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STATE-OF-THE-ART REVIEW

Clinical Implications of COVID-19-Related Endothelial Dysfunction

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ABSTRACT

Endothelial dysfunction represents a measurable and early manifestation of vascular disease. Emerging evidence suggests cardiovascular risk remains elevated after COVID-19 infection for at least 12 months, regardless of cardiovascular disease status prior to infection. We review the relationship between the severity of endothelial dysfunction and the severity of acute COVID-19 illness, the degree of impairment following recovery in both those with and without postacute sequalae SARS-CoV-2 infection, and current therapeutic efforts targeting endothelial function in patients following COVID-19 infection. We identify gaps in the literature to highlight specific areas where clinical research efforts hold promise for progress in understanding the connections between endothelial function, COVID-19, and clinical outcomes that will lead to beneficial therapeutics. (JACC Adv 2024;3:101070) © 2024 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

The concept of acute symptomatic (further simplified to acute, hereafter) respiratory infections increasing at least near-term cardiovascular risk is not novel.[1] Respiratory viruses, particularly influenza A, and community-acquired bacterial pneumonias are well-known to increase near-term risk of myocardial infarction and stroke.[2,3]

Recent data also show that COVID-19 infection has pro-atherogenic effects, and even mildly symptomatic infections are associated with increased cardiovascular risk for up to 12 months following infection.[4,5] However, unlike influenza and bacterial pneumonias, COVID-19 directly induces inflammation of the endothelium[6] and reports have emerged showing persistent impairment of vascular endothelial function for an extended period postacute infection.[4,7-11]

These data implicate COVID-19-induced endothelial dysfunction as a mechanistic culprit in adverse vascular events, both during and after COVID-19 infection. In this review, we explore our current knowledge and understanding of mechanisms of endothelial dysfunction-driven COVID-19 infection, both during acute symptomatic infection and following convalescence, and how this might modify the risk for major adverse cardiovascular events in patients during and following infection. Additionally, we examine the association of endothelial dysfunction with the severity of acute COVID-19 infection and postacute sequalae SARS-CoV-2 infection (PASC), which is primarily informed by symptomatic original and alpha strain infections. We assess current efforts to target vascular dysfunction in these individuals and in individuals infected with variant strains.

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ABBREVIATIONS AND ACRONYMS

ADMA = asymmetric dimethylarginine

cf-mDNA = cell-free mitochondrial DNA

FMD = flow-mediated dilation

Lp299v = *Lactobacillus plantarum 299v*

PASC = postacute sequelae SARS-CoV-2 infection

TLR9 = toll-like receptor 9

Based on this data, we identify key gaps in our current knowledge and areas for further investigation.

PATHOPHYSIOLOGY OF COVID-19-ASSOCIATED ENDOTHELIAL DYSFUNCTION

Endothelial dysfunction arises from multiple cellular and molecular pathways in COVID-19 (**Central Illustration**). Generation of reactive oxygen species leads to platelet activation and subsequent thrombosis.[12] This is evident in studies of intramyocardial endothelial cells where several nicotinamide adenine dinucleotide phosphate oxidase enzymes, producers of reactive oxygen species were upregulated in deceased COVID-19 patients.[13] Further, decreased amounts of nitric oxide and prostacyclin promote vasoconstriction.[12] Lastly, impaired barrier function from cytokine activation leads to basement membrane degradation, endothelial cell death, and loss of vessel integrity.[12] This phenotype manifests clinically across many organ systems, leading to deep vein thromboses, pulmonary embolisms, acute kidney injuries, ischemic strokes, arterial occlusions, and acute respiratory distress syndrome.[12]

MEASURING VASCULAR ENDOTHELIAL FUNCTION IN HUMANS

Due to the systemic nature of endothelial dysfunction, noninvasive techniques to measure endothelial function and vascular stiffness (eg, brachial artery flow-mediated dilation [FMD], digital pulse arterial tonometry, and peripheral arterial tonometry) have emerged as viable alternatives to invasive measurements. Noninvasive measures strongly correlate with dysfunction detected in the coronary bed and predict future adverse cardiovascular events in those both with and without prevalent cardiovascular disease.[14,15]

A body of literature now exists focusing on: 1) the association between the severity of acute COVID-19 infection and the severity of concomitant endothelial dysfunction; 2) determining the extent and time course of prolonged endothelial dysfunction post-COVID-19 infection; and 3) the association between PASC and endothelial dysfunction.[16]

ENDOTHELIAL DYSFUNCTION, CLINICAL IMPLICATIONS, AND CLINICAL SEVERITY OF ACUTE COVID-19 INFECTION

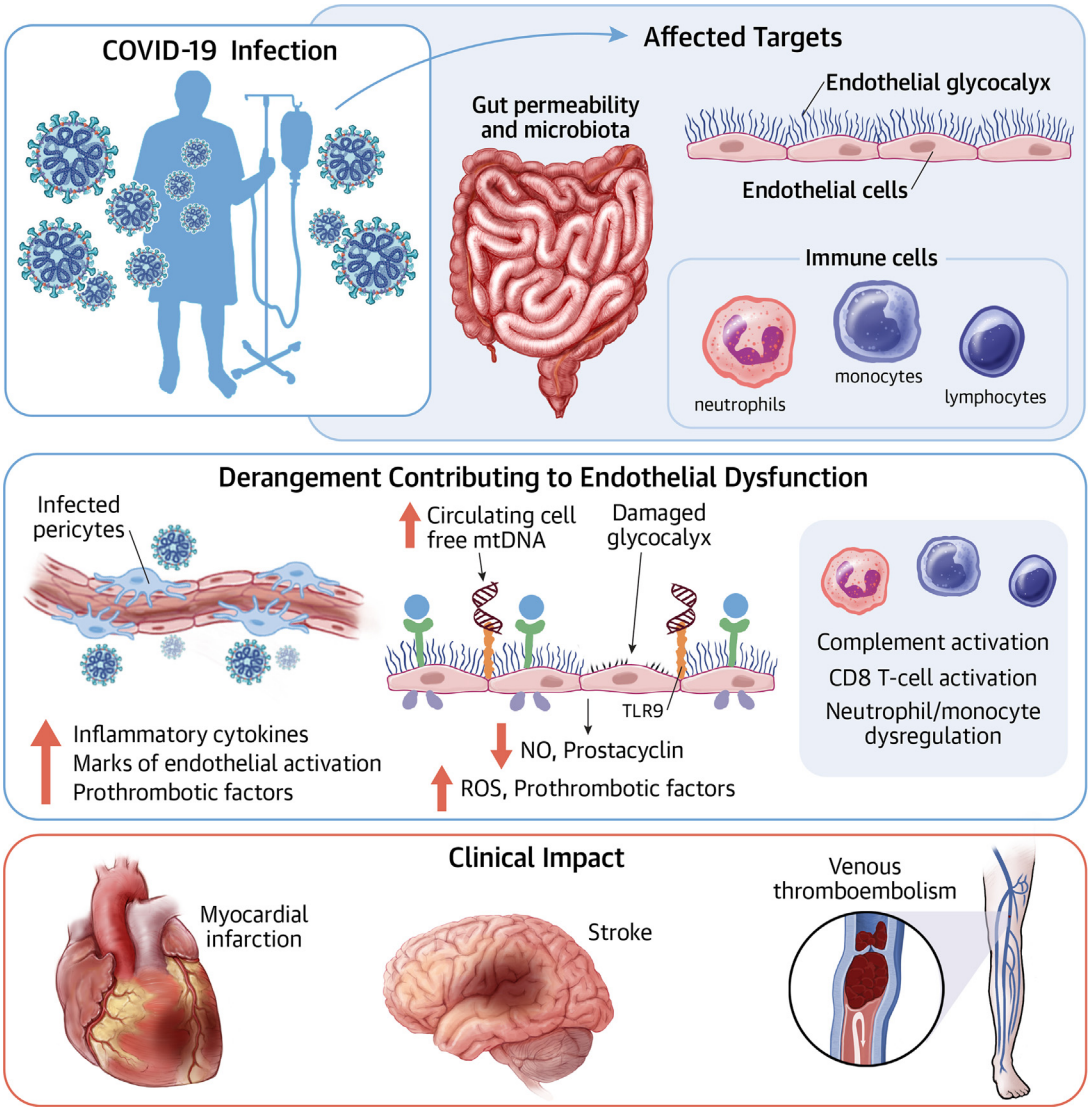
Severity of acute COVID-19 correlates with increasing endothelial activation and fibrosis.[17] Exposing human umbilical vein endothelial cells to plasma from patients with acute COVID-19 infection activates expression of genes involved in active acute inflammatory responses, cell motility, and cell stress responses.[18] Circulating levels of biomarkers of endothelial activation and oxidative stress are elevated to a greater extent with increasing severity of disease when compared to other non-COVID-19 respiratory infections.[19,20] von Willebrand Factor antigen and P-selectin are elevated above the normal range in hospitalized ward patients compared to healthy individuals and are significantly elevated in intensive care unit (ICU) patients compared to non-ICU patients.[20] Furthermore, P-selectin positively correlates with D-dimer levels and is elevated with other known markers of thrombosis, such as fibrinogen, prothrombin time, endothelial protein-C receptor, plasminogen activator inhibitor, and tissue plasminogen activator,[21,22] although inhibiting P-selectin does not necessarily blunt the severity of infection.[23] Whether greater severity of endothelial activation mechanistically drives disease severity or occurs as an "end-product" of severe infection remains unclear. While cell-free mitochondrial DNA (cf-mDNA) shows potential,[19] determining whether these and other endothelial activation biomarkers could be used to better predict severe disease and adverse outcomes merits consideration for future investigation.

Patients with acute COVID-19 infection from original and variant strains also have significantly impaired endothelial-dependent vasodilation by

HIGHLIGHTS

- Cardiovascular risk remains elevated after COVID-19 infection, and this elevated risk is likely related in significant part to COVID-19 related endothelial dysfunction.
- Severity of acute COVID-19 correlates with increased endothelial activation and dysfunction.
- Patients with and without postacute sequalae SARS-CoV-2 infection have impaired endothelial function after acute COVID-19 infection, with current data suggesting greater impairment in those with postacute sequalae SARS-CoV-2 infection.
- Clinical trials leveraging endothelial function are needed for accurate barometry of disease severity.

CENTRAL ILLUSTRATION **COVID-19 Infection to Clinical Vascular Disease**



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IL = interleukin; NO = nitric oxide; ROS = reactive oxygen species; TLR9 = toll-like receptor-9; VTE = venous thromboembolic event.

brachial artery FMD, suggesting brachial FMD could be used for risk stratification of COVID-19 patients.[24,25] To date, the results of these studies have been mixed. Brachial FMD and carotid intima-media thickness measured in 211 COVID-19 hospitalized patients with variant strains did not predict ICU admission, mechanical ventilation, or death during hospitalization.[26] A smaller 27-subject study also failed to demonstrate predictive value for brachial FMD.[27] Conversely, a 180-subject study of those with delta-strain infection found FMD% ≤3.43% predicted mortality and a longer hospital stay for patients.[28]

TABLE 1 Studies of Vascular Endothelial Dysfunction in Patients After Acute Infection With and Without PASC

Lead Author	Design	Patient Populations	Time From Acute Illness	Formal PASC Diagnosis	Timeframe of Study
Ambrosino[7]	Case-control	133 convalescent COVID-19 patients (81.2% males, mean age 61.6 years) vs 133 matched controls (80.5% males, mean age 60.4 years)	16.7 ± 18.5 d from swab negativization	No	December 2020-June 2021
Charfeddine[8]	Cross-sectional	798 COVID positive 68.934 ± 3.1 [28-186] days from symptoms. Mean age 49.94 ± 14.2 years, 60.5% women. 618 patients (77.4%) had PASC	2 wk to 6 mo from diagnosis of acute infection	Yes	January 2021-May 2021
Jud[9]	Cross-sectional	14 COVID-19 positive vs 14 healthy control vs 14 ASCVD control	6 mo and 1 y from acute infection	No	September 2020-March 2021 (patients diagnosed between March 2020-April 2020)
Tehrani[11]	Prospective observational	12 COVID-19 patients treated with NIMV or IMV vs matched healthy controls	3 mo after disease	No	May 2020 -June 2020
Ratchford[24]	Cross-sectional	Controls (5 males, 15 females, 23.0 ± 1.3 years) vs COVID-19 positive 3-4 wk prior (4 males, 7 females, 20.2 ± 1.1 years)	25 ± 5 d since symptom onset (n = 10) and 24 ± 6 d after their positive testing date (n = 11)	No	February 2020-November 2020
Oikonomou[25]	Prospective cohort	73 COVID-19 positive (37% admitted in ICU) vs healthy historic-control group	1 and 6 mo after the acute phase of COVID-19	Yes	November 2020-March 2021
Nishijima[32]	Ex vivo, cross-sectional	Control (n = 19, 44 ± 3 years) vs controls with cardiovascular risk factors (n = 28, 42 ± 1 years) vs controls with severe risk factors (n = 13, 64 ± 2 years) vs COVID-19 positive (n = 26, 48 ± 3 years)	Unknown	No	Unable to discern
Mejia-Renteria[33]	Prospective observational	20 COVID-19 patients (9.5 d from symptoms, group 1) vs 50 COVID-19 patients (101.5 d from symptoms, group 2) vs 72 matched controls	101.5 d from symptoms	No	June 2020 -November 2020
Moretta[34]	Prospective observational	55 convalescent COVID-19 patients (83.6% men, mean age 60.1 years). 41.8% had RCE	2 mo from swab negativization	Yes	Unable to discern
Santoro[35]	Prospective observational	658 patients in COVID-19 group. home-care vs hospital-no oxygen vs hospital-oxygen vs hospital-HFNC, NIMV, IMV, ECMO	3 mo from infection after negative test	No	April 2020 -March 2021
Szeghy[36]	Cross-sectional	15 COVID positive (20 ± 1 years) vs healthy controls (23 ± 1 years)	3-4 wks after a positive test result	No	Unable to discern

Summary of studies reporting measurements of endothelial dysfunction post-COVID-19 infection in patients with and without PASC. ASCVD = atherosclerotic cardiovascular disease; ECMO = extracorporeal membrane oxygenation; EQI = endothelial quality index; FMD = flow-mediated dilation; HFNC = high-flow nasal cannula; IMT = intima-media thickness; IMV = invasive-mechanical ventilation; N/A = not available; NIMV = noninvasive mechanical ventilation; NMD = nitroglycerin-mediated dilation; PASC = postacute sequalae SARS-CoV-2 infection; PORH = postocclusion reactive hyperemia; PPG = photoplethysmography; PWV = pulse-wave velocity; RCE = reduced cognitive efficiency; RHI = reactive hyperemia index.

Continued on the next page

Whether these findings were specific to COVID-19 patients is unclear as the study lacked a matching hospitalized, non-COVID-19 population. A study of 94 ICU patients and non-ICU patients with delta COVID-19 showed that ICU patients had significantly lower FMD% vs non-ICU patients, and patients who died compared to those who were discharged alive also had significantly reduced FMD percentages.[29] As a measure of microvascular function rather than large vessel function, iontophoresis of acetylcholine

TABLE 1 Continued

Likely COVID-19 Variant Studied	Vascular Bed	Endothelial Function/Vascular Stiffness Marker	Endpoint	Findings
Alpha	Brachial artery	FMD	Percent change in brachial artery diameter from baseline to the maximum value registered during reactive hyperemia induced by forearm ischemia	A lower FMD percentage was found in COVID-19 group vs controls (3.2% ± 2.6% vs 6.4% ± 4.1%)
Alpha	Finger capillaries	PORH	EQI ≥2: Good endothelial function EQI <2: Endothelial dysfunction	Endothelial dysfunction (EQI <2), female gender, and severe clinical status at acute infection were independent risk factors of PASC
Original	Brachial artery	FMD, NMD, pulse-wave velocity PWV, Augmentation Index, IMT	Change in measurements of endothelial function	COVID-19 group had higher PWV, augmentation index, IMT vs healthy controls. COVID-19 and ASCVD groups had comparably altered FMD and NMD.
Original	Forearm	Acetylcholine and nitroprusside iontophoresis coupled with laser Doppler	Endothelium-dependent microvascular reactivity	Three months after disease onset, surviving COVID-19 patients had reduced acetylcholine-mediated vasodilation vs controls
Original	Brachial artery	FMD	Absolute change in brachial artery dilation	Less absolute change in brachial artery diameter from baseline to peak in COVID-19 positive (total control: 0.30 ± 0.12 mm, total COVID-19: 0.12 ± 0.07 mm)
Alpha	Brachial artery	FMD	Percent change in brachial artery dilation	Impaired FMD percentage in PASC group (1.65% ± 2.31%) vs control (6.51% ± 2.91%). FMD remained impaired compared to control (6.48% ± 3.08%) at 1- and 6-mo post discharge.
Unknown	Fresh human microvessels from adipose or atrial appendages.	Endothelial-dependent dilation to acetylcholine using video microscopy.	Endothelial-dependent dilation	Endothelial-dependent dilation is impaired for months after COVID-19. The degree of endothelial dysfunction is inversely related to time after COVID-19 infection.
Original	Finger capillaries	PPG	Changes in pulsatile arterial volume at the tip of the fingers from baseline to reactive hyperemia	RHI was significantly lower in group 2 compared to both group 1 and controls (0.53 ± 0.23 group 2 vs 0.72 ± 0.26 group 1 vs 0.79 ± 0.23 in control)
Unknown	Brachial artery	FMD	Percent change in brachial artery dilation	FMD values were significantly lower in RCE patients as compared to non-RCE (2.25% ± 1.94 % vs 3.90% ± 2.40%)
Original/ alpha	Brachial artery	FMD	Absolute change in brachial artery dilation	Significant linear trend of FMD reduction with increase in COVID-19 category/severity
Unknown	Internal carotid artery	Carotid ultrasounds and pulse wave analysis by tonometry	Carotid artery stiffness, carotid IMT and aortic augmentation	Group differences were observed for carotid stiffness (control, 5 ± 1 m s^{-1}; COVID-19, 6 ± 1 m s^{-1}), aortic augmentation (control, 3% ± 13%; COVID-19, 13% ± 9%). Carotid IMT was similar between groups (control, 0.42 ± 0.06 mm; COVID-19, 0.44 ± 0.08 mm).

coupled with laser Doppler demonstrates impaired endothelial-dependent microvascular reactivity in critical COVID-19 infections when compared to bacterial pneumonia patients without COVID-19.[30] Young healthy adults with more mild infections may develop microvascular dysfunction as measured by this technique.[31]

Reasons for the differences in findings between studies may relate to heterogeneity of the populations studied, differences in disease severity classification, differences in exposures to therapeutics, differences in strain, or differences in technique in measuring endothelial function. Greater standardization in patient selection and outcome measurements may help better determine if greater severity of endothelial dysfunction can reliably and reproducibly predict outcomes in acutely infected patients.

TABLE 2 Future Directions for Clinical Investigations of Endothelial Function and COVID-19

Predictive value of endothelial function measures and biomarkers for predictions of risk at the time of acute infection.
Impact of specific COVID-19 variants on endothelial function post-COVID-19.
Impact of vaccination status on the ability of COVID-19 to impair endothelial function both during and following infection.
Mechanisms connecting impaired endothelial function post-COVID-19 to PASC symptoms.
"Omics"-based studies connect phenotypical endothelial function to changes in the epigenome, transcriptome, and proteome to help determine mechanisms of effect and additional therapeutic targets.
Severity and extent of impaired endothelial function in patients with and without PASC.
Connections between PASC symptoms and measures of endothelial function.
Therapeutics targeting vascular endothelial health and their impact on PASC symptoms.

PASC = postacute sequalae SARS-CoV-2 infection.

ENDOTHELIAL DYSFUNCTION POST-COVID-19 INFECTION AND ITS CLINICAL IMPLICATIONS

Emerging data showing elevated risk of adverse cardiovascular events up to at least 12 months following recovery from acute COVID-19 infection, along with the known mechanistic relationship between endothelial dysfunction and adverse cardiovascular events, have fueled interest in the studies to define the mechanisms and severity of endothelial dysfunction in this patient population.[4] The development or persistence of clinical sequelae after 4 weeks of an acute COVID-19 infection is called PASC, informally known as "long COVID."[7] Data suggest that both macrovascular and microvascular endothelial dysfunction and vascular stiffening are present in patients weeks to months after an acute infection with and without a formal diagnosis of PASC, particularly in those with nonrespiratory symptoms.[7,8,24,25,30,32,33] **Table 1** summarizes the studies to date that studied macrovascular and microvascular endothelial function in patients with post-COVID-19 infection with and without PASC.

ENDOTHELIAL FUNCTION IN PATIENTS WITH PASC. In patients diagnosed with PASC from the alpha variant, brachial FMD is impaired for up to 6 months, though it remains to be seen if improvement in FMD correlates with clinical improvement.[25] Peripheral arterial tonometry data shows increased vascular stiffness is an independent risk factor for PASC from the alpha variant.[8] PASC patients who exhibit impaired executive function, verbal long-term memory, and spatial long-term memory have impaired brachial artery FMD, with greater cognitive impairments correlating with greater FMD impairments.[34]

ENDOTHELIAL FUNCTION IN PATIENTS POST-COVID-19 INFECTION WITHOUT PASC. In patients without a formal diagnosis of PASC but who have been studied for more than 4 weeks after recovering from acute infection from the original and variant strains, results are similar to those with PASC. Studies focusing on inflammatory and endothelial activation biomarkers similarly show residual inflammation and endothelial activation at least 6 months post-COVID-19 infection.[37] These patients have higher sustained values of C-reactive protein, erythrocyte sedimentation rate, IL-6, β-2 glycoprotein antibodies, endothelin-1, and angiopoietin-2 compared to healthy controls.[9,38] Compared to healthy controls, post-COVID-19 patients infected with the original strain also demonstrate inflammatory dysregulation with increased levels of asymmetric dimethylarginine, symmetric dimethylarginine, kynurenine/tryptophan ratios, von Willebrand Factor antigen and activity, and CD31+/CD42b-endothelial microparticles.[9] These patients also have lower circulating levels of L-arginine and ornithine.[9] In patients with mild disease who did not require hospitalization, increased levels of syndecan-1 (a sign of persistent glycocalyx injury and shedding) are seen for a median time of 88 days after disease onset.[6]

Studies of human microvessels (resistance arterioles approximately 100 μM in diameter) from adipose or atrial appendage biopsies demonstrate impaired endothelium-dependent dilation to flow and acetylcholine stimulation as far out as 16 months after resolution of COVID-19 infection, with the degree of endothelial dysfunction inversely associated with the time from the last positive test.[32] These effects may not be as profound in younger individuals with mild disease without risk factors for severe COVID-19 infection.[31]

In contrast, in large vessels, healthy young adults post-COVID-19 infection with the original strain, without comorbidities, show impaired brachial FMD and increased vascular stiffness compared to controls without prior infection.[24,39] These impairments extend out to 18 months postacute infection.[39] FMD data also demonstrate that endothelium-dependent vasodilation is impaired post-COVID-19 in patients infected with the original strain and remains impaired for at least 6 months posthospital discharge, particularly in men.[7,24] Backward multivariable regression analysis confirmed that impaired FMD was most strongly predicted by history of COVID-19 infection, even with infection 2 months prior to measurement.[10] A larger study of 658 patients 3 months after resolution of their infection with the original or alpha strain showed a positive correlation of FMD impairment with severity of infection.[35] Compared to never-infected healthy controls, post-

COVID-19 patients infected with the original strain also have impaired acetylcholine-mediated (endothelial dependent) vasodilation at least 3 months after disease onset.[11] Additionally, patients with post-COVID-19 infection from the original strain also show increased arterial stiffness to a level similar to patients with long established atherosclerotic cardiovascular disease.[9,40] Patients post-COVID-19 also show increased arterial stiffness by ultrasound and increased aortic augmentation via carotid pulse wave analysis despite infection approximately 1 month prior.[36]

CLINICAL IMPLICATIONS OF ENDOTHELIAL DYSFUNCTION AND POST-COVID-19 IN PATIENTS WITH AND WITHOUT PASC. Taken together, current data suggest that, regardless of PASC, patients post-COVID-19 have impaired endothelium-dependent vasodilation and increased arterial stiffness that may extend at least 18 months postacute infection. Patients hospitalized with severe COVID-19 have significant microvascular dysfunction determined by coronary flow velocity, and this dysfunction lasted up to 3 months in patients who had persistent symptoms from COVID-19.[41,42] These data are confirmed by cardiac magnetic resonance imaging and cardiac positron emission tomography studies showing a reduction in myocardial perfusion reserve.[43,44]

These data linking impaired endothelial function with prior COVID infection provide biological plausibility for the increase in myocardial infarction and stroke associated with COVID-19 infection that extends well past recovery from the infection.[39] In addition, residual inflammation postinfection appears to correlate with peak oxygen consumption (VO_2) and exercise capacity, which could be related to inflammatory-mediated chronotropic incompetence, sympathetic overactivation, and vascular dysfunction.[45,46] Patients with elevated markers of inflammation and cellular damage also show persistent fatigue and muscle weakness independent of concomitant disease.[47] Together, these data provide justification for testing therapeutics that improve vascular function, restore reverse adverse vascular remodeling, and reduce vascular inflammation to reduce long-term cardiovascular sequelae from COVID-19 infection.

TREATING COVID-19-RELATED VASCULAR DYSFUNCTION

To date, there are limited data on the efficacy of any interventions to improve endothelial function in acute infections and post-COVID-19. Corticosteroids have been implicated in the stabilization of the glycocalyx in response to COVID-19-induced endothelitis during acute infections, and the use of heparin sulfate mimetics has been suggested to help restore damage to the endothelial glycocalyx in COVID-19 patients.[48,49] Imatinib, an Abelson tyrosine-protein kinase inhibitor, may offer survival benefits to hospitalized individuals by decreasing the concentrations of IL-6, procalcitonin, E-selectin, and TNF-alpha, but failed to show any reduction in time to discontinuation of ventilation or supplemental oxygen, and survival differences were attenuated after adjusting for baseline characteristics.[50,51] HMG-CoA reductase inhibitors, while known to have protective endothelial effects, show conflicting evidence for protection against adverse outcomes of COVID-19 in patients at high risk for cardiovascular disease.[52,53] Data on the effects of both vector- and mRNA-based COVID-19 vaccination on endothelial function are limited and conflicting, and as a result, they are not discussed in this paper, which focuses primarily on the postacute COVID-19 syndrome stemming from acute infection.

For endothelial dysfunction implicated in post-COVID-19, 7 clinical trials are listed on ClinicalTrials.gov testing the impact of interventions on post-COVID-19 endothelial function. Three of these trials (NCT05185934, NCT05252923, and NCT05371925) are using glycocalyx-targeted interventions (sulodexide, a low-molecular-weight heparin, and Endocalyx, a dietary supplement). One study uses a combination of atorvastatin, ascorbic acid, folate, nicorandil, and nevibulol (NCT04631536), while an additional study uses an antioxidant derived from sea urchin eggs (NCT05531019).

Interestingly, there are 3 clinical trials listed on ClinicalTrials.gov (NCT04813718, NCT05227170, and NCT05556733) that aim to target the gut microbiota to improve endothelial function post-COVID-19. The concept of targeting the gut microbiota to improve cardiovascular health is well-founded in prior animal and human studies.[54,55] The intestinal immune system plays a critical role in systemic immunity, and its interaction with the systemic immune system plays a crucial role in determining the severity and outcomes of common pulmonary infections.[56,57] The composition of the gut microbiota impacts the efficacy of COVID-19 vaccination,[58] and SARS-CoV-2 infection alters the composition and metabolism of the gut microbiome, correlating with systemic host immune responses and inflammatory markers.[56,57] Pathological alterations in the composition of the gut microbiota have been observed for at least 6 months postinfection and are associated with greater residual

systemic inflammation and PASC symptoms, matching well with the pattern of impaired endothelial function in these patients.[59]

We and others have previously shown that supplementation with the probiotic *Lactobacillus plantarum 299v (Lp299v)* improves endothelium-dependent vasodilation and reduces inflammation in current smokers and individuals with coronary artery disease, known chronic hyperinflammatory states.[54,60] Lp299v reduces monocyte adhesion to the endothelium and suppresses both expression of IL-6 and toll-like receptor 9 (TLR9) activity.[60,61] TLR9 is activated by unmethylated CpG sequences in DNA molecules, present in mitochondrial DNA (mDNA), which increases inflammatory states and has been shown to be important to endothelial cell damage in COVID-19.[62]

IL-6 is elevated in patients with PASC and strongly correlates with TLR9 activation in disease states with high circulating cf-mDNA levels.[9,38] These data suggest *Lp299v* could potentially suppress inflammation and improve nitric oxide bioavailability and endothelial function in patients with post-COVID-19 endothelial dysfunction. This hypothesis is being tested in clinical trial NCT05227170.

KNOWLEDGE GAPS AND FUTURE DIRECTIONS

Multiple knowledge gaps remain that can be addressed with clinical studies. We summarize these gaps in **Table 2**. Whether endothelial function can be used as a barometer of disease severity or to predict risk of clinical decompensation, and, if so, which combination of measurements has the best predictive value, remains unclear. This work could be considerably augmented and better translated into patient care with additional larger studies leveraging plasma, endothelial cells, and microvessels obtained directly from patients during and following the COVID-19 infection. Using these samples, integrated epigenomic, genomic, transcriptomic, and proteomic approaches may yield additional pathophysiological insights that are directly translatable into the testing of novel biomarkers and therapeutics. The National Institutes of Health's PASC Infection Initiative should significantly help to enable and accelerate these types of studies with large enough study populations to assure valid and reproducible findings.

Additionally, the timeline and severity of impairment during convalescence from COVID-19 remain unclear. A better understanding of time course and severity will ultimately allow for better targeting of therapeutics, particularly for those recovering from COVID-19. Our evidence base for impaired endothelial function post-COVID-19 infection is derived mostly from individuals who were infected with either the original or alpha variant of the virus, and it is unclear how this evidence applies to currently prevalent variants. Careful attention to the timelines of infections and variants likely involved in future studies will help better inform the impact of newer variants on the vascular endothelium and cardiovascular risk. Data on patients infected specifically with the delta, omicron, and more recent variants are needed to determine if the impact on endothelial function has changed with the predominant variant. Additionally, whether vaccination status blunts acute and postinfection-associated endothelial dysfunction remains unknown. Finally, despite the global nature of COVID-19 pandemic, data isolated via an English-only search limits the ability to comprehensively include studies reported internationally.

While endothelial dysfunction appears to be present in patients with PASC, and its severity appears to correlate with PASC symptom burden, the mechanistic connections between endothelial dysfunction in PASC and PASC symptoms remain unclear. Clearer connection of PASC symptoms to endothelial dysfunction will require multidisciplinary approaches, including input from research-focused and clinical experts in infectious disease, neurology, pulmonology, and vascular physiology. Finally, there remains a paucity of data on: 1) interventions that improve endothelial function in those previously infected with COVID-19; and 2) data showing that therapeutic strategies targeting endothelial function in these patients reduce their excess risk of cardiovascular events.[4,63]

CONCLUSIONS

Endothelial dysfunction induced by COVID-19 appears to play an important part in the disease process and its complications. Multiple local and systemic mechanisms have been identified as contributing to COVID-19-induced endothelial dysfunction. Impaired endothelial function appears to contribute to morbidity and mortality both during acute infection and following infection. Additional work delineating targetable mechanisms of disease and the connections between endothelial dysfunction and the pathophysiology of PASC performed in humans and with human blood and tissue samples promises to significantly improve our understanding of the connections between endothelial dysfunction and clinical outcomes in COVID-19.

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