CARDIOVASCULAR, PULMONARY, AND RENAL PHENOTYPES IN COVID-19 SURVIVORS (CAPRICORN-19 STUDY)

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STUDY SUMMARY

Title	**CARDIOPULMONARY AND RENAL PHENOTYPES IN COVID-19 SURVIVORS**
Short Title	CAPRICORN Study
Methodology	Longitudinal cohort
Study Duration	Approximately 5 years
Study Center(s)	Single center conducted at the University of Pennsylvania
Objectives	**Primary objective**: To assess organ specific phenotypes and biomarker profiles to characterize specific cardiovascular, pulmonary, renal and other abnormalities and their role in functional status, quality of life and prognosis in COVID-19 survivors. **Specific objectives**: (1) To characterize cardiac and extracardiac phenotypes in COVID-19 survivors (compared to control subjects without a history of symptomatic COVID-19), and their relationship with: (a) quality of life; (b) functional status; (c) aerobic capacity; (d) prognosis. (2) To determine if the severity of COVID-19 is associated with measures of organ-specific structure and function after recovery. (3) To perform prospective follow-up of the cohort for the development of death, heart failure-related hospitalizations, and other adverse cardiovascular outcomes. (4) To establish a biorepository for future discovery.
Number of Subjects	200 COVID-19 survivors and 80 controls are expected to be enrolled at Penn Medicine.

Main Inclusion and Exclusion Criteria	Inclusion criteria: • Subjects aged 18 years and older, male or female; • Prior diagnosis of COVID-19 (acute symptoms consistent with COVID-19, confirmed by PCR, serologic or rapid antigen test) for the COVID-19 survivors group, or no confirmed or suspected prior history of symptomatic COVID-19 for the control group at the time of enrollment. Exclusion criteria: • Pregnancy at the time of the index COVID-19 episode or at the time of the first enrollment in the study; potential participants who become pregnant after the index COVID-19 episode will not be invited to participate until at least 3 months post-delivery. Inability to provide informed consent. • Known history of CVD (defined as HF, MI, coronary revascularization, serious arrhythmia, stroke, greater than moderate valvular heart disease, any cardiomyopathy, or PAD), glomerular disease or polycystic kidney disease prior to enrollment. • End stage kidney disease (ESKD; defined as requiring persistent renal replacement therapy or prior kidney transplant) prior to enrollment • History of chronic kidney disease (CKD) with eGFR <30 mL/min/1.73m2 prior to enrollment. In participants without a history of CKD, a measurement of eGFR will not be required for enrollment.

Study procedures	• The phenotyping protocol will include brief questionnaires, a brief ultrasound examination, arterial tonometry, measurements of endothelial function, a 6-minute walk test, pulmonary function tests, Short Physical Performance Battery, impedance cardiography, blood, urine and breath condensate collection and 24-hour blood pressure monitoring. A subset of participants will be asked to complete a cardiopulmonary supine bike exercise test. All tests except for VO2 testing will be repeated at approximately 18-month intervals for a total of 3 tests
Statistical Methodology	Statistical techniques will include t-tests, linear regression models, mixed models, network analysis, pathway analysis, and machine-learning approaches. The relationship between phenotypic profiles and incident risk will be assessed using a combination of Cox regression, Poisson regression, and Kaplan-Meier survival analyses, as needed. Corrections for multiple comparisons will be performed with false discovery rate correction, Bonferroni correction, or principal-components analysis-based correction, as appropriate.
Data and Safety Monitoring Plan	The Investigators will be responsible for the data quality management and the ongoing safety of subjects.

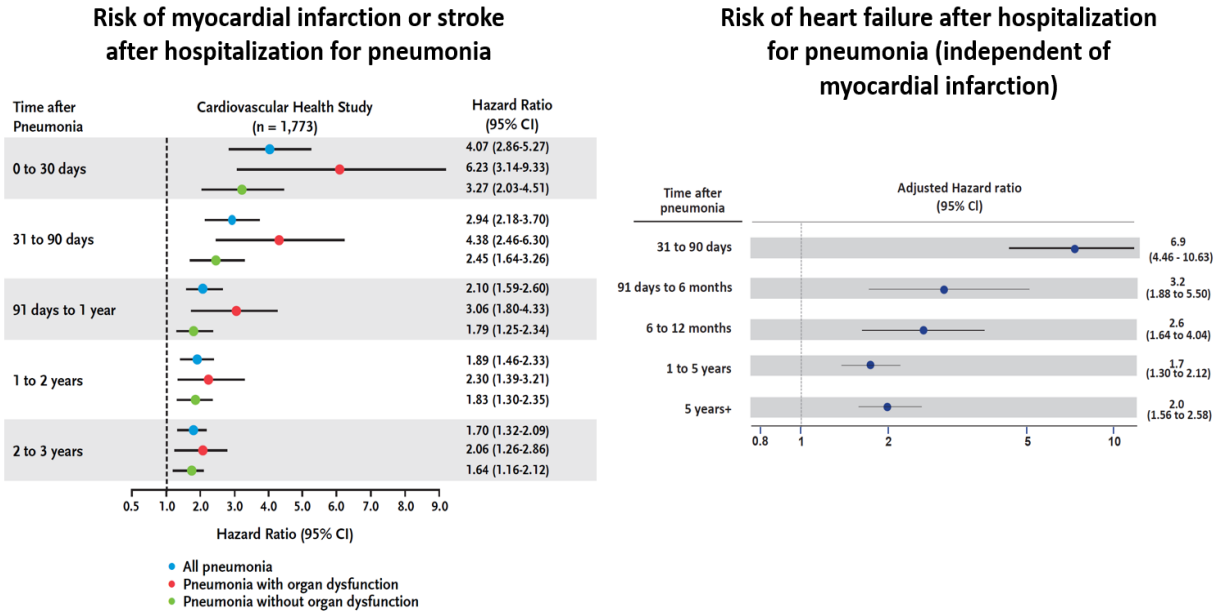
Background and Study Rationale

1 Introduction

A1. With the ongoing spread of coronavirus disease 2019 (COVID-19), there is a growing population of COVID-19 survivors. COVID-19 is a public health crisis. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for the COVID-19 pandemic, is associated with a high risk of pneumonia and multiorgan dysfunction.[1-4] Despite containment and mitigation efforts, COVID-19 continues to spread. As the case fatality rate for COVID-19 is estimated at <1%,[5] it follows that along with the pandemic increase of COVID-19 cases, there will be a marked increase of COVID-19 survivors in the general population.

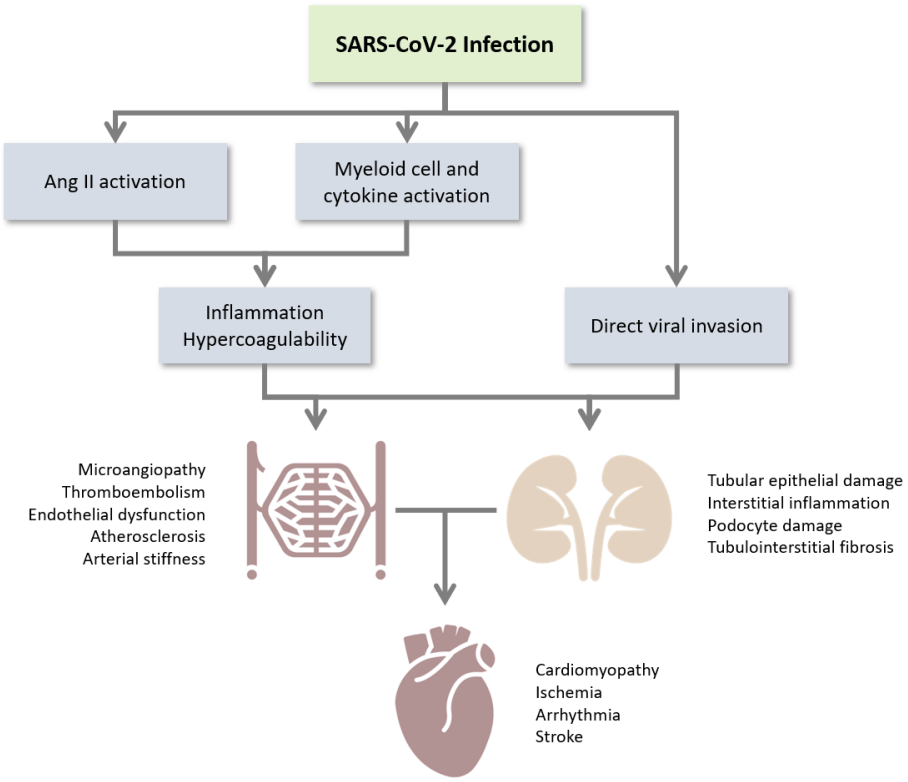
A2. COVID-19 survivors may be at an elevated risk of major adverse cardiovascular events (MACE). Pneumonia is the prominent feature of COVID-19.[1] Over the last several years, we and others demonstrated that survivors of pneumonia (irrespective of etiology), instead of going back to their pre-infection baseline health trajectories, enter a trajectory of increased risk of MACE,[6-20] including new onset heart failure, myocardial infarction (MI), and stroke, as demonstrated in **Figure 1**. For example, we identified 591 pneumonia cases in the Cardiovascular Health Study (CHS) and 680 pneumonia cases in the Atherosclerosis Risk in Communities (ARIC) study.[6,7] In CHS, 206 participants (35%) experienced a MACE during the 10 years after their pneumonia hospitalization. Of these, 104 (51%) experienced MI, 35 (17%) experienced stroke, and 67 (33%) experienced fatal coronary heart disease events. Between 91 days and 1 year following hospitalization, compared to matched controls, the HR for MACE was 2.10 (95% confidence interval [CI] 1.59-2.60); during the second year after hospitalization, the HR was 1.89 (95% CI 1.46-2.33). We found similar risk of MACE following pneumonia hospitalization in ARIC. In both cohorts, we observed that the risk associated with pneumonia was markedly higher than the risk associated with traditional risk factors (diabetes, hypertension, and smoking, among others).

Figure 1. Adjusted risk of myocardial infarction or stroke (left panel) and heart failure (independent of myocardial infarction; right panel) after hospitalization for pneumonia in the Cardiovascular Health Study. Adapted from **Corrales-Medina et al. *JAMA*.** 2015, 313(3):264-74 and **Corrales-Medina… Chirinos JA. *Am Heart J*.** 2015; 306–312.e6



A3. There is a multifaceted pathophysiologic relationship between pneumonia and cardiovascular (CV) disease. As previously discussed,[9] the pathogenesis of CV complications of acute pneumonia is complex, and involves systemic inflammation, endothelial dysfunction, increased arterial stiffness, abnormal arterial hemodynamics, abnormalities in coronary perfusion, and direct myocardial damage from a variety of factors. Importantly, some of these factors may result not only in acute complications, but also in long-term consequences for CV health. Moreover, systemic inflammation may persist for months after resolution of the acute event.[21] Inflammatory processes govern the progression of atherosclerosis from fatty streaks to advanced atherosclerotic plaques and their complication and evolution into clinical CV events such as MI and stroke.[22] Acute and chronic inflammation can lead to endothelial dysfunction, increase arterial stiffness and impair pulsatile arterial hemodynamics (all of which can mediate CV risk independently of atherosclerosis).[23] Even in the absence of systemic inflammation, progression of vascular and target organ damage initially sustained during acute pneumonia events could mediate long-term CV risk.

Figure 2. Conceptual model: Overview of the association of COVID-19 with microvascular and macrovascular disease, kidney dysfunction, and cardiovascular events



A4. Because of its unique pathophysiology, COVID-19 may distinctly predispose patients to chronic pulmonary disease, MACE and micro- and macrovascular damage/dysfunction. COVID-19 may predispose patients to particularly elevated risk of CV damage due to its distinctive proinflammatory and prothrombotic pathophysiology (**Figure 2**). In the acute phase, COVID-19 is associated with a relatively high incidence of thromboembolic events, MI, stroke, and severe arrhythmias.[24,25] Hyperinflammation and micro- and/or macrovascular thromboembolic or *in situ* thrombotic complications have been observed acutely and sub-acutely in COVID-19 in the vasculature of the lungs, spleen, brain, gut, kidneys, and periphery,[26-28] even with therapeutic anticoagulation.[28] Thromboses have been identified several weeks following critical illness, suggesting a protracted duration of pro-thrombotic risk post-hospitalization. Importantly, viral inclusion bodies within endothelial cells and sequestered mononuclear and polymorphonuclear cellular infiltration of the endothelium are seen in COVID-19, along with evidence of endothelial cell apoptosis.[37] This is consistent with the presence of endotheliitis, which could lead to acute and chronic vascular dysfunction resulting in target organ dysfunction and increased CV risk. Thromboembolic disease may also result in reduced aerobic capacity and exercise intolerance due to potentially chronically effects on the pulmonary vasculature.[29,30]

Furthermore, studies demonstrate the potential for persistent restrictive lung disease several months following recovery from acute COVID-19[31] and possible risk of pulmonary fibrosis.[32]

A5. COVID-19 may also predispose patients to clinical and subclinical kidney target organ damage, which could mediate long-term CV risk. Hospitalization for pneumonia of all causes is associated with a persistently elevated risk of subsequent CKD.[33,34] For example, in a Swedish cohort of 284,198 men born from 1952-1956, there were 5,822 initial pneumonia episodes observed over 37 years of follow up.[33] In the year following the first episode, pneumonia was associated with a 14.5-fold (95% confidence interval [CI] 10.41-20.32) elevated hazard of incident CKD; there was a 5.2-fold (95% CI 3.91-6.93) elevated hazard of incident CKD in the first five years following pneumonia. COVID-19 is particularly associated with a high incidence of acute kidney injury.[35,36] The mechanisms of acute kidney injury in COVID-19 seem to be largely immune- and coagulopathy-mediated, including marked interstitial inflammation, microangiopathy with peritubular and glomerular erythrocyte aggregation and fibrin thrombi, endothelial damage, and tubular necrosis.[35,37] The presence or severity of acute kidney injury is often missed in hospitalized patients, and many patients may have subclinical kidney injury that is not identified by typical measurements (i.e. creatinine-based eGFR) during hospitalization.[38,39] Even among individuals without evidence of acute or persistent kidney damage while hospitalized, the hyperimmune and coagulopathic mechanisms of COVID-19 may predispose COVID-19 survivors to an elevated risk of subsequent decline in kidney function. Our group has preliminary data demonstrating a strong association of decline in kidney function with elevated 1- and 3-year risk of MACE, regardless of baseline kidney function (see **A8. Preliminary Data**). Thus, decline in kidney function among COVID-19 survivors may be an important mediator of future CV risk in these patients.

A6. Several interrelated mechanisms of COVID-19 may be linked to acute multiorgan dysfunction and intermediate- and long-term target organ damage in COVID-19 survivors. In comparison to other bacterial and viral pneumoniae, inflammation and hypercoagulability in COVID-19 may be related to its unique interaction with the renin-angiotensin system (RAS). Angiotensin-converting enzyme 2 (ACE2), a counter-regulatory component of the RAS, facilitates SARS-CoV-2 entry into host cells in the respiratory tract.[40] ACE converts angiotensin I (Ang I) to Ang II, which acts on the Ang II type 1 receptor (AT_1R), resulting in vasoconstriction, sodium and fluid retention by the kidney, oxidative stress, inflammation, fibrosis, and impaired fibrinolysis.[41,42] In direct opposition to the cascade of physiological effects of the ACE/Ang II pathway, the net effect of the ACE2/Ang-(1-7) pathway is vasodilation and anti-inflammation. ACE2 hydrolyzes Ang II, converting it to Ang-(1-7). The conversion of Ang II to Ang-(1-7) diminishes the availability of Ang II to bind AT_1R, forestalling the vasoconstrictive, proinflammatory, and prothrombotic effects of AT_1R activation.[41-45] Additionally, Ang-(1-7) acts on the Mas receptor, resulting in vasodilation, natriuresis, and a reduction in oxidative stress and inflammation.[46,47] Collectively, Ang-(1-7) counteracts the pro-inflammatory and pro-fibrotic effects of Ang II in the heart and kidneys.[48,49] In animal models, unopposed Ang II in the setting of diminished ACE2 upregulated inflammatory cytokines interferon-γ, interleukin-6, and monocyte chemoattractant protein-1, and increased phosphorylation of extracellular signal-regulated protein kinase (ERK)1/2 and c-Jun N-terminal kinase (JNK)1/2 signaling pathways.[50] Furthermore, AT_1R activation by Ang II has pro-thrombotic effects, including enhanced platelet activation and impaired fibrinolysis, resulting in hypercoagulability.[44,51,52] Ang II also stimulates plasminogen activator inhibitor-1, further promoting thrombus production.[42] The pro-thrombotic effects of Ang II are downregulated by ACE2-mediated conversion into Ang-(1-7) and subsequent Ang-(1-7) signaling via MasR. ACE2 is more prominently expressed in the kidneys than in the lungs, and direct viral invasion of the kidneys may promote systemic abnormalities in RAS activity.[53,54] Dysregulation of this pathway may contribute to the hyperinflammation, hypercoagulability, and endothelial dysfunction in COVID-19, and may mediate both acute and long-term target organ CV and kidney damage.[55,56]

A7. The incidence, severity, and mechanisms of enduring CV effects of COVID-19 are unknown, and urgently require prospective, systematic evaluation. The current proposal aims to prospectively evaluate the natural history of cardiovascular and kidney disease in COVID-19 survivors, and to identify risk factors for adverse mid- and long-term outcomes in these patients. Most studies evaluating COVID-19 hospitalizations rely upon administrative datasets, and are thus prone to misclassification of outcomes, unmeasured confounding, and confounding by indication for testing (e.g., those with underlying comorbidities are more likely to undergo frequent testing, whereas those without underlying comorbidities may not undergo follow-up testing at all). Administrative and retrospective studies also rely upon patients receiving all of their care within the same health system and do not adequately account for outmigration (e.g., due to change in insurance or change in geographic location) or loss to follow up, which may be exacerbated in the current setting due to high rates of unemployment and other economic pressures surrounding the COVID-19 pandemic. Administrative studies will also lack detailed, prospective, quantitative assessment of vascular function, a likely important mechanistic contributor to MACE in these patients. Moreover, to the degree that COVID-19 is a new disease and the mechanisms that may mediate its relationship with subsequent CV risk are unknown, it is essential to couple a careful assessment of incident CV events with deep mechanistic phenotyping of vascular and renal structure and function. Our team is experienced with these approaches and has been applying them in other settings (most notably, pneumonia of all cause, heart failure (HF) with preserved ejection fraction, and CKD). Our team also has a track record of translation of mechanistic phenotypes to the testing of novel candidate therapies. We aim to apply our experience and skills in the most expeditious fashion to contribute to a better understanding of intermediate- and long-term CV risk in the rapidly growing population of COVID-19 survivors.

2 Study Objectives

Primary objective: To assess organ specific phenotypes and biomarker profiles to characterize specific cardiovascular, pulmonary, renal and other abnormalities and their role in functional status, quality of life and prognosis in COVID-19 survivors.

Specific objectives:

(1) To characterize cardiac and extracardiac phenotypes in COVID-19 survivors, and their relationship with: (a) quality of life; (b) functional status; (c) aerobic capacity; (d) prognosis.
(2) To determine if the severity of COVID-19 is associated with measures of organ-specific structure and function after recovery.
(3) To perform prospective follow-up of the cohort for the development of death, heart failure-related hospitalizations, and other adverse cardiovascular outcomes.
(4) To establish a biorepository for future discovery.

3 Investigational Plan

3.1 General Design

This is a prospective cohort study of 200 COVID-19 survivors and 80 control subjects without a known history of symptomatic COVID-19. Among survivors, we will prioritize subjects who had their index infection within the last 12 months. However, will allow for index infections acquired outside of this timeframe to facilitate enrollment. We will collect detailed measures of cardiovascular, pulmonary, renal structure and function, along with various other measures, as follows:

(1) Endothelial function: flow-mediated brachial artery dilation and venous endothelial cell harvesting for molecular analyses.

(2) Arterial stiffness: carotid-femoral pulse wave velocity and carotid artery stiffness measurements.
(3) Central pulse pressure and measurements of stroke volume and arterial compliance by impedance cardiography.
(4) Subclinical atherosclerosis: carotid intima-media thickness and ankle-brachial index.
(5) Pulmonary Function tests
(6) Microvascular function: forearm ischemic vasodilation, and microvascular imaging with sublingual in vivo microscopy
(7) A brief focused ultrasound test (liver, kidney, skeletal muscle, cardiac and carotid ultrasound), performed by a trained research team member.
(8) Biosample collection (blood, urine and breath condensate)
(9) A 6-minute walk test
(10) A Kansas City Cardiomyopathy Questionnaire (KCCQ)
(11) A subset of questions from the PROMIS Questionnaire to evaluate mental and physical function
(12) A long COVID symptom Questionnaire
(13) EQ-5D-5L Questionnaire
(14) Social determinants of health questionnaire
(15) Short Physical Performance Battery (SPPB)
(16) In a subset of participants, we will also perform supine bicycle exercise tests to assess aerobic capacity (i.e., peak oxygen consumption, VO_2).

These tests are brief and in combination will take about ~5 hours. These assessments will be performed upon enrollment (baseline visit) and will be repeated at approximately 18-month intervals for a total of 3 tests (except for bicycle testing, which will only occur at baseline). Participants will also be followed prospectively for incident cardiovascular events via a combination of medical record reviews and phone calls *as needed in order to adjudicate specific outcomes. Adjudication of all-cause death may be assisted using data from the Social Security Death Index.* Expert clinical adjudicators will identify MACE (incident HF, MI, coronary revascularization, serious arrhythmia, stroke, peripheral artery disease [PAD], or death) by detailed review of hospitalization records.

Given availability of staff and/or Center for Human Phenomics Sciences staff to complete study procedures such as endothelial cell harvesting, supine bike VO2 testing, and ultrasound, some participants may be asked to have their procedures done in different days to enhance data completeness and feasibility of scheduling.

3.2 Inclusion Criteria

- Subjects aged 18 years and older, male or female;
- Prior diagnosis of COVID-19 (acute symptoms consistent with COVID-19, confirmed by PCR, serologic or rapid antigen test) for the COVID-19 survivors group, or no confirmed or suspected prior history of symptomatic COVID-19 for the control group at the time of enrollment.

3.3 Exclusion Criteria

- Pregnant women;
- Inability to provide informed consent.
- History of CVD (defined as HF, MI, coronary revascularization, serious arrhythmia, stroke, greater than moderate valvular disease, any cardiomyopathy, or PAD), glomerular disease or polycystic kidney disease prior to enrollment.

- End stage kidney disease (ESKD; defined as requiring persistent renal replacement therapy or prior kidney transplant) prior to enrollment
- History of chronic kidney disease (CKD) with eGFR <30 mL/min/1.73m2 prior to enrollment. In participants without a history of CKD, a measurement of eGFR will not be required for enrollment.

3.4 Subject Recruitment

We will screen patients who received care at Penn Medicine for acute COVID-19 as well as patients referred to the post-COVID-19 clinic. We will also consider interested participants who reach out to us as a result of advertisements or other outreach efforts. Patients who meet the inclusion criteria will be identified and participants will be approached by a member of the study team for participation in the study. The study team will answer questions the subject has regarding participation, prior to signing the consent document.

3.5 Vulnerable Populations:

Children, pregnant women, fetuses, neonates, and prisoners are not included in this research study.

3.6 Study Procedures

3.6.1 Visit 1

A member of the study team will approach the patient for participation in the study and review the informed consent document with them. The study team will answer questions the subject has regarding participation, prior to signing the consent document.

After signing consent for participation in the study, the subject will undergo a series of brief procedures designed to obtain key phenotypes of various organ systems. All study procedures will be performed by a trained research team member. In combination, these procedures take ~2 hours.

Quality of Life Assessments: Subjects will then be given time to complete the short version of the Kansas City Cardiomyopathy Questionnaire (KCCQ), EQ-5D-5L, PROMIS Questionnaire and the long COVID symptom and impact tools.[57]

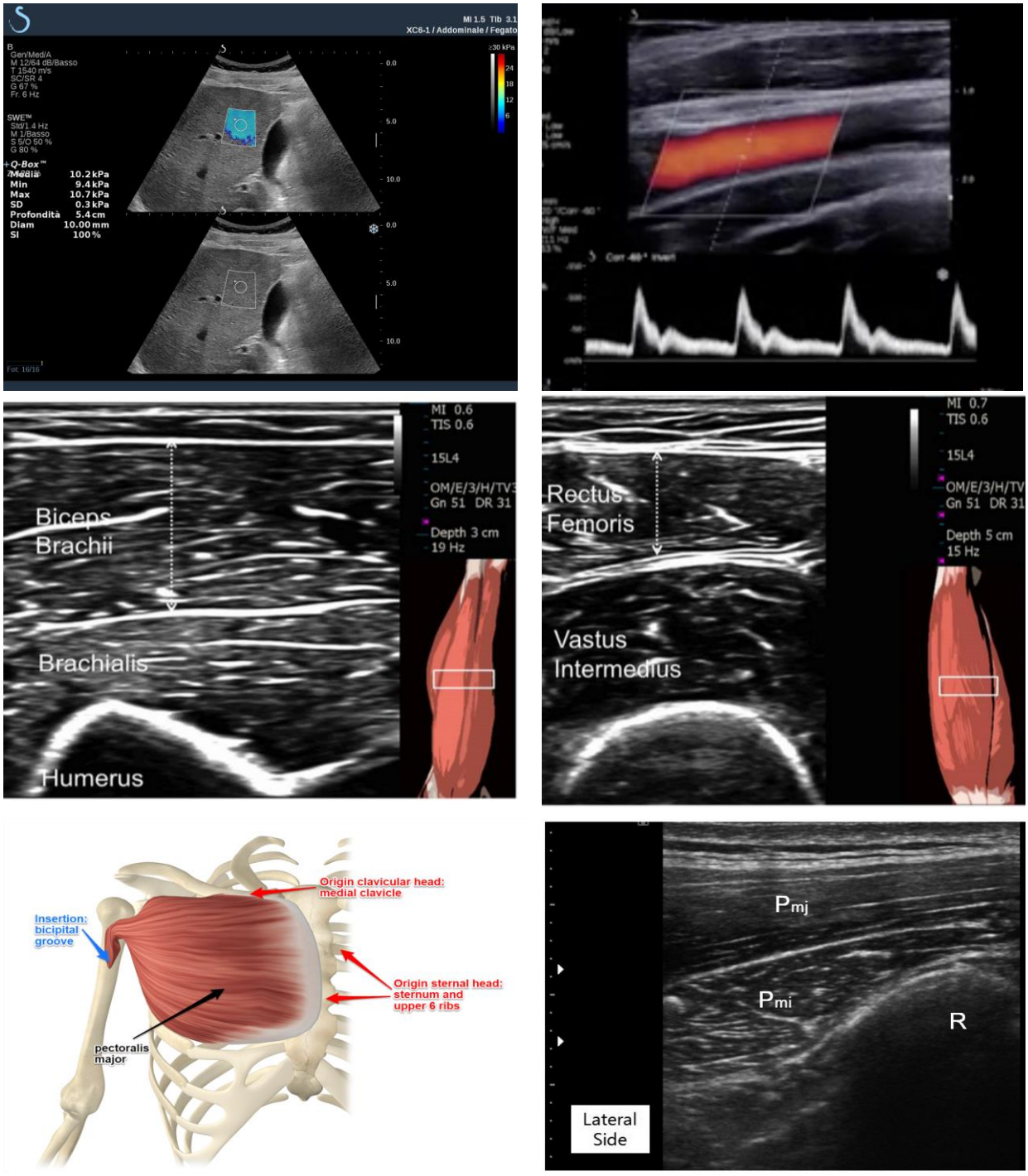
Brief ultrasound protocol: we will use an Airexplorer Mach30 (SuperSonic Imagine; France; 510K K191007) ultrasound device. The ultrasound protocol will include the interrogations listed in **Table 1** and schematized in **Figure 1**. This ultrasound protocol is brief and in will take ~ approximately 30 minutes in total. In patients in whom these areas are not accessible (amputation, bandages covering skin in areas of interrogation) or if time does not permit, the respective ultrasound acquisitions will not be performed. This procedure carries no known risks.

Table 1. Ultrasound scanning protocol

Acquisition	Description	Phenotypes of interest	Probe
Liver and kidney	Liver shear wave elastography in the supine to slightly left lateral decubitus position, with the probe on the lower right intercostal spaces, mid axillary line. B-mode, color Doppler and shear wave elastography acquisitions, followed by	Liver stiffness, echogenicity	Curved probe

	unilateral kidney shear wave elastography from the anterolateral abdominal approach		
Pectoralis major, femoris rectus, and biceps thickness	**Pectoralis Major:** Supine position. Right arm behind the neck. Probe on the right mid-axillary line, at the level of the angle of Lewis. Minimal compression. **Femoris Rectus:** Supine position. Knee extended and relaxed. Midpoint between the right patella and anterosuperior iliac spine. Minimal compression. **Biceps:** Supine position. Arms extended and relaxed. Midpoint between the acromion and elbow. Minimal compression. B-mode, color Doppler and shear wave elastography acquisitions.	Muscle thickness (sarcopenia), stiffness, echogenicity, and perfusion	High-frequency linear array probe
Carotid ultrasound	Right common carotid B-mode with color Doppler, single plane.	Carotid distensibility, wall stiffness, intima-media thickness, pulsatility, wave intensity	High-frequency linear array probe
Limited Cardiac and lung Ultrasound	Limited parasternal, 4-chamber and 2-chamber view clips, mitral inflow and mitral/tricuspid annular tissue Doppler interrogations Limited lung scan for lung B lines	Diastolic function, myocardial strain and stiffness	Cardiac probe

Figure 1. Sample images of liver elastography, carotid flow, rectus femoris, and pectoralis major thickness.



Pmj=pectoralis major; Pmi=pectoralis minor; R=rib

Carotid images will be processed offline to assess the following metrics:

1) *Carotid intima media thickness (CIMT)*: We will measure CC-IMT in near and far walls and in the anterior, lateral and posterior projections using automated edge-detection software (Carotid Studio; Quipu, Italy). A carefully trained technician will measure CIMT at these sites as the distance between the intimal-luminal and the medial-adventitial interfaces of the carotid artery wall,[58] as done previously by our group.[59-62] CIMT will be computed as the mean of all measurements.
2) *Carotid stiffness*: We will perform time-resolved semi-automated wall tracking of the common carotid lumen diameter using view that best demonstrates both the near and far walls using automated edge-detection software (Carotid Studio; Quipu, Italy). As recently reviewed by our group, when performed assessments of local stiffness it is essential to avoid confounding by wave reflections.[63] Carotid distension waveforms will be used along with calibrated carotid tonometry waveforms to calculate local carotid PWV (a measure of wall stiffness), using an expression equivalent to the Bramwell-Hill equation, which combines pressure and diameter (ln(Dia)P method), which is not susceptible to wave reflections: $PWV = \sqrt{\frac{1}{2\rho}\frac{dP}{dln(Dia)}}$, where PWV is calculated from the slope of the linear segment of the loop constructed from plotting pressure as a function of the natural logarithm of diameter[64].

Impedance Cardiography (ICG): Study subjects will undergo a resting impedance cardiogram (Z-logic; Exxer, Buenos Aires, Argentina) for the evaluation of cardiac output, stroke volume, and cardiac contractility, which will allow the estimation of arterial compliance and other parameters of interest. ICG will be performed depending on time and equipment availability. ICG is a form of plethysmography that utilizes changes in thoracic electrical impedance to estimate changes in blood volume in the aorta and changes in fluid volume in the thorax. The ICG procedure involves the placement of electrical leads (similar to ECG leads) on the patient's neck and chest. A low-amplitude, high-frequency alternating current is delivered from some sensors while other sensors detect instantaneous changes in voltage. As suggested by Ohm's law, when a constant current is applied to the thorax, the changes in voltage are directly proportional to the changes in measured impedance. The overall thoracic electrical impedance, called base impedance (Z0) is the sum of the impedances of the components of the thorax, including fat, cardiac and skeletal muscle, lung and vascular tissue, bone, and air. Changes from thoracic electrical impedance occur due to changes in lung volumes with respiration and changes in the volume and velocity of blood in the great vessels during systole and diastole. The rapidly changing component of chest impedance is filtered to remove the respiratory variation, leaving the impedance changes due to ventricular ejection. These impedance changes have been shown to be accurate predictors of stroke volume, and with the use of mathematical algorithms, ICG allows for accurate non-invasive measurements of stroke volume and cardiac output. [65,66] This procedure is non-invasive and does not produce pain or discomfort to the subject.

Pulmonary Function Testing: We will perform a basic expired spirometry and measurements of CO diffusion capacity (DLCO) and lung volumes.[67] We will utilize an FDA-approved Easyone Pro Lab Respiratory Analysis System (NDD systems Andover, Massachusetts; 510K K161534). In this test, subjects perform a forced expiration into a disposable probe meter (spirometry), primarily to determined forced vital capacity and forced expiratory capacity in 1 s (FEV1). As recommended, DLCO measurements will be performed in duplicate for enhanced accuracy. In these measurements, participants will inspire CO gas one deep inspiration, followed by an ~8-10 second breath-hold, as tolerated, followed by normal breathing into the disposable mouthpiece for ~2 minutes, during which the machine measures the washout time of CO from the alveoli. Lung volumes will be performed by measuring the washout time of inhalation of 100% oxygen.

This test will in total ~20 minutes. Pulmonary function tests will not be performed if participants are receiving >2L/min of supplemental oxygen.

Aerobic capacity: In a subset of participants who agree to additional testing, have no contraindications to exercise testing (such elevated fall risk or severely elevated blood pressure >180/100) and contingent on availability of equipment, personnel and funding, we will use a supine bicycle exercise protocol in conjunction with expired gas analysis to assess oxygen consumption (VO_2) during exercise. This subset of subjects will perform a maximal exertion limited exercise test using a graded-exercise protocol. We will use a supine cycle ergometer designed for stress echocardiography (Stress Echo Ergometer 1505, Medical Positioning, Inc, Kansas City, MO). Subjects will undergo expired gas analysis with a Parvo Medics True One 2400 device (Parvo Medics, Sandy, UT) or equivalent. Resistance began at 15 W for 3 minutes, increasing to 25 W for 3 minutes, and then increasing by 25 W every 3 minutes thereafter. Breath-by-breath information will be recorded. We will use custom-designed software already developed in Matlab (MathWorks, Natick, MA) at our lab for offline processing and quantification of all exercise data.[68] All data quantification will be blinded to treatment. Given costs considerations and funding availability, we will offer this additional component to former participants of the FERMIN trial (NCT04517396) but can be expanded to other participants as additional funding becomes available.

Arterial tonometry*:* Carotid-femoral PWV will be measured with arterial applanation tonometry, which considered the clinical gold-standard method for the assessment of large artery stiffness.[63,69,70] We will use a commercially available system (SphygmoCor CVMS, AtCor Medical) with a high-fidelity applanation tonometer (Millar Instruments). This involves sequential recordings of carotid and femoral arterial waveforms using a pen-like high-fidelity applanation tonometer placed on top of the artery on the body surface, along with electrocardiographic measurements so that the R wave of the QRS can be used as a time-reference point to compare both recordings and compute the pulse transit time between the carotid and femoral locations. Surface measurements of distance between carotid and femoral sites are performed to estimate intra-aortic path length (80% of the direct body surface distance between the sites). Carotid-femoral PWV is computed as distance/transit time. Radial and carotid applanation tonometry will be performed as well. Brachial arterial pressures will be measured with a fully validated oscillometric device (Omron HEM 907-XL BP monitor; Omron Healthcare; 510K K032305 or BP+ device, 510K K121266). Arm circumference will be measured, and the appropriate cuff size will be placed. Radial waveforms will be calibrated with brachial systolic and diastolic pressures. Radial pressures will be used to calibrate the carotid pressure waveform, which serves as a surrogate of the central pressure waveform, and will be used to obtain central pulse pressure.

Flow mediated dilation (FMD): Brachial artery FMD and hyperemic flow velocity, which evaluate conduit artery endothelial function and microvascular function, respectively,[71,72] are important indicators of vascular health and predictors of CV risk.[73,74] FMD will be measured using a semi-automated device equipped with an *ad hoc* vascular ultrasound probe (UNEX Corporation, Nagoya, Japan). In this test, a brachial cuff is inflated to 50 mmHg above the systolic blood pressure, which is measured in advance. The inflation is held for 5 minutes, and the cuff is then deflated. The maximum diameter of the blood vessel of the same region is tracked continuously after deflation. The FMD is calculated as follows: FMD (%) = (maximum diameter − diameter at rest) × 100/diameter at rest. Hyperemic brachial artery flow velocity will be determined by using pulsed wave Doppler to detect the peak flow velocity during the first 15 seconds post-cuff release.[74-76] During the test, we will use wireless near-infrared spectroscopy (NIRS) devices to measure microvascular oxygenation as feasible. We will use a wireless PortaMon device and a wireless PortaLite device (Artinis medical systems; The Netherlands) to measure forearm

oxygenation at rest and during the FMD procedure. The NIRS devices are research-only devices and will not be used to make medical decisions.

Ankle-brachial index (ABI): ABI uses non-invasive oscillometric blood pressure measurements in the ankle and upper arm to provide a measure of overt or clinically occult peripheral arterial disease. It is a well-established predictor of cardiovascular events and death.[77-80] We will perform automated ABI measurements using a VaSera Device (Fukuda-Denshi; Japan; 510K K115121).

Sublingual microscopy*:* Sublingual microcirculation will be visualized using sublingual capillaroscopy using a hand-held sublingual microscope (Microscan device; Microvision Medical; Amsterdam, Netherlands) by a trained technician.[81] This is a research-only device and will not be used to make medical decisions. At least five videos of the sublingual microcirculation will be recorded from different positions and will undergo appropriate quality control.[81,82] Videos will be analyzed using dedicated software (AVA 4.3C software; Microvision Medical; Amsterdam, Netherlands).[83] We will calculate the Becker score,[81] which is based on the principle that density of the vessels is proportional to the number of vessels crossing arbitrary lines. In this score, three equidistant horizontal and three equidistant vertical lines are superimposed on the microscopic image and vessel density is calculated as the number of vessels crossing the lines divided by the total length of the lines. Microvascular density, our primary metric of microvascular health, will be computed as the density of vessels <20 μm in diameter. We will also compute the proportion of perfused vessels as follows: 100 × (total number of vessels - [no flow + intermittent flow])/total number of vessels. Perfused vessel density, an estimate of functional capillary density, will be calculated by multiplying vessel density by the proportion of perfused vessels.[81,84]

6-minute walk test: If they are able, participants will be asked to perform a 6-minute walk test. During this test, participants are asked to walk as far as possible during a 6-minute time interval. A corridor will be selected in which the participants can walk with minimal interference. During the procedure, one of the study staff members will supervise the test. Verbal prompts will be given, as suggested by the American Thoracic Society 2002 guidelines on the 6-minute walk procedure.[85] During the test, we will use wireless near-infrared spectroscopy (NIRS) devices to measure microvascular oxygenation as feasible. We will use two wireless portamon devices and the wireless Brite device (Artinis medical systems; The Netherlands) to measure forearm, calf and brain oxygenation at rest and during the 6-minute walk test. The NIRS devices are research-only devices and will not be used to make medical decisions.

The 6-minute walk test is an important phenotype because it offers an objective, well validated metric of exercise capacity. During this test, each patient determines the intensity of their exercise, and the test has been performed in thousands of older persons and thousands of patients with heart failure or cardiomyopathy without serious adverse events. [85] In order to be prudent, however, we will exclude patients with exertional chest pain, worsening chest pain during the previous month or a history of recent myocardial infarction (within a month), a resting heart rate of more than 120, a systolic blood pressure of more than 180 mm Hg, and a diastolic blood pressure of more than 100 mm Hg. These are consistent with current guidelines [85].

Thermal images: thermal imaging patterns depend on vascular and neural function and have been associated with cardiovascular risk factors.[86] For exploratory purposes, we will acquire thermal images of the face and bilateral hands will be obtained using a commercially available compact thermal camera (FLIR C2; FLIR, Wilsonville, OR). This device is for research only and will not be used to make medical decisions. Non-contact thermal imaging is used to detect patterns of skin surface heat emissions from patients with various conditions, which has been shown to correlate with cardiovascular disease risk. This is a non-contact procedure that is not

associated with any known risks.

Breath condensate collection: Exhaled breath condensate (EBC) analysis is a developing field with tremendous promise to advance personalized, non-invasive health diagnostics as new optimized analytical instrumentation platforms and detection methods are developed.[87-93] Exhaled breath contains potentially valuable metabolomic content due to gas exchange with blood at the pulmonary alveolar membrane interface. Various disease states often significantly change the entire pattern and relative abundances of volatile organic compounds (VOCs) in exhaled breath.[87-93] We will collect the condensed exhaled fraction of alveolar air for about 5-8 minutes using a commercially available device (Turbo AAV 14; MEDIVAC, Parma, Italy). This system utilizes plastic air collectors coupled with a condenser equipped with a CO_2 detector that routes collection of air (via an electronic valve) only when CO_2 levels are consistent with alveolar air (as opposed to dead space air). This device is for research only and will not be used to make medical decisions. We will use only new or sterilized plastic collection tubes. Exhaled air condensate will be aliquoted and frozen at -80°C. This procedure carries no known risks.

Urine collection: In all participants, 10-mL of urine will be collected and aliquoted.

Venous endothelial cell harvesting: endothelial cells will be collected from the antecubital vein using 4 soft, flexible, sterile 0.021 inch J-wires which are briefly advanced through the standard intravenous catheter. A trained Research Nurse will place the intravenous catheter and advance the J-wire to obtain the cells. Once the J-wire is advanced and pulled back through the venous catheter, the distal portion of the wire is transferred to a 50-ml conical tube containing a buffer solution for analyses. Analyses will include RNA sequencing to assess gene expression and fixing for immunohistochemistry. This procedure will be optional depending on the availability of a trained research nurse at the time of the visit.

Venous blood collection: In all participants, we will collect 40 mL of venous blood, which will be aliquoted as plasma, serum, buffy coat (for future DNA extraction and potential future genetic testing as funding becomes available) and PAX gene tubes (for future RNA extraction).

24-hour ambulatory blood pressure monitoring: Ambulatory BP monitoring will be performed using the SunTech Oscar2 ambulatory BP monitor to obtain 24-hour brachial blood pressure measurements as well as estimates of central blood pressure [94]. Prior to placement of the monitor, resting BP will be measured on both arms while sitting and orthostatic blood pressure will be checked in one arm with a standing blood pressure checked approximately 3 minutes after rising. If the difference in systolic BP is <10mmHg the non-dominant arm will be used for monitoring; however, if the difference in systolic BP is ≥10mmHg the arm with higher pressure will be used. The mid-arm circumference will be measured and recorded and a cuff appropriate for the arm circumference will be used. Participants will be instructed to engage in usual daily activities and avoid strenuous exercise for the 24-hours in which the ABPM is in place. BP will be measured every 30 minutes for 24- hours. Participants will keep a diary documenting time to bed, time waking up, medications taken, and any other events of significance.

Short Physical Performance Battery (SPPB): SPPB is a well-established and objective assessment tool to evaluate lower extremity function and mobility. It is predictive of all-cause mortality[95] and incident disability[96] and has been associated with incident cardiovascular events[97] in older adults. A study team member will administer 3 physical tests and assess the participant's balance, gait, strength, and endurance by examining their ability to stand for up to 10 seconds with feet positioned in three ways (together side-by-side, semi-tandem and tandem), time to walk 4 meters, and time to rise from a chair and return to the seated position 5 times. Each test is scored on a 0 to 4 scale using previously validated norms and summed for

an overall score range of 0 to 12, with 0 indicating the lowest physical performance, and scores of 12 indicating the highest performance.[97]

Other data collection:

We will perform:

- A research-grade review and quantification of available cardiac imaging studies (echocardiograms, cardiac MRIs, CT scans) in Dr. Chirinos' imaging core lab.
- A medical record review including symptoms, past medical history, imaging, laboratory values, results of physical exams, vital signs, hemodynamic values, and other test results,w with special emphasis will be placed on collection of data related to the severity of the COVID-19 episode.
- We will also register data collected routinely in the post-COVID-19 clinic.
- The severity of the index COVID-19 episode will be determined using a hierarchical global rank score[98-100] in which all subjects will be ranked based on the characteristics of their index COVID-19 episode according to 4 factors: (1) the number of days supported by mechanical ventilation (invasive or non-invasive) or extracorporeal membrane oxygenation; (2) The inspired concentration of oxygen/percent oxygen saturation (FiO_2/SpO_2**)** ratio area under the curve during the longest hospital admission; (3) For participants who didn't get hospitalized, we will use a modified SOFA score that includes the cardiac, respiratory, coagulation and renal domains of the SOFA score because these components can be easily and reliably adjudicated using electronic medical record review. For the respiratory component of the SOFA score, we will apply a modified score used in which the arterial oxygen saturation is used instead of measured arterial oxygen saturation.

Feasibility: all measurements will be acquired only whenever feasible. Some of these measurements may be non-feasible due to patient-specific factors including anatomic factors, technical difficulty and/or logistical issues. When measurements are not feasible, the reason will be systematically recorded.

3.6.2 Follow-up

A member of the study team will perform reviews of the subject's electronic health record at regular intervals. Subjects will be contacted via telephone to assess whether they have been hospitalized or sustained any other adverse cardiovascular events. MACE will be defined as (1) acute coronary syndrome, (2) hospitalization for HF, (3) serious cardiac arrhythmia, (4) PAD, (5) cerebrovascular events, and (6) CV death.[74,101-106] Event ascertainment will be an iterative process implemented every six months that will begin with querying each participant about the interval occurrence of any of the outcome events or any hospitalizations. Positive indications from study participants about a hospitalization or possible event will be followed by collection of selected medical records from the inpatient setting. Records will include administrative hospital codes and copies of relevant portions of the medical record, including admission and discharge notes, progress notes, laboratory studies, ECGs, radiographic studies, and any other diagnostic testing or procedure notes. Individual events will be defined as described in **Table 2**:

Table 2. Summary of adjudicated outcome events	
Outcome Event	**Summary of Definitions**
Acute Coronary Syndrome	New universal definition of MI by the American Heart Association.[107] *Acute MI types 1-3:* Acute myocardial injury with clinical evidence of acute myocardial ischemia and with detection of a rise and/or fall of cardiac troponin (cTn) values with at least 1 value above the 99th percentile upper reference limit (URL) and at least 1 of the following:

	• Symptoms of myocardial ischemia • New ischemic ECG changes • Development of pathological Q waves • Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality in a pattern consistent with an ischemic etiology • Identification of a coronary thrombus by angiography or autopsy (not for types 2 or 3 MIs). *Percutaneous coronary intervention (PCI)–related MI (type 4a MI) and Coronary artery bypass grafting (CABG)–related MI (type 5 MI)*: • Coronary procedure-related MI ≤48 hours post-procedure, cTn >5-fold (type 4a MI) or >10-fold increase (type 5 MI) of the 99th percentile URL in patients with normal baseline values. Patients with elevated preprocedural cTn values must manifest a change from the baseline value of >20%. • In addition, at least 1 of the following: - New ischemic ECG changes (this criterion is related to type 4a MI only) - Development of new pathological Q waves - Imaging evidence of loss of viable myocardium that is presumed to be new - Angiographic findings consistent with a procedural flow-limiting complication such as coronary dissection, occlusion of a major epicardial artery or graft, or disruption of collateral flow - Isolated development of new pathological Q waves if cTn values are elevated and rising *Types 4b (MI stent thrombosis) and type 4c MI (restenosis)*: must meet type 1 MI criteria.
Coronary Revascularization	Percutaneous angioplasty or stent placement for obstructive coronary artery disease or CABG
Hospitalization for HF	Hospitalization for clinical symptoms of HF in the setting of objective confirmatory evidence including radiographs, evidence of pulmonary congestion/edema, physical examination, invasive hemodynamic monitoring, or echocardiographic confirmation. Admission must include administration of intravenous diuretics, escalation of diuretic doses and/or use of inotropes, volume removal by ultrafiltration or renal replacement therapy for decongestion, or mechanical circulatory support. Relevant echocardiographic information will be reviewed to determine if the event is consistent with HF with reduced ejection fraction (EF <40%), mid-range EF (40-49%), or preserved EF (≥50%).[108]
Incident Serious Cardiac Arrhythmia	Categorized as follows: • Resuscitated cardiac arrest • Ventricular fibrillation • Sustained ventricular tachycardia • Selected instances of non-sustained ventricular tachycardia • Initial episode of atrial fibrillation or flutter, severe bradycardia or heart block • Postoperative tachyarrhythmias following CABG or other cardiac surgery
Peripheral arterial disease	One of the following must be present: • PAD with resultant amputation • Surgical or percutaneous revascularization procedures such as angioplasty and artery-artery bypass graft
Cerebrovascular Events	Categorized as follows: • Intraparenchymal hemorrhage • Subarachnoid hemorrhage • Large-vessel cerebral infarction • Cardioembolic cerebral infarction • Small-vessel cerebral infarction • Cerebral infarction not otherwise specified • Transient ischemic attack: Acute disturbance of focal neurological or monocular function with symptoms lasting less than 24 hours and thought to be due to arterial thrombotic or embolic vascular disease

Death Events	*Cardiovascular death*: • Atherosclerotic coronary heart disease death • Cerebrovascular death • Other atherosclerotic disease death (non-coronary/non-stroke) • Sudden cardiac death • Other CVD death (including death from HF) *Other Death*

3.7 Unscheduled Visits

We anticipate no unscheduled visits. We anticipate that the only situation in which this would occur is if a study subject requests a meeting with the investigator to discuss any issues related to the study.

3.8 Subject Withdrawal

Subjects may withdraw from the study at any time without impact to their care. The Investigator may also withdraw subjects who violate the study plan, to protect the subject for reasons related to safety, or for administrative reasons. It will be documented whether or not each subject completes the study.

4 Statistical Plan

4.1 Data analysis plan

To characterize cardiac and extracardiac phenotypes in COVID-19 survivors, we will use latent class analysis with finite mixture modeling to classify individuals into mutually exclusive and exhaustive subgroups, maximizing within-group similarities and between-group differences on the basis of baseline covariates [109-112]. Latent class models will be compared across successive numbers of subgroups and the optimal number of groups will be determined using the parametric bootstrap likelihood ratio test, Akaike's Information Criterion, Bayesian Information Criterion, and sample-size adjusted BIC [113,114]. We will use other clustering methods for corroboration of our identified phenotypes, including Gaussian mixture models via the expectation–maximization algorithm [79], and consensus clustering [115] to evaluate the stability of the discovered clusters. Descriptive statistics will compare patients across phenotypes using Student's t-test, Wilcoxon rank sum, or Chi-square test, as appropriate. We will then assess for differences in quality of life and functional status across phenotypes using multinomial logistic regression. We will separately analyze the trajectory of each of the quantitative measures of vascular health and kidney function using mixed effects growth curve models.[116] Where necessary, we will supplement these analyses with marginal models estimated by generalized estimating equations.[117] We will assess for varying length of follow-up among subgroups to evaluate for any potential bias this may introduce. We will assess for effect modification by sex and race due to potentially important differences in COVID-19 outcomes[118-120] and trajectory of vascular and kidney health.[121,122] We will also use methods that classify participants according to their vascular and kidney health trajectories, using each individual parameter and extended to multiple parameters. We will use the latent class mixed effects model[123,124] to analyze trajectories of vascular and kidney health within our study population. The latent class mixed effects model introduces an additional variable to represent class membership, which can be modeled through a multinomial logistic regression.

The endpoint of MACE will be evaluated as a time-to-event endpoint from the date of discharge from the index hospitalization for COVID-19 and from the date of the first study visit, accordingly. We will examine the associations of severity of hospitalization, baseline covariates, and post-discharge cardiac and extra-cardiac phenotypes with this failure-time event using Kaplan-Meier curves and Cox proportional hazards regression [125]. We will use the multivariable Cox model to

estimate the effects of the primary exposure adjusting for baseline covariates. We will use time by covariate interaction terms and examine residual plots to assess the adequacy of model fit [126]. If the proportional hazards assumption is violated, a time by predictor interaction term will be added to the model to allow for changes in the HR over time. Secondary analyses will evaluate death prior to the event as a competing risk, including incorporation of the composite outcome with the cumulative incidence function [127,128]. We will assess for effect modification by sex and race due to sex- and race-related differences in COVID-19 outcomes [118-120] and risk of MACE [121,129].

4.2 Power calculations

We will enroll 200 COVID-19 survivors following recovery from their hospitalization and 80 controls. For analyses of trajectory of vascular and kidney health, accounting for multiplicity across multiple outcome parameters at an alpha at 0.0025,[130] we will have >80% power to observe, for example, a mean difference in slope of PWV of 0.8 m/s and eGFR of 4 mL/min/1.73m^2 per 18-month visit interval across COVID-19 survivors and non-COVID-19 controls, assuming intraclass correlation coefficient of 0.1 and 20% loss to follow-up.[131] For determining the association of hospitalization severity with risk of MACE, the most conservative endpoint in the study, we assumed a cumulative event rate of 5% over 5 years of follow-up [6,7], accounting for our exclusion of individuals with prior CVD. Assuming a retention rate of 80%, we will have 90% power at an alpha of 0.05 to detect a regression coefficient of 0.9 in the Cox model [132,133]. In analyses evaluating for sub-phenotypes and risk factors for MACE, using a conservative Bonferroni approach to account for multiplicity across several risk factors [130], we will have over 90% power at an alpha of 0.005 to detect a regression coefficient of 0.9 for every standard deviation difference in parameter. Power calculations were performed using PASS16 [134].

5 Study Administration, Data Handling, and Record Keeping

All information obtained as part of protocol will be maintained in locked research files. Electronic data will be stored in a password secured database (REDCap). This database will be available only with secure high-level password protection to safeguard the privacy of the participants. Any source documents for each patient involved in the protocol, other than the patient's electronic medical chart, will be maintained in a centralized, secured location.

Data collected will contain protected health information (PHI) including name, date of birth, and medical record number. Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Research data will be stored in a centralized, secure location (locked cabinets in the PI's research space).

Participant data, blood, and urine samples will be stored for future use. These stored elements will be used only for research purposes. A randomly assigned number rather than name will identify all collected samples and data. PHI will be treated with strict confidentiality and stored in a secured, limited access area. A secure database of patient information will be maintained. PHI will not be disclosed or shared with any parties outside of the research team unless required by law or by regulatory processes at Penn (such as audits).

6 Study Monitoring, Auditing, and Inspecting

6.1 Study Monitoring Plan

The study PI will be responsible for ensuring the ongoing quality and integrity of the research study.

6.2 Auditing and Inspecting

The Investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc).

7 Ethical Considerations

The protocol, amendments and continuing reviews will be submitted for review by the Penn IRB and other appropriate reviewing entities.

7.1 Risks

There are minor risks to subjects who choose to participate in the study. The ultrasound examination, breath collection, arterial tonometry, NIRS, thermal imaging, and sublingual capillaroscopy have no known risks.

Venipuncture: According to the 2010 WHO guidelines on phlebotomy, major risks associated with venipuncture include hematoma at the site of venipuncture in 2-3%, and vasovagal reactions and fainting in 1%.

Endothelial cell harvesting: The risks associated with the procurement of venous endothelial cells are low and are analogous to the risks that occur with placement of a venous catheter, which include pain, superficial phlebitis/venous blood clots and infection.

During various procedures (echocardiography, arterial tonometry), we will use adhesive electrodes attached to the participant's skin to record the electrical signal from the heart. These may occasionally cause skin itching and irritation.

Risks of pulmonary function tests: Our pulmonary function tests will not involve challenging (such as methacholine administration) and thus are very low risk. A potential risk is dizziness during forced maneuvers or shortness of breath during the 8-10 breath hold required for DLCO measurements. The tests may rarely cause sore chest muscles. There is a very low risk of pneumothorax from forced expiration.

Risks of exercise testing*:* This test is used extensively for research purposes with minimal risk to subjects. The most significant risks of the test are dysrhythmias or other cardiovascular complications, which are extremely rare. These procedures will be performed by qualified personnel according to established American Heart Association Guidelines.[135,136] Non-revascularized myocardial ischemia, which may increase the risk of complications during exercise testing, is an exclusion criterion for the study. Furthermore, the procedure is voluntary and intended to only be performed in a subset of participants in the study and will not be performed in any participants with a contraindication to exercise, such as elevated risk of falls (as assessed by the investigators) or severely elevated blood pressure (>180/100).

Subjects may feel uncomfortable as a result of pushing themselves during the maximal effort exercise test. Subjects will likely feel short of breath and fatigued as a result of the exercise test. Various other complaints, such as nausea, lightheadedness, and other aches and pains are also possible as a result of the maximal effort exercise study. Although exercise testing may result in exhaustion, rarely do people develop abnormal heart rate or heart complications during exercise tests. The risk of this happening is the same as if the participant would exert themselves during stressful situations or during exercise elsewhere.

We will perform ECG, heart rate, and blood pressures monitoring during our exercise test. In addition to the blood pressure (generally increases) and heart rate (generally increases) changes during exercise, we will also monitor arterial saturation. This will be done non-invasively using a pulse oximeter. Of note, oxygen levels can decrease with exercise, even in individuals without significant cardiopulmonary disease.[137] If the arterial saturation falls to below 88% ("severe exercise induced hypoxemia"[138]), we will alert the patient's primary care provider as this may prompt consideration for additional or alternative causes for arterial hypoxemia.

Risks of FMD measurements: The cuff inflation used for FMD may cause temporary bruising. The inflation itself may cause tingling in the hand/digits, which resolves after deflation.

Risks of ambulatory blood pressure monitoring: There is minimal physical risk from non-invasive ambulatory blood pressure monitoring. A potential discomfort will be the squeezing from inflation of the blood pressure cuff, which may cause sleep disturbance for some participants.

Risks of SPPB: The gait and balance tests may lead to falls and the repeat chair stand test require a vigorous effort. However, these tests were found to be efficient, practical, and safe to administer to thousands of older persons in a home setting without any injury or adverse outcome.[139]

Risks of impedance cardiography: this procedure is not associated with any known risks. Electrodes or electrode gel may cause irritation or itching.

7.2 Benefits

There may be no direct benefit to subjects as a result of their participation in the study. However, society in general may benefit as a result of scientific discoveries obtained during the course of this study. However, this study may provide novel information leading to the discovery and better understanding of long-term complications in COVID-19 survivors, which may in turn lead to newer approaches for their treatment or prevention. Results of research procedures will be shared with the patient or his/her providers upon request.

7.3 Informed Consent Process / HIPAA Authorization

All subjects for this study will be provided with an IRB-approved consent form providing sufficient information for subjects to make an informed decision about their participation in this study. The formal consent of a subject, including signing the IRB-approved consent form, will be required before that subject undergoes any study procedure. Participants able to perform exercise testing will be offered the option to undergo VO2 testing.

7.4 Reporting of Incidental Findings

The tests in this study will be quantified for research purposes and not for clinical interpretation purposes. However, when incidental findings are encountered in study procedures, the study PIs will communicate these to study subjects and providers as necessary, based on their clinical judgement. These findings include but are not limited to, pulmonary function testing results and abnormalities discovered during ultrasound acquisition. If a PI is unable to contact a subject via phone, they may choose to mail a letter or send a message via MyPennMedicine.

8 Study Finances

8.1 Funding Source

This study will be funded by the Investigators' discretionary funds. NIH funding will also be pursued.

8.2 Subject Stipends or Payments

Participants will receive $250. Those additionally undergoing VO2 testing will receive an additional $150.

9 Publication Plan

The Investigator and study team will prepare and lead publications arising from this study. The data may also become a part of collaborative endeavors and/or scientific consortia from which joint publications with other groups may result. As such, subjects are being consented for their data (devoid of PHI) to be shared with other investigators outside of Penn.

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