRVI, heart rate variability triangular index; hs-CRP, high-sensitivity C-reactive protein; IMT, intima-media thickness; LF/HF ratio, low frequency/high frequency ratio; MBP, mean blood pressure; SBP, systolic blood pressure; and TPR total peripheral resistance.

P value with Bonferroni correction:

*$P<0.05$ versus Group D. Precise *P* values are reported in Table S6.

DISCUSSION

The findings of the present study suggest that COVID-19 infection could involve several parts of the arterial bed and could be linked with autonomic dysfunction and could be partially reversed after the acute phase of the disease. The more time had passed since the onset of disease, the lower the stiffness of the elastic arteries.

Vascular Dysfunction in Patients With COVID-19

A position statement from the European Society of Hypertension Working Group on Vascular Structure and Function and the Association for Research into Arterial Structure and Physiology Society[5] highlighted the link between inflammation, arterial stiffening, and cardiovascular events. This link seems to be present in several chronic inflammatory diseases,[5] including IBD, which is characterized by mild/moderate chronic inflammation, peaks of severe acute inflammation during relapses and an increase in elastic and muscular artery stiffness.[21] Interestingly, aortic stiffness is dependent upon the severity and duration of the disease in IBD and is potentially improved after the reduction of inflammation in IBD and other models of inflammation.[5,6,22] Considering that COVID-19 is a systemic disease characterized by a peak

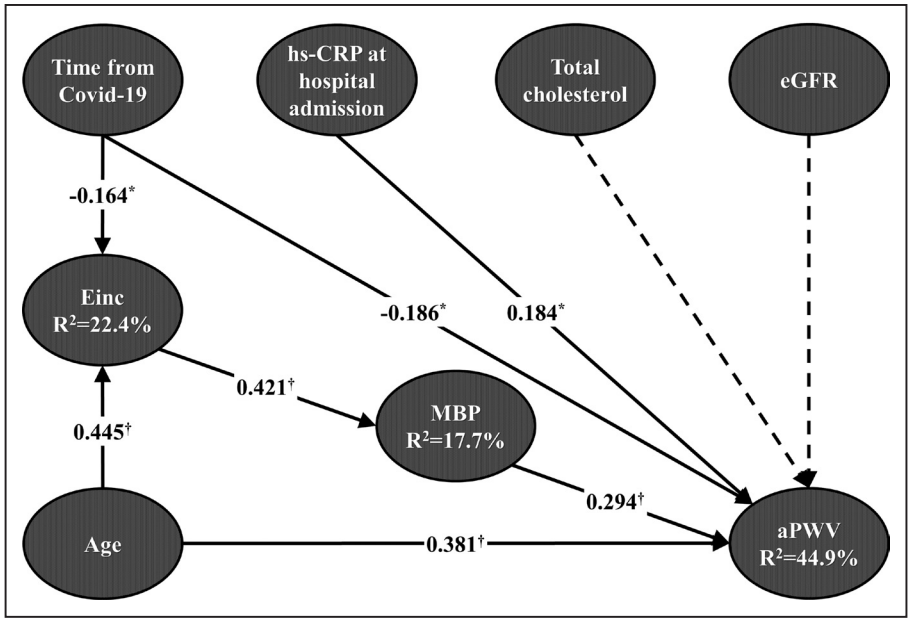

Figure 2. Partial least squares structural equation modeling performed in 90 patients with COVID-19 enrolled in Study-1 using a path weighting scheme.

Effect of COVID-19 on vascular function. hs-CRP (high-sensitivity C-reactive protein) was measured at hospital admission for COVID-19, and all other variables were measured at the time of the vascular visit. Age, time passed from COVID-19, hs-CRP, total cholesterol, and estimated glomerular filtration rate (eGFR) were included as independent variables; carotid incremental Young elastic modulus (Einc) and mean blood pressure (MBP) had a dual relationship as both independent and dependent variables; aortic pulse wave velocity (aPWV) was a dependent variable. Significant (*P<0.05; †P<0.001) direct effects are reported as continuous black lines; nonsignificant direct effects are reported as dotted lines. Arrows indicate the direction of the effects tested in the model. R^2 indicates the variance explained by the model. For instance, 44.9% of the variance in aPWV (dependent variable) was explained by the model.

of severe acute inflammation and an altered immune response that can potentially lead to mild chronic inflammation after the resolution of the acute phase of the disease, the arterial phenotype of patients with COVID-19 could resemble that observed in patients with IBD.

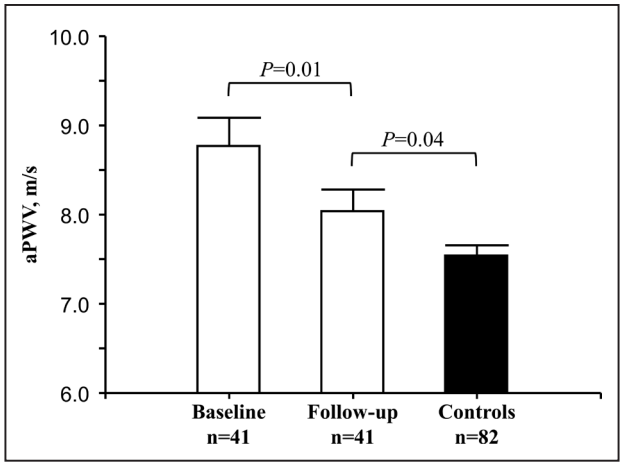

Figure 3. Longitudinal Study-2.

Aortic pulse wave velocity (aPWV) was measured in 41 patients with COVID-19 (empty bars) and 82 control subjects (black bars). Within the COVID-19 group, aPWV was measured at baseline and at the end of follow-up (21±5 and 48±6 wk after the onset of the disease, respectively). Bars represent means and SEMs. Significance was determined by 1-way ANOVA with repeated measures within the COVID-19 group and by 1-way ANOVA between the patients with COVID-19 at the end of follow-up and the control subjects.

An important question is whether vascular dysfunction in patients with COVID-19 is (1) reverted in parallel with the resolution of the acute phase or (2) sustained by persistent alterations in the arterial wall. In previous studies, comparing patients with controls, aPWV seemed to be higher (+30%) during COVID-19 than in the early post-acute phase of disease (+13%–20%).[1,2,4] In the present study, we evaluated the vascular function of patients with COVID-19 for the first time in a longitudinal study and reported that not only aPWV but also carotid Einc, subendocardial viability ratio, heart rate variability triangular index, and BRS were significantly improved after 27 weeks of follow-up, whereas bPWV and carotid distensibility were slightly, but not significantly, improved during follow-up. Our findings suggest that the stiffening of elastic arteries following COVID-19 infection could be at least partially reversible. This is clinically of interest because, in other populations, increased aortic stiffness leads to an increased risk of cardiovascular events,[23] whereas its reduction has the opposite effect.[24] Accordingly, in patients with COVID-19, arterial stiffness seems to influence outcomes in the short term, since the brachial-ankle PWV is higher in nonsurvivors.[1]

The improvements in subendocardial viability ratio (an index of myocardial oxygen supply and demand) and heart rate variability triangular index (an independent predictor of cardiovascular and all-cause mortality)[17] during follow-up in Study-2 suggest that heart

disease in COVID-19 could be improved in parallel with vascular disease after the acute phase of COVID-19. However, we also reported that aPWV is reduced, but not fully reverted, in patients with COVID-19 with a longer recovery compared with controls (Figure 3). Therefore, considering that (1) COVID-19 survivors frequently experience long-term symptoms,[25] (2) more than half of our patients had symptoms of the disease 25 to 48 weeks after infection (Table S3), and (3) the higher the number of symptoms was at the time of the vascular study, the higher the aPWV, our data could suggest residual structural damage of the arterial wall. However, we cannot exclude that a chronic low-grade inflammatory process persists after the acute phase of COVID-19, leading, in turn, to a progressive increase in arterial stiffness in the long-term. Moreover, residual confounding and effects of deconditioning associated with hospitalization could also be involved. Large longitudinal studies and a longer follow-up are needed to evaluate whether vascular dysfunction is fully reverted or whether there are long-term post-COVID-19 vascular effects. In the latter case, aPWV in patients with COVID-19 at the end of follow-up could represent the bottom of a J curve.

Potential Mechanisms of Vascular Dysfunction

Several studies have reported endotheliitis and endothelial dysfunction during COVID-19.[26–30] These alterations are linked with subintimal inflammation, hemorrhage and thrombosis, altered vascular tone, edema, and increased levels of matrix metalloproteinases,[21] thus suggesting that the vascular disease of COVID-19 could be both functional and structural.[31] In this regard, interleukin 1, a cytokine involved in pathogenic inflammation in COVID-19 and in the arterial stiffening process in patients with inflammatory diseases, could play a role, since it leads to the development of oxidative stress and stimulates hypertrophy and change in the phenotype of vascular smooth muscle cells.[5]

Functional and structural alterations of the arterial wall could be involved. In this regard, the elastic properties of the biomaterial of the arterial wall can be altered as a consequence of both endothelial dysfunction and reduced vascular smooth muscle cell relaxation (ie, functional arterial stiffening), as well as changes in the biomaterial of the arterial wall (ie, for the production of uncoiled stiff collagen and degradation of elastin; structural arterial stiffness). Interestingly, in the present study, the increase in Einc seemed to be partially reversible in patients with COVID-19, since it was significantly reduced during follow-up in Study-2 (Table S5). This finding may suggest that the functional alterations of the arterial wall could be, at least partially, reverted in the mid-term in patients with COVID-19. This hypothesis should be assessed in future studies.

Baroreflex Function in Patients With COVID-19

Recent studies reported baroreflex dysfunction in the early phase of infection with SARS-CoV-2.[32] Our findings suggest that baroreflex dysfunction could be restored after COVID-19, since BRS was improved during follow-up. Furthermore, BRS and aPWV were inversely correlated in COVID-19 patients. These results are of clinical interest since baroreflex dysfunction is associated with increased cardiovascular risk[33] and is linked with arterial stiffening.[34] SARS-CoV-2 can lead to reduced BRS affecting several portions of the baroreflex arc. Coronaviruses have a neurotoxic effect through direct hematogenous and neural propagation.[35] SARS-CoV-2 may also have a direct toxic effect on the central nervous system by binding ACE2 (angiotensin-converting enzyme 2) expressed in the capillary endothelium of the blood brain barrier.[36] Interestingly, the results of PLS-SEM suggest the presence of an additive mechanism by which SARS-CoV-2 could lead to altered baroreceptor function, as a consequence of the increased arterial stiffness and the reduced stimulation of the carotid baroreceptors (which are mechanosensors sensitive to the stretching of the arterial wall and not to changes in BP per se). We have previously observed this pathway in patients with metabolic syndrome, which is another model of inflammation and increased arterial stiffness.[37]

METHODOLOGICAL ISSUES

The present study has several strengths. First, considering that the vascular disease of COVID-19 can potentially affect several portions of the vascular bed (large elastic arteries, muscular arteries, and peripheral resistance vessels), as well as the autonomic control of BP and heart rate, we defined aPWV as the primary outcome and reported data on all these portions of the cardiovascular system. To the best of our knowledge, this is the first study designed to elucidate the mid- and long-term effects of COVID-19 on several measures of vascular function and to examine the link between vascular and autonomic function in patients with COVID-19. Second, we used well-validated devices for measuring: (1) BP, using an oscillometric device recommended by European Society of Hypertension,[9] and (2) PWV,[11] as well as for calculating MBP[12] and BRS.[14] Finally, the sample size of Study-1 (90 patients with COVID-19 and 180 matched control subjects) was sufficient to compare aPWV between patients with COVID-19 and control subjects using a multiple-group logistic regression analysis adjusted for the matching variables (age, sex, and body mass index) and major confounders (MBP, eGFR, and total cholesterol). The sample size was also sufficient for mediation analysis using PLS-SEM since, according to Cohen et al[19] and Hair et al,[20] 9 arrows pointing at a construct (independent variables) and 90 patients allowed for the detection of a minimum 25% variance (R^2) of the dependent variable explained by the model (variance of aPWV explained by the model reported in Figure 2: 44.9%).

This study also has some limitations. First, Study-1 was a cross-sectional study; therefore, a causal relationship cannot be established for any of the observed correlations except for the link between hs-CRP measured at hospital admission for COVID-19 and aPWV measured 12 to 48 weeks later, at the

time of the vascular study. Nonetheless, according to the literature,[38] we used a method (PLS-SEM) also used in cross-sectional studies and constructed a mediation model to examine the hypothetical association of COVID-19 with several measures of vascular function. The findings show strengths of association, temporality, consistency, biological plausibility, and gradient coherence with previous studies. Therefore, it is probable that our findings reflect a biological phenomenon.[39] Moreover, the main results of Study-1 were confirmed in Study-2 (ie, the longitudinal study). Nevertheless, larger longitudinal studies are needed to confirm our results. Second, control subjects and patients with COVID-19 were taken from different populations. We attempted to minimize bias by (1) excluding patients with COVID-19 and control subjects with conditions associated with arterial stiffening or the use of drugs that could potentially modify vascular function from this study, (2) employing an expert operator blind to clinical data for the measurement of hemodynamic parameters, (3) including control subjects matched for age, sex, and body mass index, and (4) performing multivariate analyses adjusted for major confounders. Third, although aPWV was not associated with monthly salary, education or marital status in the subgroup of 83 patients with COVID-19 with available data on these measures of social deprivation, which are also potential determinants of the need for hospitalization, we cannot exclude a confounding effect of other measures of social deprivation, including housing, household characteristics, and transportation costs. Finally, specific analyses are needed in patients with conditions associated with arterial stiffening excluded from our study (ie, individuals with diabetes, chronic kidney disease, dyslipidemia, stroke, or ischemic heart disease; current or former smokers and hypertensive subjects on treatment for hypertension). Since several drugs used to treat these cardiometabolic diseases (ie, antihypertensive, hypolipidemic, and antidiabetic drugs)[40–43] may also affect arterial stiffness, future studies should also focus on their role in vascular function in COVID-19 patients in the long-term (whether there will be a reinfection or not).

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Disclosures

None.

Supplemental Material

Supplemental Methods
Tables S1–S6

APPENDIX

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