Post-COVID-19 illness trajectory: a multisystem investigation.

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Abstract

Background: The pathophysiology and trajectory of multiorgan involvement in post-COVID-19 syndrome is uncertain.

Methods: A prospective, multicenter, longitudinal, cohort study involving post-COVID-19 patients enrolled in-hospital or early post-discharge (visit 1) and re-evaluated 28-60 days post-discharge (visit 2). Multisystem investigations included chest computed tomography with pulmonary and coronary angiography, cardiovascular and renal magnetic resonance imaging, digital electrocardiography, and multisystem biomarkers. The primary outcome was the adjudicated likelihood of myocarditis.

Results: 161 patients (mean age 55 years, 43% female) and 27 controls with similar age, sex, ethnicity, and vascular risk factors were enrolled from 22 May 2020 to 2 July 2021 and had a primary outcome evaluation. Compared to controls, at 28-60 days post-discharge, patients with COVID-19 had persisting evidence of cardio-renal involvement, systemic inflammation, and hemostasis pathway activation. Myocarditis was adjudicated as being not likely (n=17; 10%), unlikely (n=56; 35%), probable (n=67; 42%) or very likely (n=21; 13%). Acute kidney injury (odds ratio, 95% confidence interval: 3.40 (1.13, 11.84); p=0.038) and low hemoglobin A1c (0.26 (0.07, 0.87); p=0.035) were multivariable associates of adjudicated myocarditis. During convalescence, compared to controls, COVID-19 was associated with worse health-related quality of life (EQ5D-5L) (p<0.001), illness perception (p<0.001), anxiety and depression (p<0.001), physical activity (p<0.001) and predicted maximal oxygen utilization (ml/kg/min) (p<0.001). These measures were associated with adjudicated myocarditis.

Conclusions: The illness trajectory of COVID-19 includes persisting cardio-renal inflammation, lung damage and hemostasis activation. Adjudicated myocarditis occurred in one in eight hospitalized patients and was associated with impairments in health status, physical and psychological wellbeing during community convalescence.

Public registration: ClinicalTrials.gov identifier is NCT04403607.

Background

Self-reporting1–4 and population studies5–7 of post-COVID-19 illness trajectory have found that symptoms, such as fatigue, breathlessness, and exercise intolerance are common. At the outset of the COVID-19 pandemic, clinical studies lacked a prospective evaluation of disease pathogenesis and/or health status, and selectively recalled patients3,7. Few prospective studies have been reported8–12, and multisystem imaging with clinical outcomes and contemporary controls were lacking. Pre-existing disease complicates attribution of causal inferences in COVID-19 and, since the heart, lungs and kidneys are deep organs, clinical evaluation is challenging. Accordingly, the pathophysiology and clinical significance of post-COVID-19 syndromes remain uncertain7.

The pathogenesis of multiorgan inflammation in COVID-19 may involve direct virus invasion through binding angiotensin converting enzyme 2 (ACE2)13,14, cardio-renal inflammation15, endothelial dysfunction15, thrombotic microvascular angiopathy16, stress cardiomyopathy15, and drug toxicity15. These distinct mechanisms define subgroups with multiorgan involvement (endotypes) in COVID-19. Myocarditis may cause longer-term morbidity and mortality17. Prior studies using cardiovascular magnetic resonance imaging in COVID-19 have reported imaging features of myocardial inflammation in 27%–60%18,19 of patients. These studies involved case selection based on troponin elevation and retrospective recall18,19. Lack of coronary artery imaging is also a limitation for attributing the etiology of myocardial injury, which becomes susceptible to ascertainment bias.

Based on the cardiovascular tropism of SARS-CoV-215, we hypothesized, firstly, that the illness trajectory of post-COVID-19 syndromes involved hemostatic pathway activation and systemic inflammation during convalescence, second, cardio-renal involvement associates with pre-existing cardiovascular disease and, third, adjudicated myocarditis post-COVID-19 associates with persisting impairments in health status, physical and psychological wellbeing. Disease mechanisms were investigated using multisystem imaging and biomarkers and their changes over time. Health status and physical function were serially assessed using validated patient reported outcome measures.

Methods

Design

This study involved a prospective, observational, multicenter, longitudinal, secondary care cohort design to assess the time-course of multiorgan injury in survivors of COVID-19 during convalescence. Clinical information, a 12-lead digital ECG, blood and urine biomarkers, and patient reported outcome measures were acquired at enrolment (visit 1) and again during convalescence, 28–60 days post-discharge (visit 2). Chest computed tomography (CT), including pulmonary and coronary angiography, and cardio-renal MRI were acquired at the second visit.

Setting

The study involved three hospitals in the West of Scotland (population 2.2 million) - the Queen Elizabeth University Hospital, the Royal Infirmary in Glasgow, and the Royal Alexandra Hospital in Paisley.

Participant identification
Patients who received hospital care for COVID-19, with or without admission, and were alive, were prospectively screened in real time using an electronic healthcare information system (TrakCare®, InterSystems®, USA) and daily hospital reports identifying inpatients with laboratory-positive results for COVID-19.

**Eligibility criteria**

The inclusion criteria were: (1) age ≥18 years old; (2) history of an unplanned hospital visit e.g., emergency department, or hospitalization >24 hours for COVID-19 confirmed by a clinical diagnosis, laboratory test (e.g., polymerase chain reaction (PCR)), and/or a radiological test (e.g. CT chest or chest radiograph); (3) ability to comply with study procedures; and (4) ability to provide written informed consent. The imaging results were reported by accredited radiologists according to contemporary, national guidelines.

The exclusion criteria were: (1) contra-indication to magnetic resonance (MR) imaging (e.g., severe claustrophobia, metallic foreign body); and (2) lack of informed consent.

**Screening**

A screening log was prospectively completed. The reasons for being ineligible, including lack of inclusion criteria and/or presence of exclusion criteria, were recorded.

**Diagnosis of COVID-19**

A diagnosis of COVID-19 was based on either laboratory evidence of SARS-CoV-2 infection using a PCR test (Roche Cobas 6800 or Seegene SARS-CoV-2 PCR) on a biospecimen or a radiological and clinical diagnosis of COVID-19 but biospecimen negative.

**Diagnosis of myocardial injury**

The diagnosis of myocardial injury aligned with the Fourth Universal Definition of Myocardial Infarction. Troponin I was measured in hospitalized patients using the Abbott Architect STAT TnI assay (sex-specific >99th percentile upper reference limit: female: >16 ng/L, male: >34 ng/L).

**Diagnosis of acute kidney injury**

Acute kidney injury (AKI) was defined as any stage of AKI (1-3) during COVID-19 hospitalization using categorization with the Kidney Disease: Improving Global Outcomes (KDIGO) criteria (Supplement).

**Research schedule**

The protocol involved two visits. The first visit involved informed consent and baseline assessments during the initial hospitalization, or as soon as possible after discharge. The second visit occurred 28–60 days post-discharge. This window was positioned to reflect the convalescent phase and give sufficient scope to schedule the patients.

The procedures involved prospective collection of clinical data and a time-course of research investigations. Clinical data included demographics, medical and cardiovascular history, findings from clinical examinations, laboratory and radiological tests, cardiology tests (including an electrocardiogram (ECG) and an echocardiogram if available) and treatment. The research investigations at both visits included blood and urine samples, a 12-lead digital ECG (Beneheart R3, Mindray, Huntingdon, UK), health status questionnaires, and assessments of adverse events (Supplement). Heart, lung, and kidney imaging were acquired at the second visit.

**Electrocardiology**

SARS-CoV-2 infection and treatment may cause alterations in heart rate and rhythm, and ventricular repolarization. The changes may be specific for myocarditis e.g., concave ST-elevation, or non-specific e.g., ventricular arrhythmias. Digital ECGs were acquired, de-identified and provided to the University of Glasgow Electrocardiology Core Laboratory for automated analysis and adjudication. The ECG features of myopericarditis were predefined according to contemporary criteria.

**Biomarkers**

Blood and urine samples were collected at enrolment (visit 1) and 28–60 days post discharge (visit 2). Circulating biomarkers of cardiac injury (troponin I, N-terminal (NT)-pro hormone brain natriuretic peptide (NT-proBNP), inflammation (C-reactive protein, ferritin), thrombosis (TCT ratio, D-Dimer, fibrinogen, Factor VIII, antithrombin, protein C, protein S), endothelial activation (von Willebrand factor (vWF):GP1bR, vWF:Ag) and renal function (serum creatinine, glomerular filtration rate (GFR) was estimated using the Chronic Kidney Disease Epidemiology (CKD-EPI) equation and urinary albumin: creatinine ratio), and their...
changes over time, were investigated. The measurements were undertaken in a central laboratory, blinded to the other clinical data. The methodology is described in the Supplement.

**Multimodality imaging**

**CT**

A 320-detector CT scanner (Aquilion ONE, Canon Medical Systems Corp.) provided full heart coverage within a single heartbeat. Intravenous metoprolol was used where required to control the heart rate (target 60 beats/min) and sublingual glyceryl trinitrate was given to all patients immediately before the scan acquisition. An initial low radiation dose helical scan of the thorax was acquired for comprehensive assessment of the lungs. A contrast bolus timing scan was acquired to provide information on cardiopulmonary transit times. Non-contrast and contrast-enhanced angiographic breath-hold ECG-gated volumes were acquired and timed for optimum pulmonary and systemic arterial (coronary) opacification. Patients with severe renal dysfunction underwent non-contrast CT.

Coronary CT angiography provided information on the presence and extent of coronary calcification (calcium score), coronary artery disease, and whether any coronary artery disease was obstructive (flow-limiting) including the Coronary Artery Disease - Reporting and Data System (CAD-RADS) score\(^5\). The functional significance of coronary artery disease was evaluated using fractional flow reserve CT (FFR\(_{CT}\), HeartFlow, Redwood City, CA). A FFR\(_{CT}\) ≤ 0.80 defined obstructive coronary artery disease, taking the lowest value in the vessel. FFR\(_{CT}\) measurements were taken at prespecified points using standard coronary segment definitions as a reference\(^6\). Median FFR\(_{CT}\) values were calculated for the left anterior descending, circumflex, and right coronary arteries, respectively, in combination with subsidiary vessels (i.e., diagonal arteries, obtuse marginal arteries). Patient-level FFR\(_{CT}\) values included all these coronary arteries.

Pulmonary vascular imaging assessed arterial thrombus (embolism)\(^7\). CT was used to delineate pulmonary features associated with COVID infection e.g., atelectasis, reticulation and/or architectural distortion, ground-glass opacity, and pre-existing lung damage e.g., emphysema. Cardiac and extra-cardiac incidental findings were reported and managed according to local standards of care.

**Cardiovascular MRI**

Cardiovascular MRI was undertaken to measure heart structure and function and assess for persisting evidence of myocardial injury and/or myocardial infarction using multi-parametric techniques\(^8\). MRI was acquired in a single reference site for all patients using a research-dedicated 3.0 Tesla (3T) scanner (MAGNETOM Prisma, Siemens Healthineers, Erlangen, Germany) with two 18-channel surface coils placed anteriorly and a 32-channel spine coil placed posteriorly. All patients underwent protocol-directed MRI in the convalescent phase, 28–60 days after discharge. The scan protocol included cine-imaging of cardiac anatomy and function and myocardial tissue characterization using multiparametric techniques. They included 1) mapping myocardial native longitudinal relaxation time (T1 in milliseconds) using the modified Look-Locker inversion recovery technique (T1-mapping) before and after intravenous administration of gadolinium contrast media (0.15 mmol/kg of Magnevist, Bayer Healthcare), 2) mapping transverse relaxation time (T2 in milliseconds), 3) first pass contrast-enhanced perfusion and 4) late gadolinium-enhancement imaging. Specific details on the MRI protocol are provided in the Supplement.

The expert consensus recommendations for the MRI diagnostic criteria of non-ischemic myocardial inflammation (modified Lake Louise criteria) were used to diagnose definite myocardial inflammation (abnormal T2 and T1 (native T1, late gadolinium enhancement or extracellular volume)) or probable myocardial inflammation (abnormal: T2 or T1)\(^1,7,24\) (Supplement). Reference ranges derived from the UK Biobank were used to interpret cardiac structure and function\(^30\), and contemporary local reference ranges specifically derived using the 3T MRI scanner (MAGNETOM Prisma) were used to define thresholds for localized abnormalities in myocardial T1- and T2- relaxation times. To limit selection bias, patients with severe renal dysfunction (GFR <45 ml/kg/m\(^2\)) were not excluded. They were eligible for MRI with or without contrast media according to the site Radiology protocol.

**Renal MRI**

Multi-parametric renal MRI included anatomical imaging and tissue characterization by measurement of native T1 and T2. The volume (ml), and native T1 (ms) and T2 (ms) in regions of interest obtained within the cortex and medulla of each kidney were recorded, and the averaged value of these parameters for both kidneys was then determined. Corticomedullary differentiation reflects a difference in tissue contrast on T1-weighted imaging due to a shorter T1 relaxation time of the cortex relative to the medulla, this being attributed to differences in water content between the two tissues\(^31,32\). Corticomedullary differentiation, reported here as a ratio of T1 cortex divided by T1 medulla\(^32\), may diminish in kidney disease\(^31\).

**Blinding**

The patients and the outcome assessors were blinded. Outcome assessments, including laboratory, MRI and CT analyses, and endpoint adjudication were undertaken by blinded researchers. The patients completed the health status questionnaires before undergoing the scans and they were unaware of the test results.

**Outcomes**

**Primary outcome**
The predefined primary outcome was a diagnosis of myocarditis (myocardial inflammation), an endotype of acute myocardial injury.

The diagnostic criteria for myocarditis included relevant clinical findings and test results (Supplement)\textsuperscript{17}. Positive clinical findings included chest pain, pericarditic or pseudo-ischemic in nature, new onset breathlessness, subacute/chronic breathlessness, palpitations, unexplained arrhythmia, syncope, aborted sudden cardiac death, or unexplained cardiogenic shock. Positive test findings included 1) ECG features, 2) elevated troponin I (sex-specific >99\textsuperscript{th} percentile upper reference limit: female: >16 ng/L, male: >34 ng/L; Abbott Architect STAT TnI assay); 3) functional and structural abnormalities on cardiac imaging (echocardiography, angiography, or MRI), and 4) tissue characterization MRI, including myocardial edema and late gadolinium enhancement with a distribution in alignment with the modified Lake Louise diagnostic criteria for myocarditis\textsuperscript{29}. Acute and chronic myocardial pathology can be identified, discriminated, and quantified using MRI.

Myocarditis was clinically suspected if at least 1 clinical finding and at least 1 diagnostic test criterion from different categories, in the absence of: (1) angiographically detectable coronary artery disease (coronary stenosis ≥ 50%); (2) known pre-existing cardiovascular disease or extra-cardiac causes that could explain the syndrome (e.g., valve disease, congenital heart disease, hyperthyroidism, etc.). Suspicion increases with a rising number of fulfilled criteria. If the patient was asymptomatic, at least 2 diagnostic criteria were required.

Adjudication of the primary outcome

A diagnosis of myocarditis is susceptible to confounding through ascertainment bias. Recent studies in COVID-19 have not implemented the modified Lake Louise diagnostic criteria\textsuperscript{18,19}. Accordingly, we pre-specified an adjudication procedure for the primary outcome, involving a panel of cardiologists with specialty accreditation. The reviews were undertaken according to a prespecified charter.

Consultant cardiologists (n=14) who were independent of the research team were invited as assessors. They were initially provided with information on the European Society of Cardiology Working Group on Myocardial and Pericardial Disease position statement on myocarditis\textsuperscript{17}, a charter, and training cases. The cardiologists were blind to the identity of the patients and independent of their clinical care. The adjudications were coordinated by a researcher (A.M.) using Teams (Microsoft, Seattle, USA) software.

Each cardiologist independently assessed the clinical data, including the medical history, biomarkers, ECG, and radiology reports for the CT chest, CT pulmonary angiogram, coronary CT angiogram, and cardiac MRI. Deidentified source clinical data e.g., scan images, were made available on request. The cardiologists determined the likelihood (not likely / unlikely / probable / very likely) of myocardial inflammation (myocarditis). The final diagnosis was based on the median likelihood based on the adjudications of 5 cardiologists. Their determinations were also categorized in binary form (not/unlikely = no; probable/very = yes).

Secondary outcomes

The differential etiology of myocardial injury/inflammation was adjudicated as a secondary outcome. The potential endotypes were:

1) SARS-CoV-2 myocarditis,

2) Acute stress cardiomyopathy,

3) Myocardial ischemia/impaired perfusion as a stressor of inflammation,

4) Infective myopericarditis (non-COVID infection),

5) Drug-induced (toxic) myocardial inflammation,

6) Idiopathic myocardial ± pericardial inflammation.

The endotypes of acute myocardial injury, including the type of myocardial infarction according to the 4\textsuperscript{th} Universal Definition of MI\textsuperscript{22}, and myocarditis (myocardial inflammation, ischemia or stress cardiomyopathy)\textsuperscript{17,29}, were secondary outcomes.

Renal outcomes

Renal function was assessed using convalescent eGFR (CKD-EPI\textsuperscript{24}) and albuminuria. Multi-parametric renal MRI at 28-60 days provided information on renal parenchymal disease.

Health status and patient reported outcome measures

Questionnaires were completed by participants at enrolment (visit 1) and 28–60 days after the last episode of hospital care (visit 2), blind to the other research data. Self-reported health status was assessed using the generic EuroQOL EQ-5D-5L questionnaire and the Brief Illness Perception Questionnaire (Brief-IPQ)\textsuperscript{33,34}. The Patient Health Questionnaire-4 (PHQ–4) was utilized to assess for anxiety and depressive disorders\textsuperscript{35}. The Duke Activity Status Index (DASI) was used to assess predicted maximal oxygen utilization (ml/kg/min), a measure of aerobic capacity, and functional capacity, a higher score reflects greater
physical function. The International Physical Activity Questionnaire - Short Form (IPAQ-SF) measures the types and intensity of physical activity and sitting time that people do as part of their daily lives. The score reflects total physical activity in metabolic equivalent minutes per week.

**Longitudinal follow-up**

The participants were invited to give consent for clinical outcome assessment during follow-up using electronic health record linkage without direct contact.

**Statistics**

The statistical analyses were pre-defined in a Statistical Analysis Plan.

**Sample size calculation**

The primary outcome was myocarditis (myocardial inflammation), and the primary analysis determined the proportion of patients with the primary outcome by visit 2. The likelihood of myocarditis was determined based on the median likelihood from the clinical adjudication committee. To detect an association between a history of pre-existing cardiovascular disease and incident myocardial inflammation (myocarditis), we assumed a 25% prevalence of prior cardiovascular disease in the study population, and the incidence of myocardial inflammation in those with/without prior cardiovascular disease to be 33% and 10%, respectively. To have 80% power to detect this difference we calculated that 140 participants (35 with cardiac problems, 105 without) with complete data would be required. Anticipating that 10-15% of the participants may have incomplete imaging e.g., artefact or claustrophobia, the target sample size was 160 to complete the imaging visit.

Cardiovascular disease status was prespecified and defined by (1) a prior history of cardiovascular disease, and (2) treatment. The associations between the circulating concentrations of mechanistic biomarkers, patient reported outcome measures, and their changes over time, and the primary and secondary outcomes were assessed. Missing data are reported. Significance tests with 2-sided p-values are accompanied by confidence intervals for estimated effect sizes and measures of association. The widths of the confidence intervals have not been adjusted for multiplicity. The p-values for subgroup differences were calculated using the Fisher Exact test and the Kruskal-Wallis test, for categorical and continuous data, respectively. A p-value of 0.05 was taken as statistically significant.

**Trial management and timelines**

The study was conducted in line with the current Guidelines for Good Clinical Practice in Clinical Trials and STrengthening the Reporting of OBservational studies in Epidemiology guidelines, and coordinated by a Study Management Group. A Scientific Steering Group had oversight of the study.

**Ethics**

The study was approved by the UK National Research Ethics Service (Reference 20/NS/0066).

**Sources of Funding**

This was an investigator-initiated clinical study that was funded by the Chief Scientist Office of the Scottish Government (COV/GLA/Portfolio project number 311300). The funder had no role in the design, conduct (non-voting TSC member), data analysis and interpretation, manuscript writing, or dissemination of the results. C.B, C.D., N.S., R.M.T. were supported by the British Heart Foundation (RE/18/6134217).

The MRI study involved technologies provided by Siemens Healthcare and the National Institutes of Health. HeartFlow (HeartFlow, Redwood City, CA) provided FFR<sub>CT</sub>. The study was co-sponsored by NHS Greater Glasgow & Clyde Health Board and the University of Glasgow.

**Data and code availability**

The datasets that support the findings of this study are available from the corresponding author upon reasonable request. Statistical code will be made available by the corresponding author upon reasonable request.

**Registration**

ClinicalTrials.gov: NCT04403607.

**Results**
One thousand three hundred and six patients were screened between 22 May 2020 and 16 March 2021 and 267 patients provided written informed consent. The flow diagram is shown in Figure 1 and clinical cases are illustrated in the Supplement.

One hundred and sixty-one patients were evaluated at 28-60 days after the last episode of hospital care. Their average age was 55 years, 88% were white, 43% were female, 47% had a history of cardiovascular disease or treatment, 40% were in the highest quintile of deprivation and 22% were healthcare workers (Table 1 and Supplement). Clinical disease severity scores are described in Table 1. Two (1.2%) patients had received a single dose of SARS-CoV-2 vaccine prior to hospitalization (Supplement, Table 2). Regarding COVID-19 therapy, 68.9% received oxygen, 55.3% received steroids, 26.1% received antiviral drug therapy, 19.3% received non-invasive respiratory support and 8.7% received invasive ventilation.

**Comparison with controls**

Twenty-seven control patients with similar age, sex, ethnicity, and cardiovascular risk factors underwent the same research procedures during a single visit between 13 April to 2 July 2021. Their characteristics are described in Table 1. Compared to controls, COVID-19 patients had multisystem differences in keeping with acute illness.

**Multisystem investigations: comparisons with controls**

In post-COVID-19 patients, compared to controls, the heart, lung and kidney imaging, electrocardiography and multisystem biomarkers revealed multiple persisting abnormalities (Table 2).

At 28-60 days post-discharge (visit 2), CT chest abnormalities were common: 44.7% had ground glass opacities and/or consolidation, 23.9% had ≥20% of the total lung area abnormal by visual estimation and 3.3% had pulmonary arterial thrombus. In the post-COVID-19 patients, the minimum patient-level FFR$_{CT}$ was lower than in the control group (minimum FFR$_{CT}$: 0.80 (0.10) vs. 0.85 (0.08); p<0.001) consistent with flow-limiting coronary artery disease. MRI revealed persisting differences for left and right ventricular ejection fraction, contractility (strain), volumes, myocardial tissue characteristics, including late gadolinium enhancement in one in five patients mainly with a non-ischemic distribution, increased myocardial extracellular volume and pericardial thickening (Table 2). Renal MRI findings at 28-60 days post-discharge were similar between COVID-19 patients and controls (Table 2).

Circulating concentrations of C-reactive protein, ferritin, D-Dimers, fibrinogen, Factor VIII, and von Willebrand factor were higher in post-COVID-19 patients at enrolment compared to controls consistent with hemostatic pathway activation (Table 2). At 28-60 days post-discharge, Factor VIII concentration remained high. Circulating concentrations of NT-proBNP were higher in COVID-19 patients at enrolment and 28-60 days post-discharge. Urine albumin: creatine ratio and eGFR were not statistically different between the groups.

**Primary outcome**

A diagnosis of myocarditis was adjudicated as being not likely (n=17; 10%), unlikely (n=56; 35%), probable (n=67; 42%) or very likely (n=21; 13%). Adjudicated likelihood of myocarditis was associated with typical radiological features of COVID-19 (p=0.027), intensive care (p=0.045) and invasive ventilation (p=0.047), but there were no associations with demographic characteristics, cardiovascular history, or standard care blood results obtained during the index hospitalization (Table 1).

**Multisystem phenotyping and adjudicated myocarditis**

*Electrocardiology*

Premature ventricular contractions associated with the likelihood of myocarditis (Table 2).

*CT chest, coronary and pulmonary angiography*

Myocarditis did not appear to be associated with the extent or nature of lung involvement or coronary artery disease (Table 2).

*Cardiovascular magnetic resonance imaging*

Evidence was found of associations between the adjudicated likelihood of myocarditis and reduced left ventricular ejection fraction in females, myocardial inflammation, extracellular volume, late gadolinium enhancement (non-ischemic distribution) and the diagnostic criteria (Lake Louise) for myocardial inflammation (Table 2). Distinct patterns of myocardial pathology revealed by late gadolinium enhancement imaging are shown in the Supplement.

*Renal magnetic resonance imaging*

The adjudicated likelihood of myocarditis was associated with acute kidney injury during the initial admission. The average renal medulla T1 (ms), an imaging marker of inflammation in the left and right kidneys, associated with adjudicated myocarditis. No differences were observed for the averaged renal volumes, cortex tissue characteristics (T1, T2 ms), or renal function at 28-60 days.

*Biochemical and hematological markers*
At 28-60 days, protein S was inversely associated with the adjudicated likelihood of myocarditis. We found no other evidence of associations between the adjudicated myocarditis and biochemical and hematological markers of inflammation, hemostatic pathway activation, myocardial injury, or left ventricular dysfunction (Table 2).

**Adjudicated cause of myocarditis**

The etiology of myocardial inflammation was also adjudicated. SARS-COV-2 myocarditis was determined as being probable (66.7%) or very likely (33.3%) in all patients with adjudicated myocarditis (p<0.001) (Supplement, Table 3). Impaired myocardial blood flow as a stressor of inflammation was determined as probable in 6 (6.8%) patients with myocarditis adjudicated to be either probable or very likely (p<0.001).

**Multivariable associates of adjudicated myocarditis**

Univariate and multivariable associations between selected demographic and clinical measures at baseline (visit 1) and an adjudication of myocarditis being probable or very likely were assessed with logistic regression models (Table 3). Univariable associates of adjudicated myocarditis were female sex (odds ratio, 95% confidence interval: 1.92 (1.02, 3.70); p=0.045), healthcare worker (2.24 (1.03, 5.10); p=0.046), acute kidney injury (3.42 (1.25, 10.98); p=0.024), and hemoglobin A1c (per 1% difference: 0.25 (0.07, 0.77); p=0.020). After age and sex adjustment, acute kidney injury (3.40 (1.13, 11.84); p=0.038) and hemoglobin A1c (0.26 (0.07, 0.87); p=0.035) were multivariable associates of adjudicated myocarditis.

**Health status**

Compared to controls, at enrolment and 28-60 days post-discharge, post-COVID-19 patients had lower health-related quality of life, enhanced illness perception, higher levels of anxiety and depression, lower levels of physical activity and lower predicted maximal oxygen utilization (ml/kg/min) (Table 4).

The adjudicated likelihood of myocarditis associated with patient reported outcome measures at 28-60 days post-discharge, including lower health-related quality of life (p=0.005), enhanced illness perception (p=0.029), enhanced depression score (p=0.030), lower physical activity (p=0.014) and lower predicted maximal oxygen utilization (ml/kg/min) (p=0.014).

**Serious adverse events**

One patient died following consent, before discharge from hospital. A further patient died within 60 days of discharge (prior to visit 2). The causes of death and hospital readmission are listed in the Supplement.

**Discussion**

This prospective multicenter study characterized the illness trajectory of patients who survived hospitalization for COVID-19 during community convalescence. The study provided serial measurements of multisystem pathology coupled with patient-reported health status and aerobic exercise capacity.

Our results bridge a knowledge gap between post-COVID-19 syndromes and objective evidence of disease. We found that the illness trajectory of post-COVID-19 syndrome involved hemostatic pathway activation initially, including increases in circulating concentrations of fibrinogen and factor VIII and a reduction in protein S, and endothelial activation reflected by higher circulating concentrations of VWF:GP1bR and VWF:Ag. Most of these differences resolved by 28-60 days. This time-course of resolving hemostasis activation provides pathophysiological insights to explain the results of recent randomized clinical trials of antithrombotic therapy, including the efficacy of therapeutic anticoagulation with heparin in noncritically ill, hospitalized patients with Covid-19 (comparable to our population), and the lack of efficacy of aspirin or apixaban in community patients with milder illness due to the low rate of cardiopulmonary thrombotic events. There was evidence of persisting systemic and renal inflammation and higher circulating concentrations of NTproBNP 28-60 days post-discharge. Post-COVID-19 status during convalescence associated with lingering impairments in health-related quality of life, illness perception, anxiety, depression, physical function, and predicted aerobic exercise capacity.

Incident myocarditis persisting 28-60 days post-COVID-19 affected approximately 1 in 8 patients (13%), which is lower than the percentages reported in other studies involving cardiovascular MRI (27%-60%) . On the other hand, there is a knowledge gap on the incidence of myocardial inflammation in non-COVID infection, such as influenza, and new prospective studies are needed. The prevalence of obstructive coronary artery disease derived from FFR was higher in the COVID-19 group than in the controls (Table 2). Notwithstanding, the etiology of myocarditis determined by the adjudication committee was predominately SARS-CoV-2 infection and less commonly, myocardial ischemia due to coronary artery disease (Supplement, Table 3).

Distinct from controls, one in five post-COVID-19 patients had imaging evidence of myocardial fibrosis indicative of distinct disease mechanisms, including myocarditis, microvascular thrombosis, myocardial infarction, and pre-existing scar (Supplement). Adjudicated myocarditis was also associated with premature ventricular contractions, myocardial fibrosis, pericardial thickening, and mild differences in left and right ventricular systolic function. On the other hand, hemoglobin A1c (%) was the only baseline characteristic associated with adjudicated myocarditis, but in the opposite direction to what may be expected, and so requiring validation in other cohorts. The mechanism may involve systemic inflammation leading microangiopathic hemolytic anemia and reduced red cell survival, although the lack of association with haptoglobin (Table 2) and other hematological parameters (Supplement, Table 1) does not support this possibility in our population. Reverse causality and residual confounding may be relevant.
Acute kidney injury portends mortality in COVID-19. Adjudicated myocarditis was associated with acute kidney injury during admission and the averaged native T1 (ms) in the kidney medulla 28-60 days post-discharge, reflecting multiorgan inflammation during convalescence. These associations may be explained by systemic pathophysiology i.e. inflammation, hemostatic pathway activation, microvascular dysfunction, severe COVID-19 infection, or a combination of these pathologies. Cardio-renal injury associated with persisting impairments in health-related quality of life, and poorer physical and psychological wellbeing during convalescence.

Post-COVID-19 syndrome (‘long COVID’) predominately affects females. The proportion of women increased with the likelihood of myocarditis and female sex was a univariable associate of adjudicated myocarditis, which in turn was associated with lower mental and physical wellbeing. Adjudicated myocarditis was associated with left ventricular systolic dysfunction in females. Our findings provide a pathophysiological basis for symptoms burden and exercise limitation in some female patients with myocardial involvement post-COVID-19.

Our findings support clinical evaluation for myocarditis in patients who are hospitalized with COVID-19, especially in intensive care. Troponin is a cardiac protein that is ubiquitously released from injured cardiomyocytes. A rise in circulating troponin concentration is not cause-specific and troponin may increase due to hypoxia, hypotension, ischemia, and renal failure as well as from direct myocardial toxicity. On the other hand, cardiac biomarkers are a diagnostic criterion for myocarditis and are informative for prognostication. Outside of intensive care, a selective approach for measuring cardiac biomarkers, informed by clinical findings, would seem appropriate.

Although there are no evidence-based treatments for the prevention or treatment of myocarditis in COVID-19, acute treatments, such as dexamethasone, should reduce the likelihood of myocarditis occurring. Since our findings identify myocarditis as an associate of worse physical and mental health post-COVID-19, cardio-renal involvement could be considered a therapeutic target (endpoint) in clinical trials to prevent post-COVID-19 syndrome. The RECOVERY Trial is currently investigating the effects of immunomodulatory therapies in acute COVID-19, including baricitinib and dimethyl fumarate, and the sodium-glucose cotransporter-2 inhibitor, empagliflozin, which reduces the progression of kidney disease and lower rates of clinically relevant renal events in patients with type 2 diabetes at high cardiovascular risk.

To our knowledge, the combination of systematic cardio-renal MRI and chest CT, including pulmonary and coronary angiography with FFR, during the same visit, coupled with serial assessments of multisytem biomarkers and patient reported outcome measures, is novel. FFR provided a high level of certainty for identifying flow-limiting coronary artery disease (myocardial ischemia) as a confounding associate for myocardial inflammation in this post-COVID-19 population.

Our study was designed to minimize selection bias excepting those who were unable to comply with the protocol. Use of hospital-level electronic health records in real-time facilitated an unbiased approach to screening. Troponin elevation was not an eligibility criterion and renal dysfunction was not an exclusion criterion. Our study stands apart from other studies, including those which had target populations with myocardial injury defined by troponin elevation (COVID-HEART and COVIDsortium), retrospective case selection or a sample size limiting generalizable conclusions. Since multi-organ imaging was not performed during the acute phase, some pathologies that might have been detected acutely may have resolved by 28 days. Our findings may therefore under-estimate disease burden. Most of the patients were unvaccinated. The incidence of myocarditis in hospitalized vaccinated patients warrants investigation. The definition of acute kidney injury was based on in-hospital blood tests. Endomyocardial biopsy (EMB) was not performed. Longer term follow-up is ongoing.

This study was designed but not powered to assess clinical outcomes such as rehospitalization and death. In 47,780 individuals (mean age 65 years, 55% men) hospitalized with COVID-19 and discharged alive, during a mean follow-up of 140 days, nearly a third of individuals were readmitted (14,060 of 47,780) and more than one in ten (5,875) died after discharge, with these events occurring at rates four and eight times greater, respectively, than in the matched control group.

**Limitations**

By designating imaging during the convalescent phase, the community-based participants were not anticipated to be infectious. This approach aligns with the International Severe Acute Respiratory and Emerging Infection Coronavirus Clinical Characterisation Consortium (ISARIC4C) study. Since multi-organ imaging was not performed during the acute phase, some pathologies that might have been detected acutely may have resolved by 28 days. Our findings may therefore under-estimate disease burden. Most of the patients were unvaccinated. The incidence of myocarditis in hospitalized vaccinated patients warrants investigation. The definition of acute kidney injury was based on in-hospital blood tests. Endomyocardial biopsy (EMB) was not performed. Longer term follow-up is ongoing.

**Conclusions**

The illness trajectory of COVID-19 includes persisting cardio-renal inflammation, lung involvement, and hemostatic pathway activation. Adjudicated myocarditis occurred in one in eight patients and was associated with poorer health-related quality of life, psychological wellbeing, physical activity, and predicted aerobic exercise capacity. The results support the rationale for cardio-renal therapy development for prevention of post-COVID-19 syndromes.

**Declarations**

**Disclosures**
CB is employed by the University of Glasgow which holds consultancy and research agreements with Abbott Vascular, AstraZeneca, Boehringer Ingelheim, Coroventis, GSK, HeartFlow, Menarini, Novartis, Siemens Healthcare and Somalogic. These companies had no role in the design or conduct of the study, or in the data collection, interpretation, or reporting. HeartFlow derived FFR\textsubscript{CT}. None of the other authors have any relevant disclosures.

Contributors

CB designed the study and wrote the first draft of the manuscript with KM. AMcI and AMcC developed the statistical analysis plan and performed the statistical analyses. The co-authors reviewed the manuscript drafts. Each author has individually contributed to either the delivery of the study or helped to devise aspects of the study protocol. All authors have given final approval for the current version to be published.

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Tables

Table 1. Clinical characteristics of the study population by likelihood of adjudicated myocarditis post-COVID-19.
### Demographic

| Age ±SD, years | n = 161 | n = 27 | p-value | n = 17 (10%) | n = 56 (35%) | n = 67 (42%) | n = 21 (13%) | p-value |
|---------------|---------|--------|---------|--------------|--------------|--------------|--------------|---------|
| Male sex, %   | 56.6±12.0 | 56.5±9.3 | 0.072 | 56.9±11.4 | 55.1±13.3 | 53.6±11.6 | 54.9±10.1 | 0.669 |
| Female sex, % | 44 (27.3) | 11 (40.7) | 1.000 | 13 (76.5) | 35 (62.5) | 35 (52.2) | 9 (42.9) | 0.136 |
| Most deprived SIMD Quintile (Q1), % | 61 (40.7) | 5 (18.5) | 0.058 | 4 (25.0) | 20 (37.0) | 25 (40.3) | 12 (60.0) | 0.066 |
| Healthcare worker, % | 36 (22.4) | 5 (19.2) | 1.000 | 1 (5.9) | 10 (17.9) | 10 (18.5) | 7 (33.3) | 0.132 |

### Ethnicity, %

| Value | n = 161 | n = 27 | p-value |
|-------|---------|--------|---------|
| Arab  | 4 (2.5) | 0 (0.0) | 0.738 |
| Black | 2 (1.2) | 0 (0.0) | 0.000 |
| East Asian | 4 (2.5) | 0 (0.0) | 0.000 |
| South Asian | 8 (5.0) | 2 (7.4) | 0.000 |
| West Asian | 2 (1.2) | 1 (3.7) | 0.000 |
| Latin American | 0 (0.0) | 0 (0.0) | 0.000 |
| White | 141 (87.6) | 24 (88.9) | 0.000 |

### Presenting characteristics, mean (SD)

| Value | n = 161 | n = 27 | p-value |
|-------|---------|--------|---------|
| Body mass index, kg/m² | 30.5 (7.1) | 30.6 (5.1) | 0.701 |
| Heart rate, bpm | 95 (19) | 66 (11) | <0.001 |
| Systolic blood pressure, mmHg | 129 (20) | 143 (19) | 0.003 |
| Diastolic blood pressure, mmHg | 77 (13) | 84 (14) | 0.018 |
| Peripheral oxygen saturation, % | 93 (6) | 98 (2) | <0.001 |
| Respiratory rate, min | 24 (12) | 14 (2) | <0.001 |

### WHO clinical severity score, n (%)

| Value | n = 161 | n = 27 | p-value |
|-------|---------|--------|---------|
| No evidence of infection | 0 (0.0) | 27 (100.0) | <0.001 |
| Hospitalized, no oxygen therapy | 50 (31.1) | 0 (0.0) | 0.000 |
| Oxygen therapy by mask or nasal prongs | 76 (47.2) | 0 (0.0) | 0.000 |
| Non-invasive ventilation | 20 (12.4) | 0 (0.0) | 0.000 |
| Mechanical ventilation | 5 (3.1) | 0 (0.0) | 0.000 |
| Ventilation with organ support | 10 (6.2) | 0 (0.0) | 0.000 |

### COVID-19 diagnosis, n (%)

| Value | n = 161 | n = 27 | p-value |
|-------|---------|--------|---------|
| PCR test | 158 (98.1) | - | <0.001 |
| Nosocomial | 8 (5) | 0 | 0.605 |
| Antibody test* | - | 27 (100.0) | <0.001 |

### Radiology, chest radiograph or CT scan, n (%)

| Value | n = 161 | n = 27 | p-value |
|-------|---------|--------|---------|
| Typical features of COVID-19 | 111 (75.0) | - | 0.027 |
| Atypical features of COVID-19 | 11 (7.4) | - | 2 (12.5) |
| Unlikely | 4 (2.7) | - | 2 (12.5) |
| Normal | 22 (14.9) | - | 0 (0.0) |

### Acute COVID-19 therapy, n (%)

| Value | n = 161 | n = 27 | p-value |
|-------|---------|--------|---------|
| Oxygen | 111 (68.9) | - | 14 (82.4) |
| Steroid | 89 (55.3) | - | 12 (70.6) |
| Antibiral | 42 (26.1) | - | 9 (52.9) |
| Non-invasive respiratory support | 31 (19.3) | - | 5 (29) |
| Intensive care | 24 (14.9) | - | 5 (29.4) |
| Invasive ventilation | 14 (8.7) | - | 2 (11.8) |
| Intravenous inotrope | 7 (4.3) | - | 1 (5.9) |

### Cardiovascular history, n (%)

| Value | n = 161 | n = 27 | p-value |
|-------|---------|--------|---------|
| Smoking: Never | 107 (66.5) | 17 (63.0) | 0.826 |
| Smoking: Former | 44 (27.3) | 9 (33.3) | 0.000 |
| Smoking: Current | 10 (6.2) | 1 (3.7) | 0.000 |
| Hypercholesterolaemia | 77 (47.8) | 12 (44.4) | 0.072 |
| Hypertension | 57 (35.4) | 9 (33.3) | 0.000 |
| Diabetes mellitus | 35 (21.7) | 2 (7.4) | 0.011 |
| Chronic kidney disease | 7 (4.3) | 0 (0.0) | 0.056 |
| CCS Angina Class: No angina | 156 (96.9) | 27 (100.0) | 0.000 |
| CCS Angina Class I-IV | 5 (3.1) | 0 (0.0) | 0.033 |
| Myocardial Infarction | 17 (10.6) | 0 (0.0) | 0.000 |
| Heart failure | 6 (3.7) | 0 (0.0) | 0.000 |
| Stroke or TIA | 6 (3.7) | 2 (7.4) | 0.000 |
| Peripheral venous disease | 1 (0.6) | 0 (0.0) | 0.056 |
| Previous PCI | 10 (6.2) | 0 (0.0) | 0.000 |
| Previous CABG | 2 (1.2) | 0 (0.0) | 0.000 |
| Cardiovascular disease and/or treatment | 75 (466) | 13 (48.1) | 0.000 |

### Risk scores, mean (SD)

| Value | n = 161 | n = 27 | p-value |
|-------|---------|--------|---------|
| ISARIC-4C in-hospital mortality risk, % | 12.3 (10.7) | 5.4 (6.2) | 0.0003 |
| Q-Risk 3, 10-year cardiovascular risk, % | 13.7 (11.1) | 12.5 (9.7) | 0.0000 |
| Charlson Comorbidity Index | 1.9 (1.8) | 1.3 (1.1) | 0.179 |

### Pre-existing maintenance medication, n (%)

| Value | n = 161 | n = 27 | p-value |
|-------|---------|--------|---------|
| Aspirin | 12 (7.5) | 0 (0.0) | 0.021 |
| | 3 (17.6) | 4 (7.1) | 0.462 |
Table 2. Multisystem phenotyping: serial electrocardiography, biomarkers of inflammation, metabolism, renal function, and hemostasis, and heart, lung, and kidney imaging at 28-60 days post-discharge.
| Electrocardiogram, n (%) | Myocarditis |
|-------------------------|------------|
| **Admission (n = 152)** |            |
| Myocarditis criteria    |            |
| Premature atrial contraction | 2 (1.3)  |
| Premature ventricular contraction | 3 (2.0)  |
| Arrial fibrillation or flutter, n = 9 missing | 5 (3.3)  |
| **Enrolment (n = 148)** |            |
| Myocarditis criteria    |            |
| Premature atrial contraction | 7 (4.7)  |
| Premature ventricular contraction | 1 (0.6)  |
| Arrial fibrillation or flutter | 3 (2.0)  |
| **28-60 days post-discharge (n = 144)** |            |
| Myocarditis criteria    |            |
| Premature atrial contraction | 8 (5.0)  |
| Premature ventricular contraction | 2 (1.2)  |
| Arrial fibrillation or flutter | 2 (1.3)  |
| **CT chest 28-60 days post-discharge** |            |
| Ground glass opacity and/or consolidation, n (%) | 71 (44.7) |
| Reticulation and/or architectural distortion, n (%) | 47 (29.6) |
| Atelectasis, n (%) | 13 (8.2)  |
| Pulmonary arterial thrombus, n (%) | 5 (3.3)  |
| Visual estimate of % of total lung area abnormal, mean (SD) | 14.4 (19.2) |
| <20%, n (%) | 108 (67.9) |
| ≥20%, n (%) | 38 (23.9)  |
| ≥50%, n (%) | 13 (8.2)  |
| **CT coronary angiogram 28-60 days post-discharge** |            |
| Coronary calcium - Agatston score, mean (SD) | 146 (500) |
| CADS-RADS score, n (%) | 75 (289)  |
| Level 0 | 77 (52.4) |
| Level 1 | 42 (28.6) |
| Level 2 | 9 (6.1)  |
| Level 3 | 6 (4.1)  |
| Level 4 | 13 (8.8) |
| Level 5 | 0 (0.0)  |
| Obstructive coronary artery disease, n (%) | 21 (13.7) |
| **FFRCT patient level (all coronary arteries)** |            |
| Median FFRCT, mean (SD) | 0.93 (0.03) |
| Minimum FFRCT, mean (SD) | 0.80 (0.10) |
| Minimum FFRCT ≤0.80, n (%) | 52 (38.8) |
| **Cardiovascular MRI 28-60 days post-discharge** |            |
| LV end-diastolic volume index, mean (SD), mL/m² | 76.3 (17.6) |
| LV end-systolic volume index, mean (SD), mL/m² | 35.6 (13.2) |
| LV ejection fraction, mean (SD), % | 54.0 (9.6) |
| LV ejection fraction reduced, males <48%, n (%) | 20 (22.0) |
| LV ejection fraction reduced, females <51%, n (%) | 12 (17.6) |
| LV mass index, mean (SD), g/m² | 92.2 (26.0) |
| LV global longitudinal strain, mean (SD), % | -14.2 (3.0) |
| LV global circumferential strain, mean (SD), % | -17.0 (3.3) |
| LV global radial strain, mean (SD), % | 28.2 (7.8) |
| RV end-diastolic volume index, mean (SD), mL/m² | 73.5 (17.7) |
| RV end-systolic volume index, mean (SD), mL/m² | 36.0 (11.3) |
| RV ejection fraction, mean (SD), % | 51.1 (10.3) |
| RV global longitudinal strain, mean (SD), % | -17.2 (5.9) |

**Myocardial tissue characterization**

- Increased global T1 (>1233 ms), n (%) | 56 (35.0)  |
- Increased global T2 (>44 ms), n (%) | 10 (6.2)  |
- T2 ratio (myocardium/ serratus anterior), n (%) | 1.7 (0.2) |
- Increased global extracellular volume (>27.4%), n (%) | 72 (49.7) |

**Late gadolinium enhancement**

- Myocardial late gadolinium enhancement, n (%) | 32 (20.0) |
- Ischemic distribution, n (%) | 8 (5.4)  |
- Non-ischemic distribution, n (%) | 26 (17.4) |
| Myocarditis | COVID-19 (n = 161) | Controls (n = 27) | P-value | Not likely | Unlikely | Probable | Very likely | P-value |
|------------|--------------------|------------------|---------|-----------|---------|----------|-----------|---------|
| Pericardial thickening, n (%) | 32 (22%) | 7 (12%) | 0.005 | 24 (14.9%) | 10 (11.8%) | 17 (20.1%) | 7 (3.3%) | 0.168 |
| Pericardial effusion, n (%) | 17 (10.7) | 0 (0.0) | 1.34 | 0 (0.0) | 5 (9.1) | 8 (12.1) | 4 (19.0) | 0.277 |
| Right atrial area, mean (SD), cm² | 18.8 (4.7) | 19.5 (4.2) | 0.399 | 19.2 (3.9) | 18.8 (4.9) | 18.3 (5.1) | 19.9 (3.2) | 0.253 |
| Left atrial area, mean (SD), cm² | 20.7 (4.7) | 21.8 (4.6) | 0.231 | 22.3 (5.0) | 19.9 (4.2) | 20.7 (5.1) | 21.9 (4.1) | 0.214 |

| Myocardial inflammation (Lake Louise criteria), n (%) | No evidence (0/2) | Probable (1/2) | Definite (2/2) | P-value |
|---------------------------------|------------------|----------------|----------------|---------|
| Average volume of right and left kidneys, ml | 1533 (31) | 155 (34) | 0.887 | 158 (37) | 154 (25) | 150 (33) | 153 (38) | 0.705 |
| Average cortex T1 of right and left kidneys, ms | 1545 (62) | 1515 (68) | 0.063 | 1548 (66) | 1535 (58) | 1543 (63) | 1585 (60) | 0.110 |
| Average medulla T1 of right and left kidneys, ms | 1934 (68) | 1955 (60) | 0.134 | 1935 (66) | 1924 (65) | 1925 (66) | 2008 (57) | 0.003 |
| Average T1 corticomedullary differentiation of kidneys | 0.80 (0.03) | 0.78 (0.03) | 0.000 | 0.80 (0.03) | 0.80 (0.03) | 0.80 (0.03) | 0.79 (0.02) | 0.475 |

### Biomarkers at enrolment, central laboratory

| Biomarker | Mean (SD) |
|-----------|-----------|
| eGFR, median [IQR], ml/min/1.73m² | 96 (85, 105) |
| eGFR <60 ml/min/1.73m², n (%) | 5 (3.3%) |
| CR-protein, mean (SD), mg/L | 26.3 (50.5) |
| High sensitivity tropon I, median [IQR], ng/L | 4 (2.6) |
| NT pro BNP, median [IQR], pg/mL | 115 (57, 265) |
| Ferritin, median [IQR], ug/L | 366 (203, 650) |
| Haptoglobin, mean (SD), g/L | 2.3 (1.2) |
| Total cholesterol, mean (SD), mmol/L | 4.8 (1.4) |
| Triglycerides, mean (SD), mmol/L | 2.2 (1.3) |
| HDL cholesterol, mean (SD), mmol/L | 1.1 (0.3) |
| Prothrombin time, mean (SD), s | 12.3 (3.7) |
| D-Dimer, mean (SD), mg/mL | 329 (221) |
| P-fibrinogen, mean (SD), g/L | 4.1 (1.6) |
| Factor VIII, mean (SD), IU/dL | 185 (93) |
| Antithrombin, mean (SD), IU/dL | 111 (18) |
| Protein C, mean (SD), IU/dL | 123 (30) |
| Protein S, mean (SD), IU/dL | 85 (28) |
| VWF:GPIbR, mean (SD), IU/dL | 239 (130) |
| VWF:Ag, mean (SD), IU/dL | 246 (150) |

### Biomarkers at 28-60 days post-discharge, central laboratory

| Biomarker | Mean (SD) |
|-----------|-----------|
| eGFR, median [IQR], ml/min/1.73m² | 95 (83, 105) |
| eGFR <60 ml/min/1.73m², n (%) | 7 (4.6%) |
| CR-protein, mean (SD), mg/L | 6.6 (23.3) |
| High sensitivity tropon I, median [IQR], ng/L | 2 (2.5) |
| NT pro BNP, median [IQR], pg/mL | 84 (55, 202) |
| Ferritin, median [IQR], ug/L | 144 (71, 283) |
| Haptoglobin, mean (SD), g/L | 1.3 (0.6) |
| D-Dimer, mean (SD), mg/mL | 206 (254) |
| P-fibrinogen, mean (SD), g/L | 3.7 (2.8) |
| Factor VIII, mean (SD), IU/dL | 148 (66) |
| Protein S, mean (SD), IU/dL | 99 (22) |
| VWF:GPIbR, mean (SD), IU/dL | 145 (85) |
| VWF:Ag, mean (SD), IU/dL | 173 (145) |

### Urine Biomarkers

| Biomarker | Mean (SD) |
|-----------|-----------|
| Albumin: creatinine ratio at enrolment, SD | 3.2 (8.0) |
| Albumin: creatinine ratio at 28-60 days post-discharge, mean (SD) | 4.7 (15.6) |

**Missing data in post-COVID-19 patients (admission, enrolment, 28-60 days) and controls - myopericarditis criteria - n=9, n=13, n=17, n=0; Missing data in post-COVID-19 patients at 28-60 days and controls - CT chest atelectasis, reticulisation, ground glass - n=2, n=5; pulmonary arterial thrombus - n=8, n=6; CT coronary angiogram 28-60 days and controls - Agatston score - n=7, n=4; CAD-RADS score - n=5, n=4; FFRCT - n=27, n=4; Cardiovascular magnetic resonance imaging 28-60 days post-discharge - left ventricular end-diastolic volume index, left ventricular end-diastolic volume index, left ventricular ejection fraction, left ventricular strain - n=2, n=3; right ventricular end-diastolic volume index, right ventricular systolic volume index, right ventricular ejection fraction, n=4, n=3; Global T1 - n=1, n=1; global T2 - n=1, n=1; extracellular volume - n=16, n=5; left ventricular mass - n=8, n=4; late gadolinium enhancement - n=1, n=3; ischemic distribution - n=14, n=4; non-ischemic distribution - n=12, n=4; mixed distribution - n=14, n=4; pericardial thickening - n=3, n=3; pericardial effusion - n=2, n=3; right and left atrial area - n=1, n=3; myocardial inflammation - n=1, n=0; Blood biomarkers, post-COVID-19 patients (enrolment and 28-60 days) and controls - eGFR - n=9, n=10, n=8; C-reactive protein - n=7, n=7, n=2; High sensitivity tropon I - n=17, n=34, n=2; NT pro BNP - n=6, n=10, n=2; Total cholesterol, triglycerides, HDL cholesterol - n=4, n=5, n=2; Fibrinogen - n=11, n=14, n=12; D-Dimer - n=13, n=11; Fibrinogen - n=1, n=14, n=2; Factor VIII - n=1, n=12, n=2; Antithrombin - n=1, n=14, n=2; Protein C - n=1, n=NA, n=15; Protein S - n=1, n=14, n=3; VWF:GPIbR - n=1, n=13, n=2; VWF:Ag - n=1, n=13, n=2; Abbreviations - aPTT - activated partial thromboplastin time; CAD-RADS - Coronary Artery Disease - Reporting and Data System; ECV - extracellular volume; eGFR (CKD-EPI) - estimated glomerular filtration rate using the Chronic Kidney Disease Epidemiology (CKD-EPI) equation; EF - ejection fraction; EDV - end-diastolic volume; ESV - end-systolic volume; FFRCT - fractional flow reserve computed tomography; Ha1Ac - hemoglobin A1C; HDL - high density lipoprotein; LV - left ventricle; MESA - Multi-ethnic study of atherosclerosis; NT-proBNP - N-terminal pro B-type natriuretic peptide; PT - prothrombin time; RV - right ventricle; T1 - longitudinal relaxation time; T2 - transverse relaxation time; TCT - thrombin clotting time; VWF:GPIbR - von Willebrand factor antigen.
Table 3. Univariable and multivariable associates of adjudicated myocarditis (primary outcome) including demographic characteristics (A), cardiovascular history (B), severity of COVID-19 (C), and biomarkers (D).

|                       | Univariate Odds ratio (95% CI) | p-value | Multivariable Odds Ratio (95% CI) | p-value |
|-----------------------|-------------------------------|---------|-----------------------------------|---------|
| **Demographics**      |                               |         |                                   |         |
| Age (decades)         | 0.89 (0.68, 1.16)             | 0.398   | 0.99 (0.70, 1.40)                 | 0.938   |
| Sex: Female (vs. Male)| 1.92 (1.02, 3.70)             | 0.045   | 1.75 (0.81, 3.85)                 | 0.161   |
| Ethnicity: Other (vs. white) | 2.11 (0.79, 6.25) | 0.147 | 2.11 (0.79, 6.25) | 0.147 |
| SIMD quintile 2 (vs. most deprived) | 0.49 (0.19, 1.20) | 0.120 | 0.49 (0.19, 1.20) | 0.120 |
| SIMD quintile 3 (vs. most deprived) | 0.47 (0.16, 1.33) | 0.159 | 0.47 (0.16, 1.33) | 0.159 |
| SIMD quintile 4 (vs. most deprived) | 0.58 (0.19, 1.71) | 0.319 | 0.58 (0.19, 1.71) | 0.319 |
| SIMD quintile 5 least deprived (vs. most deprived) | 1.10 (0.44, 2.87) | 0.838 | 1.10 (0.44, 2.87) | 0.838 |
| **Healthcare worker** | 2.24 (1.03, 5.10)             | 0.046   |                                   |         |
| Body mass index, kg/m² | 1.02 (0.98, 1.07)             | 0.345   |                                   |         |
| **Cardiovascular history** |                             |         |                                   |         |
| Hypertension          | 0.71 (0.37, 1.35)             | 0.297   |                                   |         |
| Chronic kidney disease | 2.14 (0.45, 15.25)            | 0.372   |                                   |         |
| Diabetes mellitus     | 0.63 (0.29, 1.34)             | 0.232   |                                   |         |
| Hypercholesterolemia  | 1.67 (0.90, 3.14)             | 0.108   |                                   |         |
| Smoking (former vs. never) | 0.96 (0.48, 1.95) | 0.911 | 0.96 (0.48, 1.95) | 0.911 |
| Smoking (current vs. never) | 3.51 (0.83, 23.97)            | 0.123   |                                   |         |
| History of cardiovascular disease | 0.74 (0.40, 1.38) | 0.343 | 0.74 (0.40, 1.38) | 0.343 |
| Q-Risk 3, 10-year cardiovascular risk, % | 0.98 (0.95, 1.02) | 0.311 | 0.98 (0.95, 1.02) | 0.311 |
| **Medical history**    |                               |         |                                   |         |
| Charlson Comorbidity Index | 0.96 (0.80, 1.14) | 0.612 | 0.96 (0.80, 1.14) | 0.612 |
| ISARIC-4C in-hospital mortality risk, % | 0.94 (0.86, 1.03) | 0.231 | 0.94 (0.86, 1.03) | 0.231 |
| WHO Score: oxygen therapy (vs. hospitalized, no oxygen) | 0.67 (0.32, 1.37) | 0.272 | 0.67 (0.32, 1.37) | 0.272 |
| WHO Score: non-invasive ventilation (vs. hospitalized, no oxygen) | 0.55 (0.19, 1.55) | 0.257 | 0.55 (0.19, 1.55) | 0.257 |
| WHO Score: invasive ventilation (vs. hospitalized, no oxygen) | 1.83 (0.54, 7.35) | 0.352 | 1.83 (0.54, 7.35) | 0.352 |
| **Acute kidney injury** | 3.42 (1.25, 9.98)             | 0.024   | 3.40 (1.13, 11.84)                | 0.038   |
| **Biomarkers (standard care)** |                           |         |                                   |         |
| Hemoglobin, g/L       | 1.00 (0.98, 1.02)             | 0.872   | 1.00 (0.98, 1.02)                 | 0.872   |
| Platelet count, x10⁹/L | 1.82 (0.74, 4.60)             | 0.195   | 1.82 (0.74, 4.60)                 | 0.195   |
| Peak white cell count, x10⁹/L | 1.52 (0.75, 3.23) | 0.256 | 1.52 (0.75, 3.23) | 0.256 |
| Lowest lymphocyte count, x10⁹/L | 1.69 (0.97, 3.13) | 0.080 | 1.69 (0.97, 3.13) | 0.080 |
| Peak D-Dimer, ng/mL   | 1.02 (0.74, 1.42)             | 0.925   | 1.02 (0.74, 1.42)                 | 0.925   |
| Peak fibrinogen, g/L  | 1.25 (0.87, 1.90)             | 0.257   | 1.25 (0.87, 1.90)                 | 0.257   |
| HbA1c, %              | 0.25 (0.07, 0.77)             | 0.020   | 0.26 (0.07, 0.87)                 | 0.035   |
| Peak creatinine, mmol/L | 1.43 (0.75, 2.87)            | 0.288   | 1.43 (0.75, 2.87)                 | 0.288   |
| Peak ferritin, mg/L   | 0.89 (0.67, 1.17)             | 0.397   | 0.89 (0.67, 1.17)                 | 0.397   |
| Peak high sensitivity troponin I, ng/L | 1.11 (0.94, 1.33) | 0.226 | 1.11 (0.94, 1.33) | 0.226 |
| Peak C-reactive protein, mg/L | 0.87 (0.68, 1.09)           | 0.223   | 0.87 (0.68, 1.09)                 | 0.223   |

Table 4. Health status, illness perception, anxiety and depression, and physical function.
| Patients, n = 161 | Controls, n = 27 | P-value | Not likely n = 17 (10%) | Unlikely n = 56 (35%) | Probable n = 67 (42%) | Very likely n = 21 (13%) | P-value |
|------------------|-----------------|---------|-------------------------|-----------------------|------------------------|--------------------------|---------|
| Health-related quality of life EQ-5D-5L score at enrolment | 0.74 (0.22) | 0.87 (0.20) | <0.001 | 0.80 (0.19) | 0.76 (0.18) | 0.73 (0.24) | 0.66 (0.25) | 0.154 |
| Health-related quality of life EQ-5D-5L score 28-60 days post-discharge | 0.77 (0.23) | 0.87 (0.20) | 0.003 | 0.85 (0.13) | 0.81 (0.20) | 0.75 (0.27) | 0.64 (0.20) | 0.005 |
| Patient assessed EQ-5D-5L score at enrolment, EQ-5D-5L score | 61.1 (22.0) | 77.1 (18.4) | <0.001 | 71.2 (18.7) | 64.2 (19.0) | 56.4 (23.1) | 59.9 (25.8) | 0.065 |
| Patient assessed EQ-5D-5L score at 28-60 days post-discharge | 72.4 (19.7) | 77.1 (18.4) | 0.179 | 75.3 (16.6) | 74.8 (17.3) | 72.5 (21.5) | 63.0 (20.9) | 0.134 |

### Illness perception, mean (SD)

| Health status, mean (SD) |
|--------------------------|
| Health-related quality of life EQ-5D-5L score at enrolment | 0.74 (0.22) | 0.87 (0.20) | <0.001 | 0.80 (0.19) | 0.76 (0.18) | 0.73 (0.24) | 0.66 (0.25) | 0.154 |
| Health-related quality of life EQ-5D-5L score 28-60 days post-discharge | 0.77 (0.23) | 0.87 (0.20) | 0.003 | 0.85 (0.13) | 0.81 (0.20) | 0.75 (0.27) | 0.64 (0.20) | 0.005 |
| Patient assessed EQ-5D-5L score at enrolment, EQ-5D-5L score | 61.1 (22.0) | 77.1 (18.4) | <0.001 | 71.2 (18.7) | 64.2 (19.0) | 56.4 (23.1) | 59.9 (25.8) | 0.065 |
| Patient assessed EQ-5D-5L score at 28-60 days post-discharge | 72.4 (19.7) | 77.1 (18.4) | 0.179 | 75.3 (16.6) | 74.8 (17.3) | 72.5 (21.5) | 63.0 (20.9) | 0.134 |

### Anxiety and depression, mean (SD)

| PHQ-4 anxiety score at enrolment | 2.11 (2.08) | 0.70 (1.51) | <0.001 | 1.53 (1.74) | 1.83 (1.83) | 2.31 (2.23) | 2.70 (2.36) | 0.347 |
| PHQ-4 anxiety score at 28-60 days post-discharge | 1.81 (2.00) | 0.70 (1.51) | 0.002 | 1.20 (1.08) | 1.43 (1.73) | 2.09 (2.23) | 2.45 (2.24) | 0.196 |
| PHQ-4 depression score at enrolment | 2.18 (1.94) | 0.44 (1.15) | <0.001 | 1.59 (1.87) | 2.06 (1.79) | 2.30 (2.01) | 2.60 (2.16) | 0.412 |
| PHQ-4 depression score at 28-60 days | 1.77 (1.90) | 0.44 (1.15) | <0.001 | 1.07 (1.10) | 1.34 (1.68) | 2.06 (2.05) | 2.55 (2.06) | 0.030 |
| PHQ-4 total score at enrolment | 4.29 (3.77) | 1.15 (2.60) | <0.001 | 3.12 (3.37) | 3.89 (3.29) | 4.61 (4.01) | 5.30 (4.35) | 0.346 |
| PHQ-4 total score at 28-60 days post-discharge | 3.58 (3.70) | 1.15 (2.60) | <0.001 | 2.27 (2.02) | 2.77 (3.11) | 4.15 (4.17) | 5.00 (3.97) | 0.054 |

### Physical function, mean (SD)

| IPAQ category at enrolment | High | Moderate | Low | P-value |
|---------------------------|------|----------|-----|---------|
| IPAQ category at enrolment | 12 (8.5) | 11 (42.3) | <0.001 | 2 (11.8) | 3 (5.9) | 4 (7.1) | 3 (16.7) | 0.441 |
| Predicted maximal O2 utilization (ml/kg/min) at enrolment | 19.7 (18.2) | 47.9 (17.5) | <0.001 | 25.7 (18.5) | 19.9 (17.7) | 18.1 (18.1) | 19.2 (19.9) | 0.250 |
| Predicted maximal O2 utilization (ml/kg/min) at 28-60 days post-discharge | 23.2 (17.6) | - (-) | 0.014 | 33.6 (18.7) | 25.1 (17.9) | 23.9 (17.4) | 14.6 (12.5) | 0.014 |

### Figures

PHQ-4 - Patient Health Questionnaire-4 ; IPAQ - International Physical Activity Questionnaire.
Figure 1

Flow diagram of the clinical study. The procedures involved screening hospitalised patients with COVID-19 and obtaining informed consent. Serial investigations were initiated in-hospital or early post-discharge (visit 1) and then repeated in association with multi-organ imaging at 28-60 days post-discharge (visit 2).

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