

The Chief Scientist Office Cardiovascular and Pulmonary Imaging in SARS Coronavirus disease-19 (CISCO-19) study

Kenneth Mangion ^{1,2}, Andrew Morrow ^{1,2}, Catherine Bagot ³, Hannah Bayes ⁴, Kevin G. Blyth ^{5,6}, Colin Church ⁵, David Corcoran^{1,2}, Christian Delles ¹, Lynsey Gillespie ⁷, Douglas Grieve ⁸, Antonia Ho ⁹, Sharon Kean¹⁰, Ninian N. Lang ^{1,2}, Vera Lennie ¹¹, David J. Lowe ¹², Peter Kellman ¹³, Peter W. Macfarlane ¹⁴, Alex McConnachie ⁹, Giles Roditi ^{1,15}, Robert Sykes ^{1,16}, Rhian M. Touyz ^{1,2}, Naveed Sattar ¹, Ryan Wereski ^{2,11,17}, Sylvia Wright ¹⁸, and Colin Berry ^{1,2*}

¹British Heart Foundation Glasgow Cardiovascular Research Centre, Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK; ²Department of Cardiology, Queen Elizabeth University Hospital, NHS Greater Glasgow and Clyde Health Board, Glasgow, UK; ³Department of Haemostasis and Thrombosis, Royal Infirmary, NHS Greater Glasgow and Clyde Health Board, Glasgow, UK; ⁴Department of Respiratory Medicine, Glasgow Royal Infirmary, NHS Greater Glasgow and Clyde Health Board, Glasgow, UK; ⁵Department of Respiratory Medicine, Queen Elizabeth University Hospital, NHS Greater Glasgow and Clyde Health Board, Glasgow, UK; ⁶Institute of Cancer Sciences, University of Glasgow, Glasgow, UK; ⁷Project Management Unit, Glasgow Clinical Research Facility, Greater Glasgow and Clyde Health Board, Glasgow, UK; ⁸Department of Respiratory Medicine, Royal Alexandra Hospital, NHS Greater Glasgow and Clyde Health Board, Glasgow, UK; ⁹MRC-University of Glasgow Centre for Virus Research, Glasgow, UK; ¹⁰Robertson Centre for Biostatistics, Institute of Health and Wellbeing, University of Glasgow, Glasgow, UK; ¹¹Department of Cardiology, University Hospital Ayr, Ayrshire and Arran Health Board, Ayr, UK; ¹²Department of Emergency Medicine, Queen Elizabeth University Hospital, NHS Greater Glasgow and Clyde Health Board, Glasgow, UK; ¹³National Heart, Lung, and Blood Institute, National Institutes of Health, DHHS, Bethesda, MD, USA; ¹⁴Electrocardiography Core Laboratory, Institute of Health and Wellbeing, University of Glasgow, UK; ¹⁵Department of Radiology, NHS Greater Glasgow and Clyde Health Board, Glasgow, UK; ¹⁶Department of Cardiology, Royal Infirmary, NHS Greater Glasgow and Clyde Health Board, Glasgow, UK; ¹⁷British Heart Foundation Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, UK; and ¹⁸Department of Pathology, Laboratory Medicine, Queen Elizabeth University Hospital, NHS Greater Glasgow and Clyde Health Board, Glasgow, UK

Received 12 June 2020; editorial decision 29 June 2020; accepted 17 July 2020; online publish-ahead-of-print 23 July 2020

Background

COVID-19 is typically a primary respiratory illness with multisystem involvement. The prevalence and clinical significance of cardiovascular and multisystem involvement in COVID-19 remain unclear.

Methods

This is a prospective, observational, multicentre, longitudinal, cohort study with minimal selection criteria and a near-consecutive approach to screening. Patients who have received hospital care for COVID-19 will be enrolled within 28 days of discharge. Myocardial injury will be diagnosed according to the peak troponin I in relation to the upper reference limit (URL, 99th centile) (Abbott Architect troponin I assay; sex-specific URL, male: >34 ng/L; female: >16 ng/L). Multisystem, multimodality imaging will be undertaken during the convalescent phase at 28 days post-discharge (Visit 2). Imaging of the heart, lung, and kidneys will include multiparametric, stress perfusion, cardiovascular magnetic resonance imaging, and computed tomography coronary angiography. Health and well-being will be assessed in the longer term. The primary outcome is the proportion of patients with a diagnosis of myocardial inflammation.

Conclusion

CISCO-19 will provide detailed insights into cardiovascular and multisystem involvement of COVID-19. Our study will inform the rationale and design of novel therapeutic and management strategies for affected patients.

Clinical trial registration

ClinicalTrials.gov identifier NCT04403607.

Keywords

SARS-CoV-2 • Myocardial inflammation • Myocardial infarction • Myocardial injury • Imaging • Biomarkers

*Corresponding author. British Heart Foundation Glasgow Cardiovascular Research Centre, Institute of Cardiovascular and Medical Sciences, 126 University Place, University of Glasgow, Glasgow G12 8TA, Scotland, UK. Tel: +44 141 330 1671 or +44 141 951 5180. Fax +44 141 330 6794. Email: colin.berry@glasgow.ac.uk

© The Author(s) 2020. Published by Oxford University Press on behalf of the European Society of Cardiology.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

Background

COVID-19 presents the most significant threat to human health in modern times.^{1–4} The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, which causes COVID-19 illness, is mediated by tropism for nasopharyngeal and pulmonary epithelial cells. Cardiovascular complications are common, affecting ~1 in 4 patients, are associated with prior cardiovascular disease, and increase the risk of death.^{5–11} Cardiovascular injury may be secondary to inflammation, hypoxia, hypotension, and thrombosis, or, potentially, through virus invasion of endothelial cells, vascular smooth muscle cells, and pericytes, leading to vascular injury or dysfunction. This mechanism may then play a primary role in the development of pathology.¹¹ The angiotensin-converting enzyme 2 (ACE2) transmembrane receptor, which normally has protective effects in the cardiovascular system, is also the receptor which mediates virus transmission in human cells, including in the cardiovascular system (Figure 1).^{12,13}

Mechanisms of SARS-CoV-2 tropism for cardiovascular cells

Coronavirus (Coronaviridae family) is named after the crown morphology of its outer membrane. SARS-CoV-1, which caused an epidemic in 2002–2003, and SARS-CoV-2, infect human cells when the surface spike (S) protein, a type 1 membrane glycoprotein,^{10,12} binds to the ACE2 transmembrane receptor protein on human cells. SARS-CoV-1 and SARS-CoV-2 have 76% similarity in their S proteins. Fusion of the coronavirus S protein with the cell surface ACE2 transmembrane receptor internalizes the virus, promulgating replication and dissemination. ACE2 is expressed in endothelial cells in the heart, lungs, kidneys, intestine, and testis,^{14–16} and is a member of the counter-regulatory axis of the renin-angiotensin-aldosterone system (RAAS).¹⁷ In its canonical function, as a carboxypeptidase, ACE2 is protective in the cardiovascular system through its role in metabolising angiotensin I (Ang I) to Ang-(1-9) and Ang II to Ang-(1-7), therefore reducing Ang II levels and generating the protective peptides Ang-(1-9) and Ang-(1-7) which act through the angiotensin type 2 receptor (AT2R)^{18,19} and the Mas receptor,²⁰ respectively.

Prior knowledge from SARS-CoV and cardiovascular involvement

Like SARS-CoV-2, SARS-CoV-1 also uses ACE2 and transmembrane protease serine 2 (TMPRSS2) as mechanisms of cell invasion. During SARS-CoV-1 infection of transgenic mice expressing human ACE2, ACE2 becomes dysregulated and depleted.²¹ In acute lung disease triggered by sepsis, ACE2 is directly protective.²² SARS patients may experience overwhelming immune and inflammatory responses, in the form of a cytokine storm, leading to left ventricular (LV) systolic dysfunction, arrhythmias, and sudden death.^{23,24} Imaging is therefore well placed to investigate the underlying pathology of myocardial injury in COVID-19 patients.

Evidence of coronavirus inoculation of cardiovascular cells and myocarditis

Chan et al.²⁵ provided preliminary evidence of coronavirus infection in pulmonary endothelial cells. Varga et al. studied the histopathology of heart, lung, kidney, and liver tissue samples from three patients who died from COVID-19. They found evidence of viral elements within endothelial cells and endotheliitis.¹¹ Recent studies suggest that systemic

manifestations of COVID-19, including hypertension, thrombosis, myocardial involvement, and kidney failure, may be due to endothelial and vascular disease.²⁶

High levels of ACE2 are expressed by pericytes in the heart¹⁶ and cardiomyocytes (7.5%, scRNA-seq data).²⁷ ACE2 may have paradoxical roles since ordinarily it regulates a vasculoprotective signalling pathway and has protective catalytic effects in the lung. On the other hand, it is a receptor for virus transmission into human cells. SARS-CoV-2 infection reduces the activity and/or protein levels of ACE2, leading to a harmful imbalance of Ang II/AT1R effect.²⁸ ACE2 overexpression enhances the stability of atherosclerotic plaque.²⁹ However, ACE2 transcript and protein levels are increased in patients with cardiovascular disease such as heart failure,¹⁶ and post-myocardial infarction (MI),³⁰ implying an increased risk of cardiac infection. The association between COVID-19 and RAAS inhibitor therapy^{31,32} and other cardiovascular medications²¹ is most probably explained by underlying cardiovascular disease.³¹ There have been a number of case reports or case series of reported COVID-19-associated myocardial injury, presenting as myocarditis^{2,5,33,34} or reverse Takotsubo.^{35,36} A number of autopsy reports identify SARS-CoV-2 RNA in the myocardium of patients dying from COVID-19-related complications (pulmonary embolism and pneumonitis³⁷, and undefined³⁸), whilst other series do not identify myocardial SARS-CoV-2 RNA in the context of death related to pneumonitis and acute respiratory distress syndrome (ARDS).^{39–42} Taken together, this suggests that there are several potential mechanisms of myocardial injury (endotypes) in severe SARS-CoV-2 infection and that further research is warranted.

Evidence of venous and pulmonary arterial thrombosis

Endothelial damage and thrombotic microvascular angiopathy may underpin systemic vascular dysfunction.¹⁰ Autopsy studies have shown evidence of this at a pulmonary microvascular level, and this may be responsible in part for the severe hypoxia present in these patients.⁴³

Evidence of renal involvement

The prevalence of renal involvement in patients with SARS-CoV-2 infection is low,^{44,45} and is hypothesized to be secondary to cytokine damage, systemic effects of the illness,⁴⁴ or interaction between the cardiopulmonary axis and renal function, as has been reported in ARDS.⁴⁶

Contemporary definitions of acute myocardial infarction or myocardial injury

Acute myocardial injury is defined as a rise in the circulating concentration of troponin above the 99th percentile of the upper reference limit and then a fall. Acute MI is myocardial injury in the context of myocardial ischaemia. Acute MI is categorized into one of five types.⁴⁷ Type 1 MI is diagnosed based on the occurrence of at least one of the following: (i) symptoms of acute myocardial ischaemia; (ii) new ischaemic ECG changes; (iii) development of pathological Q waves; (iv) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality in a pattern consistent with an ischaemic aetiology; or (v) identification of a coronary thrombus by angiography including intracoronary imaging or by autopsy. Clinical scenarios of Type 1 MI include coronary plaque rupture and plaque erosion. A diagnosis of Type 2 MI is based on evidence of an imbalance between myocardial oxygen supply and

demand unrelated to coronary thrombosis, requiring at least one of the following: (i) symptoms of acute myocardial ischaemia; (ii) new ischaemic ECG changes; (iii) development of pathological Q waves; or (iv) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality in a pattern consistent with an ischaemic aetiology.

Clinical scenarios of Type 2 MI include severe hypertension, hypoxia, and tachyarrhythmia. Type 3 MI is the classification used for patients who suffer cardiac death due to MI, and Types 4 and 5 MI are iatrogenic, consequent on MI arising from percutaneous coronary intervention (PCI) or coronary artery bypass graft (CABG), respectively.⁴⁷ The diagnosis of myocardial infarction with no obstructive coronary arteries (MINOCA) indicates that there is an ischaemic mechanism responsible for the myocyte injury. Clinical scenarios for MINOCA include MI due to atherosclerotic plaque rupture, i.e. Type 1 MI, coronary spasm, spontaneous coronary dissection, and Type 2 MI.

Myocardial injury is diagnosed based on elevated cardiac biomarkers without myocardial ischaemia. Acute myocardial injury is associated with a rise and fall of troponin, whereas chronic myocardial injury is associated with a stable troponin concentration. Myocardial injury may be primarily due to a cardiac or non-cardiac cause.⁴⁸ Cardiac causes of myocardial injury include arrhythmias and disorders of coronary vascular function. Non-cardiac causes of myocardial injury include anaemia and pulmonary embolism.⁴⁸

The plasma concentration of troponin I will be measured using the Abbott Architect assay. The sex-specific upper reference limits (99th centile) are >16 ng/L and >34 ng/L for females and males, respectively.

Rationale for multimodality imaging

MRI

Cardiovascular magnetic resonance imaging (CMR) is a non-invasive diagnostic test for myocardial pathology.^{49–51} Through tissue characterization, CMR can differentiate between myocardial inflammation (acute vs. chronic), myocardial infarction (acute vs. chronic scar), and pericarditis, and simultaneously provide information on cardiac function, blood flow, and incidental findings such as pericardial effusion.^{49,50,52,53}

In COVID-19, myocardial inflammation due to viral myocarditis, ischaemia, or stress cardiomyopathy, as well as MINOCA (Type 1 or Type 2 MI) and other cardiac complications are potentially more common than previously thought based on current crude biomarker studies. Establishing their real frequency using powerful imaging techniques is essential for optimizing risk stratification and therapy. In our study, MRI will be used to assess for and classify clinical endotypes. The endotypes of myocardial involvement are (1) myocardial inflammation, e.g. (1.1) myocarditis, (1.2) ischaemia, or (1.3) stress (Takotsubo) cardiomyopathy; (2) myocardial infarction; (3) indeterminate; or (4) none. A diagnosis of viral myocarditis typically requires endomyocardial biopsy (EMB). We do not anticipate that EMB will be undertaken in our study population; therefore, a presumptive diagnosis may be made based on the available clinical information.

The wide field of view of the chest and abdomen also permits imaging of the lungs and kidneys.

CT

A computed tomography (CT) coronary angiogram/chest protocol will image for coronary artery disease (CAD), delayed enhancement, lung perfusion, pulmonary thrombo-embolism, and parenchymal pathology.

Taken together, integration of multimodality imaging of the heart, lungs, and kidneys within a single visit represents a highly novel approach to investigate the cardiovascular complications of COVID-19. The imaging scans will also support advanced computational modelling to better understand this multisystem disease.

Hypothesis

We hypothesize that myocarditis (myocardial inflammation) is common after SARS-CoV-2 infection.

Objectives

- i. To assess the incidence, nature, time-course and clinical significance of cardiovascular involvement in patients with COVID-19.
- ii. To quantify myocardial perfusion as a measure of coronary microvascular function.
- iii. To determine whether patients with COVID-19 and pre-existing cardiovascular disease are at increased risk of cardiac involvement.
- iv. To assess the incidence of pulmonary thrombo-embolism and right ventricular dysfunction.
- v. To assess for renal involvement in patients with COVID-19 and suspected cardiac involvement.
- vi. To assess mechanisms using circulating biomarkers of cardiac injury [troponin I, N-terminal probrain natriuretic peptide (NT-proBNP)], inflammation [C-reactive protein (CRP)], vascular injury [sACE2, von Willebrand factor (vWF), and interleukin-6 (IL-6)], and thrombotic microangiopathy, and their changes over time.
- vii. To establish substudies using computational cardiovascular modelling, electrocardiography, and pathology.

Study design

The CISCO-19 study has a prospective, observational, multicentre, longitudinal, cohort design, minimal selection criteria, and a near-consecutive approach to screening.

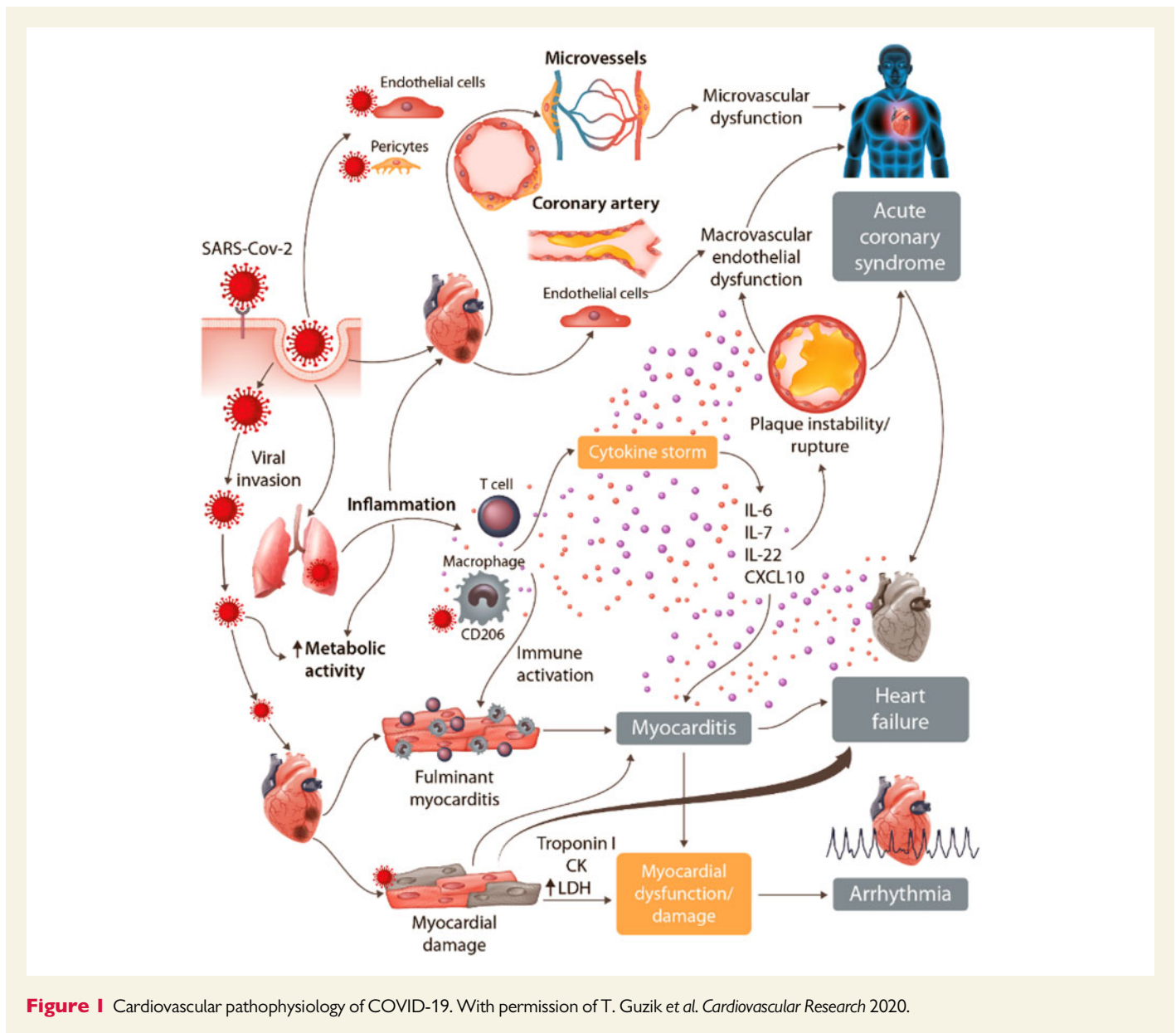
Study population

The study population will be focused on COVID-19 patients who have survived the initial, acute illness. Accordingly, the findings from our study are most relevant to patients in the convalescent phase looking forward to the longer term. We will characterize cardiac involvement evidenced by an increase in circulating high sensitivity troponin I > upper reference limit at any time during the hospital episode of care. Control data may be drawn from local cohorts, as appropriate. Patients who received hospital care with COVID-19 will be enrolled within 28 days of discharge. This enrolment strategy is intended to maximize enrolment of all-comers affected by COVID-19. Patients who die or are ineligible for other reasons will be recorded in a screening log.

Multiparametric, stress perfusion, cardiovascular MRI, CT coronary angiography (CTCA), and a 12-lead ECG will be acquired at ~28 days post-discharge. The study is designed to assess for imaging evidence of multiorgan injury (Figure 2).

Setting

The study will involve multiple centres in the West of Scotland (population 2.2 million) including the Queen Elizabeth University Hospital, the Royal Infirmary in Glasgow, and the Royal Alexandra Hospital in Paisley.



These hospitals provide secondary care services for NHS Greater Glasgow and Clyde Health Board (Figure 3).

Patient identification

Patients with a diagnosis of COVID-19 will be identified from clinical databases. The clinical pathways include, but are not limited to: (i) Emergency Medicine and in-patient wards; and (ii) laboratory records.

Eligibility criteria

The inclusion criteria are: (i) age >18 years old; (ii) history of hospital attendance or hospitalization for COVID-19, confirmed clinical diagnosis e.g. case definition according to criteria of the World Health Organisation and Health Protection Scotland), laboratory test [e.g. polymerase chain reaction (PCR)], and/or a radiological test (e.g. CT chest or chest X-ray); (iii) able to comply with study procedures; and (iv) able to provide written informed consent. The radiology results will be

reported by accredited radiologists according to contemporary, national guidelines.

The exclusion criteria are: (i) contra-indication to CMR, e.g. severe claustrophobia or metallic foreign body; (ii) contra-indication to intravenous adenosine, i.e. severe asthma, long QT syndrome, second- or third-degree atrioventricular (AV) block, and sick sinus syndrome; and (iii) lack of informed consent.

Screening

We aim to screen a near-consecutive cohort of patients diagnosed with COVID-19. A screening log will be prospectively completed. The reasons for being ineligible, including lack of inclusion criteria and/or presence of exclusion criteria, will be prospectively recorded. This information will characterize selection bias, if any.

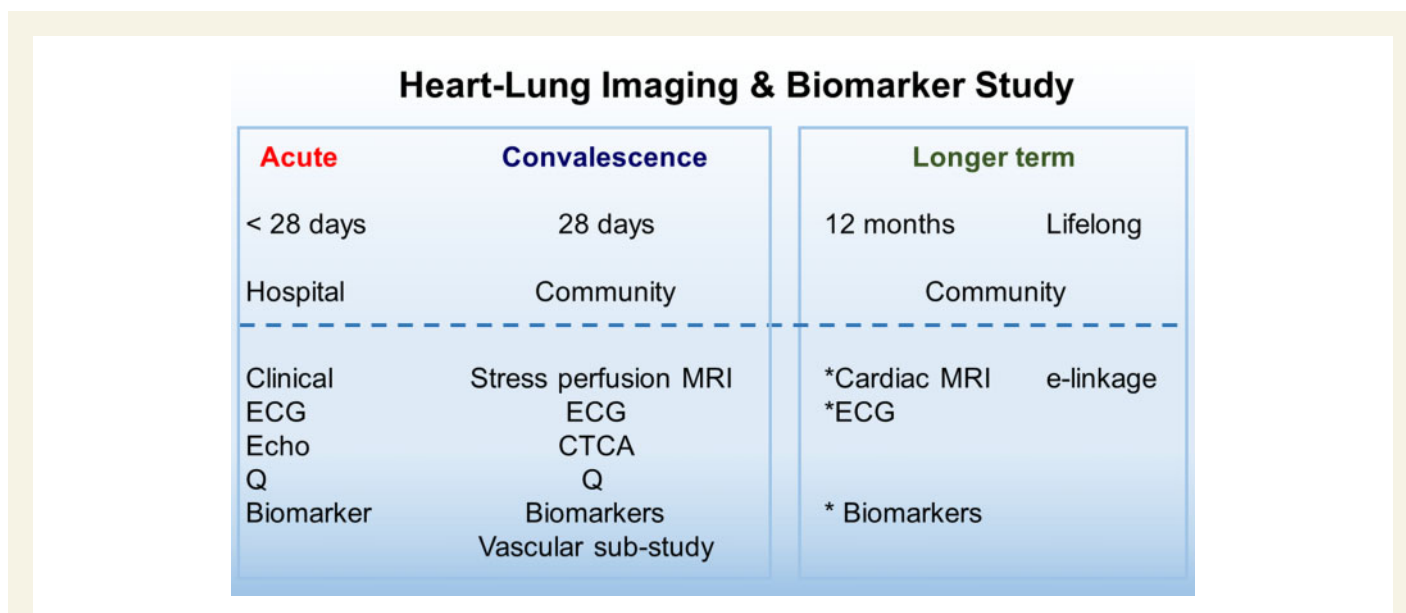


Figure 2 Schematic study design: flow diagram. The time periods are from hospital discharge. Abbreviations: CTCA, computed tomography coronary angiography; ECG, electrocardiogram; Q, questionnaires; MRI, stress perfusion cardiac magnetic resonance imaging.

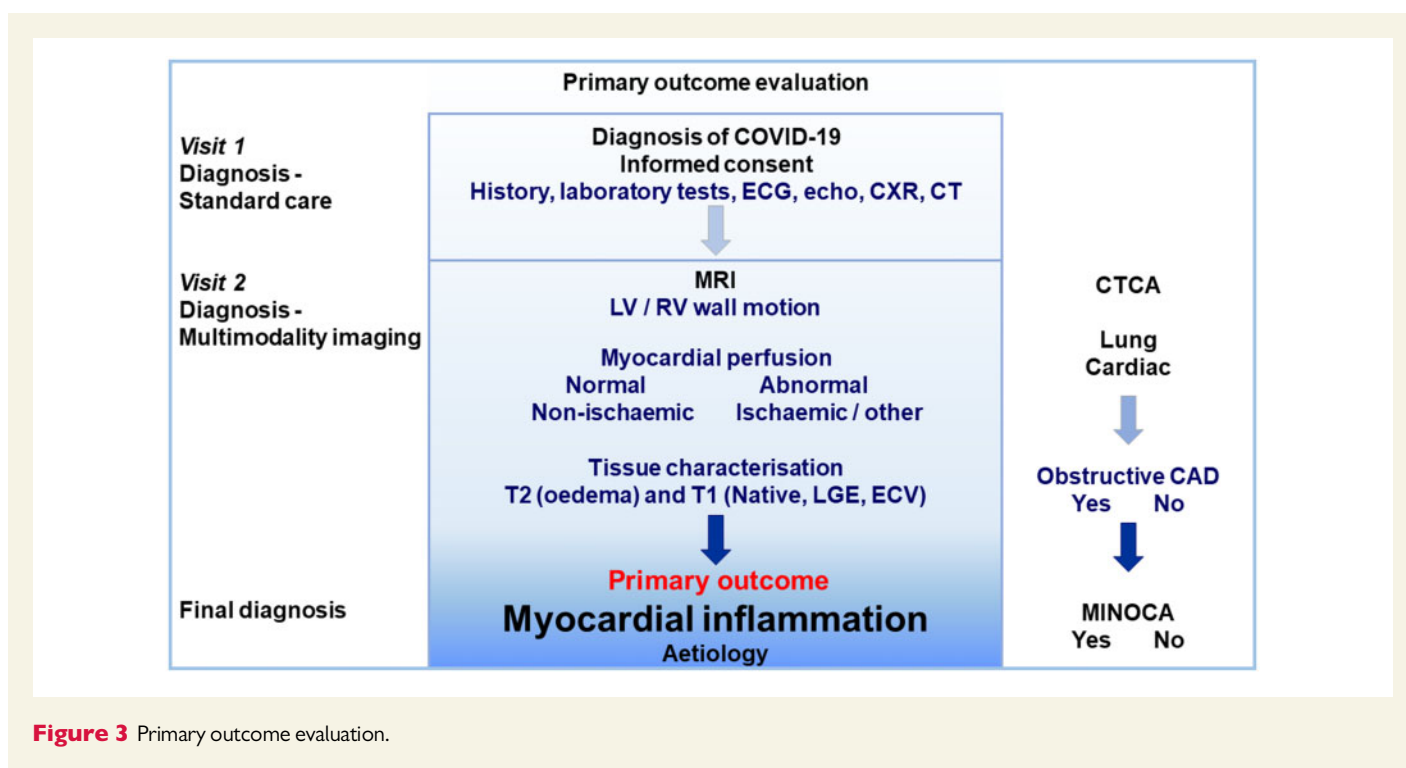


Figure 3 Primary outcome evaluation.

Recruitment

One hundred and eighty patients will be enrolled following written informed consent. If a consented patient is subsequently found to be ineligible, unless consent is withdrawn, they will remain included in the study population, including consent for long-term follow-up using linkage of electronic government and patient records (EPRs).

Diagnosis of COVID-19

A diagnosis of COVID-19 will be based either laboratory evidence of SARS-CoV-2 infection using a PCR test on a biospecimen or a radiological diagnosis consistent with COVID-19 but biospecimen negative.⁵⁴ The laboratory tests used are either the Roche Cobas 6800 or Seegene SARS-CoV-2 tests.

Study procedures

The current protocol involves two visits. The first visit involves informed consent and baseline assessments. The second visit occurs at 28 days after discharge. A third visit is intended at 1 year, contingent on additional funding and a protocol amendment being secured. A proposed vascular biology substudy involves a gluteal skin biopsy after 28 days. The participants will be invited to consent for life-long follow-up using electronic record linkage without direct contact.

The study assessments involve gathering information from standard of care procedures and research tests. The standard of care information includes demographics, medical history (including multimorbidity), limited examination, laboratory and radiological tests, cardiology tests (including an ECG and an echocardiogram if clinically indicated), and treatment. The research assessments at both visits include blood and urine samples, a 12-lead digital ECG (Beneheart R3, Mindray), health status questionnaires, and assessments of adverse events. Visit 2 involves cardiovascular imaging, including stress perfusion MRI and CTCA, and an ECG. By designating imaging during the convalescent phase, at 28 days post-discharge, the participants are not anticipated to be infectious. This approach aligns with other contemporary studies, such as the International Severe Acute Respiratory and Emerging Infection Coronavirus Clinical Characterisation Consortium-4 (ISARIC-4C) study.⁵⁵ Since MRI and CTCA are appropriately not performed during the acute phase, some pathologies that might have been detected acutely may have resolved by 28 days. Therefore, all clinical information obtained during Visits 1 and 2 will be used to inform the diagnosis of myocardial injury.

Cardiovascular disease and risk factors

Prior cardiovascular disease includes diabetes, hypertension, myocardial infarction, heart failure, stroke, and transient ischaemic attack.

Cardiovascular risk factors include age, cigarette smoking, obesity, hypertension, hyperlipidaemia, and glycated haemoglobin. Cardiovascular risk will be described using the established JBS3 and Heart Age Scores.

Outcomes

Primary outcome

The primary outcome for our study is a diagnosis of myocarditis (myocardial inflammation). The relevant endotypes are (1) myocardial inflammation due to (1.1) myocarditis, (1.2) ischaemia, or (1.3) stress (Takotsubo) cardiomyopathy, (1.4) combination; (2) myocardial infarction; (3) indeterminate; or (4) none.

Myocardial inflammation is caused by the immune response to virus infection, autoimmune disease, ischaemic injury, or toxic agents.⁴⁹ MRI provides a non-invasive approach to characterizing acute and chronic myocardial pathology. Expert consensus recommendations for the CMR-based diagnosis of definite myocardial inflammation include one T2-based criterion (global or regional increase of myocardial T2 relaxation time or an increased signal intensity in T2-weighted CMR images), with at least one T1-based criterion [increased myocardial T1, extracellular volume, or late gadolinium enhancement (LGE)].^{49,50} Having just one criterion may support a diagnosis of probable myocardial inflammation. A key attribute of the imaging protocol in our study involves the prioritized assessment of myocardial perfusion using pharmacological stress testing with intravenous adenosine. This approach is intended to permit classification of myocardial injury as being ischaemic or non-ischaemic, to facilitate classification of myocardial infarction vs. ischaemic

or non-ischaemic myocardial inflammation. Information on myocardial perfusion will provide insights into coronary microvascular dysfunction that may, potentially, occur following COVID-19.⁵¹ Information from CTCA will clarify the presence or absence of obstructive CAD and MINOCA.

Consensus-based diagnosis of the primary outcome

A panel of three or more cardiologists will assess the clinical information to make a diagnosis (endotype) and related certainty (Not/unlikely = no; Probable/very = yes) before and after disclosure of the MRI and CTCA findings. The diagnosis will be based on consensus. This approach reflects the uncertainty in determining a diagnosis in patients with myocardial injury. The diagnosis draws upon clinical information and test results, rather than any single test modality in isolation.

Secondary outcomes

A prioritized secondary outcome is the endotype for myocardial injury, including myocardial infarction type according to the 4th Universal Definition of MI,⁴⁷ and myocarditis (myocardial inflammation, ischaemia, or stress cardiomyopathy).^{49,50}

Patient-reported outcome measures

We aim to assess the impact of cardiovascular complications on health status, well-being, and physical function. We will prospectively collect patient-reported outcome measures (PROMS) in order to assess for associations with the cardiovascular complications of COVID-19, reflected by the primary and secondary outcomes. Self-reported health status will be assessed using the generic EuroQOL EQ-5D-5L questionnaire and the Brief Illness Perception Questionnaire (Brief-IPQ).^{56,57} We will utilize the Patient Health Questionnaire-4 (PHQ-4) to assess for anxiety and depressive disorders.⁵⁸ Participants will be invited to complete these questionnaires at each visit. The Duke Activity Status Index (DASI) provides a measure of functional capacity, and a higher score reflects greater physical function.⁵⁹ The International Physical Activity Questionnaire–Short Form (IPAQ-SF) measures the types of intensity of physical activity and time spent sitting that people do as part of their daily lives. The score reflects total physical activity in MET-min/week and time spent sitting.⁶⁰

Biomarkers

In order to research the mechanisms of cardio-pulmonary and renal involvement of SARS-CoV-2 infection, we will measure circulating biomarkers of inflammation (CRP, ferritin, IL-6), cardiac injury (troponin I, NT-proBNP), renin–angiotensin system (aldosterone, sACE2), and haemostasis [coagulation screen, Clauss fibrinogen, D-dimer, FVIII (one stage), VWF antigen and VWF:GP1ba, antithrombin, protein C, and free protein S)], and renal function (albumin:creatinine ratio) and their changes over time.

The measurements will be undertaken in a central laboratory, blind to the other clinical data. The associations between the circulating concentrations of these mechanistic biomarkers, including their changes over time, and the primary and secondary outcomes will be assessed.

Table 1 Secondary outcomes

Secondary outcomes	
Health status	<ul style="list-style-type: none"> • Illness perception (Brief IPQ) • Anxiety/depression (PHQ4) • EQ-5D-5L • The Duke Activity Status Index (DASI) • The International Physical Activity Questionnaire – Short Form (IPAQ-SF) • Serious adverse events (SAEs)
Cardiac MRI	<ul style="list-style-type: none"> • Impaired LV systolic function • Increased LV end-diastolic volume • Myocardial oedema (T2 map) • Myocardial inflammation (native T1 map) • Late gadolinium enhancement • Increased extracellular volume • Impaired myocardial perfusion • Impaired RV systolic function • Increased RV end-diastolic volume
CT chest and angiography	<ul style="list-style-type: none"> • Consolidation • Pulmonary artery diameter • Thrombus, main pulmonary artery • Microthrombi
Renal	<ul style="list-style-type: none"> • Urine albumin:creatinine ratio
Biomarkers	<ul style="list-style-type: none"> • Circulating biomarkers of inflammation (CRP, ferritin), cardiac injury (troponin I, NT-proBNP), vascular injury (aldosterone, sACE2, IL-6) and haemostasis [coagulation screen, Clauss fibrinogen, D-dimer, FVIII (one stage), VWF antigen and VWF:GP1ba, antithrombin, protein C, and free protein S)], and renal function (albumin:creatinine ratio)
Feasibility	<ul style="list-style-type: none"> • Participant withdrawal rate
Exploratory outcomes	<ul style="list-style-type: none"> • Vascular biology changes • Computational modelling of co-registered imaging to develop novel biomarkers • ECG abnormalities • Renal MRI—kidney size, oedema/inflammation, apparent diffusion coefficient (ADC), a quantitative parameter calculated from diffusion-weighted imaging (DWI)

Cardio-pulmonary and renal MRI

Cardiovascular MRI is the reference diagnostic method for myocardial injury, including myocarditis and acute cardiomyopathy. Stress perfusion MRI using intravenous adenosine enables dynamic imaging of myocardial blood flow during stress and rest conditions. In-line pixel mapping enables fully quantitative read-out of myocardial blood flow (mL/min/g tissue), classified at a subsegmental level (32 myocardial segments) with the percentage extent of myocardium with impaired perfusion during stress (% ischaemic burden).^{61–66} All of this information can be spatially

mapped with LV function, tissue characteristics revealed by T2-mapping, native T-mapping, LGE, and extracellular volume (ECV).⁵² In this study, the modified Lake Louise criteria will be used to diagnose definite myocardial inflammation [T2 map + T1 (native T1, LGE, or ECV abnormal)] or probable myocardial inflammation (either T2 or T1 abnormal).^{49,50} In order to limit selection bias, renal dysfunction is not an exclusion criterion. Patients with severe renal dysfunction [glomerular filtration rate (GFR) <30 mL/kg/m²] will be considered for contrast MRI according to local radiology protocols.

SARS-CoV-2 causes vascular dysfunction and microthrombotic angiopathy.¹⁰ Our scientific hypothesis is that microvascular dysfunction underlies the cardiovascular toxicity caused by this infection through primary and secondary mechanisms. Direct virus infection of injured endothelial cells, disrupting the integrity of the endothelial barrier, permits virus infection of deep tissues, causing myocarditis and nephritis. Myocarditis and ischaemia may induce cardiomyopathy leading to left and right ventricular dysfunction. Secondary consequences of COVID-19 including hypoxia, inflammation, and vasospasm impaired tissue perfusion, leading to myocardial ischaemia.

MRI will be undertaken at 3.0 Tesla (Siemens PRISMA) ~28 days after discharge. This timepoint may be considered as the subacute, convalescent phase. The rationale for undertaking the multiparametric, stress perfusion cardiovascular MRI at this timepoint is to determine whether there is persisting evidence of myocardial injury and/or MI, LV function, and incidental pathology (e.g. mural thrombus) in the convalescent phase. The patients will be advised to withhold cardiovascular medications and to avoid caffeine-containing drinks for 24 h before the scan.

MRI will also provide information for incidental findings in the chest (e.g. pulmonary arterial thrombus) and abdomen. Imaging of renal anatomy, size, and tissue characterization will be exploratory.

Computed tomography

The CT scanner has 320 detectors enabling full heart coverage within a single heartbeat (Aquilion ONE, Canon). Intravenous metoprolol will be used where required to control the heart rate (target 60 b.p.m.) and sublingual glyceryl trinitrate will be given to all patients immediately before the scan acquisition. An initial low radiation dose helical scan of the thorax will be acquired for comprehensive assessment of the lungs. A contrast bolus timing scan will be acquired which will provide information on cardiopulmonary transit times. Non-contrast and contrast-enhanced angiographic breath-hold ECG-gated volumes will be acquired timed for optimum pulmonary and systemic arterial (coronary) opacification.

CTCA will provide information on the presence and extent of coronary calcification (calcium score), CAD, and whether CAD is obstructive (flow-limiting) including the CAD-RADS score. Intracardiac thrombus will be assessed. Late enhancement ECG-gated CT will be acquired to assess for delayed enhancement (scar)⁶⁷ and ECV calculation.⁶⁸ Pulmonary vascular imaging will assess pulmonary vascular thrombosis (embolism) including CT obstruction score, cardiopulmonary transit times, and measures of raised pulmonary artery (PA) and right heart pressures [PA, caval and azygous dimensions plus hepatic inferior vena cava (IVC) reflux]. CT will also characterize pulmonary features associated with COVID infection (percentage ground-glass opacity, percentage consolidation, and CO-RADS score) plus pre-existing lung damage (percentage chronic obstructive pulmonary

disease). CT will also assess for signs of osteoporosis and sarcopenia as frailty markers.

The CT and MRI findings will be correlated with the other clinical data. Cardiac and extracardiac findings will be reported and managed according to local standards of care. Patients with severe renal dysfunction thought to be at risk of acute kidney injury as determined by local radiology clinical protocols will undergo non-contrast CT.

Natural history

In order to assess the natural history, longer term follow-up for health outcomes will be undertaken using electronic record linkage, removing the need for participants to undergo further research visits after the 12-month visit.

Exploratory analyses

The scans will be pseudoanonymized, i.e. identifiers removed and assigned a unique study number to enable linkage. The scans will be shared with research collaborators for cardiovascular modelling to better understand the relationships between tissue pathology, blood flow, and function.

COVID-19 infection and treatment are associated with changes on the ECG. The changes include alterations in heart rate, conduction, and ventricular repolarization. Drug treatment for COVID-19 may prolong the QTc interval. Whether these changes persist after the acute phase and their relationships to myocardial pathology are unknown. Paper and/or digital ECGs will be acquired, de-identified, and provided to the University of Glasgow Electrocardiography Core Laboratory for automated analysis. The ECG measures will be linked with the clinical and imaging data.

We will undertake exploratory research into the vascular biology of COVID-19 infection. This work will be undertaken in collaboration with cardiovascular scientists and virologists in the University of Glasgow. We will study the vascular biology of cells and molecules (e.g. RNA and cytokines) implicated in SARS-CoV-2. Informed consent will be obtained for post-mortem examination in the event of death during the study period. Histology samples of the heart will be examined for features of myocarditis, MI, and microvascular disease. The histopathological findings will be linked to the CMR findings.

Feasibility

This study has been developed with input from members of a multidisciplinary research team and the Scientific Strategy Group of the University of Glasgow. The study has been peer reviewed by panel members of the Chief Scientist Office of the Scottish Government. The study has been reviewed by the Patient and Public Involvement group of NHS Greater Glasgow and Clyde Health Board.

Statistical considerations

The statistical analyses will be pre-defined according to a Statistical Analysis Plan.

Sample size calculation

The primary outcome is myocarditis (myocardial inflammation). To detect an association between a history of pre-existing cardiovascular disease and the incidence of myocardial inflammation (myocarditis) at 2–4 weeks, we have assumed 25% of patients with prior cardiovascular disease and the incidence of myocardial inflammation in those with/without prior cardiac problems to be 33% and 10%, respectively. To have 80% power to detect this difference will require 140 participants (35 with cardiac problems, 105 without) to be scanned. We aim for 160 patients to attend the imaging visit, anticipating that 10–15% of the participants may have incomplete imaging data due to technical reasons such as imaging artefact or claustrophobia.

Pre-specified subgroup analyses are intended for patients without cardiovascular disease, as defined by the absence of (i) prior cardiovascular disease and (ii) obstructive CAD on CTCA. Given the public health significance of COVID-19, interim reports may be undertaken.

Blinding

Outcome assessments (endpoint adjudication) will be undertaken in blinded fashion. The primary outcome evaluation (myocardial injury endotype) will be adjudicated by a panel of cardiologists blind to the clinical status of the patient and performed according to a pre-specified charter.

Trial management and timelines

The trial will be conducted in line with the current *Guidelines for Good Clinical Practice in Clinical Trials* and STROBE guidelines.⁶⁹ The Study Management Group (SMG) includes those individuals responsible for the day-to-day management of the study including the chief investigator, project manager, and representatives from the sponsor. The role of this group will be to facilitate the progress of the study, ensure that the protocol is adhered to, and take appropriate action to safeguard participants and the quality of the study itself. Decisions about continuation or termination of the study or substantial amendments to the protocol will be the responsibility of the sponsor. A scientific steering group will oversee the study. This study is designed to be undertaken and reported rapidly in response to the global need for information about COVID-19.

Ethics

The CISCO-19 study is approved by the UK National Research Ethics Service (Reference 20/NS/0066).

Sources of funding

CISCO-19 is an investigator-initiated clinical study that is funded by the Chief Scientist Office of the Scottish Government (COV/GLA/Portfolio project number 311300). The funder has no role in the study 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. are supported by the British Heart Foundation (BHF RE/18/6134217).

The MRI study involves imaging and analyses technologies provided by Siemens Healthcare and the National Institutes of Health

The trial is co-sponsored by NHS Greater Glasgow & Clyde and the University of Glasgow.

Registration

The ClinicalTrials.gov identifier is NCT04403607.

Discussion

Our observational, multimodality, imaging cohort study will prospectively gather information on the cardiovascular complications and their clinical significance in COVID-19. A relatively unselected approach to patient enrolment will minimize selection bias beyond those who do not survive or who are unable to comply with the protocol. The findings will be generalizable to patients in the convalescent phase of the illness and informative for the natural history. On the other hand, the findings will not necessarily be generalizable to all patients with COVID-19, since our enrolment strategy focuses on survivors following the acute phase of the illness.

Cardiovascular MRI will be used to clarify clinical endotypes according to contemporary guidelines.^{49,50} Quantitative measurements of myocardial blood flow at stress and rest will enable focused research into coronary microvascular dysfunction that may be secondary to endotheliitis caused by SARS-CoV-2. CT imaging will clarify the presence and relevance of CAD and MINOCA, lung pathology, and pulmonary arterial thrombosis. Renal MRI will be undertaken on an exploratory basis to assess kidney size (differential volume) plus both global values and cortico:medullary ratios for T1, T2, and the apparent diffusion coefficient (ADC) for correlation with renal function. Renal dysfunction is not an exclusion criterion, reflecting the open approach to enrolment. Taken together with a comprehensive clinical assessment, laboratory tests (including renal function and urine albumin:creatinine ratio), and circulating biomarkers, our study will characterize multisystem involvement of SARS-CoV-2 infection.

High-sensitivity troponin is a cardiac protein that is ubiquitously released from injured cardiomyocytes. However, troponin is not cause specific, and circulating concentrations may increase due to hypoxia, hypotension, and renal failure, as well from direct cardiac toxicity. It is unclear whether cardiovascular involvement in COVID-19 is mainly secondary to severe pneumonia, or whether there is direct viral infection of the heart and blood vessels. A recent expert consensus guideline highlighted the pivotal value of CMR in the diagnosis of myocardial inflammation due to viruses, autoimmune disease, ischaemic injury, and toxic agents. MRI is diagnostically useful to identify endotypes for stratified therapy. We will assess whether this could be the case in COVID-19.

To our knowledge, CISCO-19 is the first to apply stress perfusion CMR to assess and quantify abnormalities in myocardial perfusion that may be secondary to microvascular dysfunction. The information on myocardial perfusion will help classify patients with myocardial injury into ischaemic or non-ischaemic groups. Stress perfusion MRI is not usually undertaken in patients with myocarditis, and quantitative measurements of myocardial blood flow are a key attribute of our study design. We aim to advance new knowledge into the pathogenesis of myocardial inflammation (ischaemic vs. non-ischaemic) and into the associations between the aetiology of disease and abnormalities in myocardial perfusion. Recent advances in fully quantitative, in-line pixel mapping of myocardial perfusion are uniquely enabling the quantification of myocardial blood flow in near real-time without the need for time-intensive, off-line post-processing. In addition, multimodality imaging involves multiparametric cardiovascular MRI and CTCA during the same visit. As such, our study will provide methodologically robust estimates of persisting myocardial injury and the impact on

the physical and mental well-being of the participants. The results should be helpful to inform clinical management strategies for the diagnosis and management of patients recovering from COVID-19. Our study will provide complementary information to add to the growing body of knowledge on multisystem involvement in COVID-19.

We will collect information on participants' characteristics at baseline including demographics, anthropometry, cardiovascular and medical history, and health status. The participants will be invited to give informed consent for life-long follow-up by linkage of EPRs. Using multivariable analyses, we will link the patients' characteristics at baseline to observations during longer term follow-up in order to characterize the natural history of this condition.

Our imaging research will clarify the prevalence and clinical significance of cardiopulmonary injury (notably myocardial inflammation) in patients with COVID-19, which is a major knowledge gap in the NHS. By adopting an all-comers approach, we will identify patients with myocardial inflammation that is subclinical (i.e. not diagnosed) or clinically overt. By correlating the MRI findings with troponin and other measures of cardiovascular injury, such as BNP, our results will potentially inform NHS care pathways to use these blood tests in a more directed manner for the clinical management of patients with COVID-19. Currently, there are no disease-modifying therapies for myocarditis, including due to SARS-CoV-2.

Overall, our study will add new knowledge on the natural history of COVID-19 in a comparatively unselected population. Our study will create a unique biorepository of clinical samples and images, which in turn may be exploited by scientists undertaking mechanistic research into the vascular biology of SARS-CoV-2 infection, and cardiovascular modelling.

Acknowledgements

We thank the staff and patients who are supporting this study, and the Chief Scientist Office of the Scottish Government for financial support. We also thank the CISCO-19 Study Management Group (Mrs Ammani Brown, Mrs Chloe Cowan, Ms Sharon Kean, Mr Jurgen Van-Melckebeke, Dr Kim Moran-Jones, Dr Debra Stuart, and Dr Maureen Travers) for their contributions towards the delivery of this study.

Investigators and management groups

Grant applicants

Kenneth Mangion MD/PhD, Catherine Bagot MBBS/MD, Colin Church MBChB/PhD, Christian Delles MD, Antonia Ho MBChB/PhD, David Lowe MBChB/MD, Alex McConnachie PhD, Giles Roditi MBChB, Rhian M. Touyz MBChB/PhD, Naveed Sattar MBChB/PhD, Ryan Wereski MBChB, Sylvia Wright MBChB, and Colin Berry MBChB/PhD.

Site investigators

Queen Elizabeth University Hospital

Kenneth Mangion MD/PhD, Kevin G. Blyth MBChB/PhD, Colin Church MBChB/PhD, Christian Delles MD, Stephen Dobbin MBChB, Claire Glen MBChB, Antonia Ho MBChB/PhD, David Lowe MBChB/MD, Kaitlin Mayne MBChB, Patrick Mark MBChB/PhD, Andrew Morrow MBChB, Oliver Peck MBChB, Alistair Rankin MBChB, Giles Roditi MBChB, David Stobo MBChB, Ryan Wereski MBChB, Sylvia Wright MBChB/PhD, and Colin Berry MBChB/PhD.

Royal Alexandra Hospital

Daniel Ang MBChB, Douglas Grieve MBChB/PhD, and Richard W. Mcfarlane BSc (Hons)/MBChB.

Glasgow Royal Infirmary

Catherine Bagot MBBS/MD, Hannah Bayes MBChB/PhD, Jaclyn Carberry MBChB(Hons)/BMedSci, Daniel Docherty MBChB, Fraser Graham MBChB, Javed Khan MBChB, Giles Roditi MBChB, David Stobo MBChB, Varun Sharma MBChB, and Robert Sykes MBChB/BMedSci

Glasgow Clinical Research Facility and Study Management Group

Ammani Brown BN, Patricia Clark BN, Andrew Dougherty BN, Lesley Gilmour BN, Chloe Cowan BN, Lynsey Gillespie PhD, Sharon Kean, Jurgen Van-Melckebeke BSc, Kim Moran-Jones PhD, Debra Stuart PhD, Maureen Travers PhD, Tracey Hopkins DCR/BSc (Hons)/PGd, Laura Dymock BSc (Hons), Evonne McLennan BSc (Hons)/PGc, Rosemary Woodward BSc/PGc, Fiona Savage BSc (Hons)/PGc, and Nicola Tynan BSc (Hons)/PGc.

Radiology, NHS Greater Glasgow and Clyde Health Board

Sau Lee Chang MBChB, John Dreisbach MBChB, Mhairi Dupre MBChB, Lindsey Norton MBChB, Giles Roditi MBChB, and David Stobo MBChB.

Laboratory Medicine and Biorepository, NHS Greater Glasgow and Clyde Health Board

Clare Orange BSc and Rory Gunson PhD.

Medical Physics, NHS Greater Glasgow and Clyde

Sarah Allwood-Spiers PhD, George Bruce BSc, Rosario Gonzalez-Lopez PhD, Pauline Hall Barrientos PhD, Aleksandra Radjenovic PhD, and Rebecca Stace BSc.

University of Glasgow

Institute of Cardiovascular and Medical Sciences

Colin Berry MBChB/PhD, Elaine Butler BSc, Christian Delles MD, Kenneth Mangion MD/PhD, Patrick Mark MBChB/PhD, Andrew Morrow MBChB, Naveed Sattar MBChB/PhD, Rhian Touyz MD/PhD, and Paul Welsh PhD.

Institute of Infection and Immunity

MRC Centre for Virology Research

Antonia Ho MBChB/PhD and Massimo Palmarini PhD.

Institute of Health and Wellbeing

Robertson Centre for Biostatistics

John G.F. Cleland MBChB/PhD, Sharon Kean, Alex McConnachie PhD, and Dionne Russell BSc.

Electrocardiology Core Laboratory

Peter W. Macfarlane DSc, Julie Kennedy MSc, Louise Inglis BSc (Hons), Jean Watt, Kathryn McLaren, and Shahid Latif MAppSci

Departments of Mathematics and Statistics

Nick Hill PhD, Dirk Husmeier PhD, and Xiaoyu Luo PhD

National Institutes of Health

Peter Kellman PhD, Hui Xue PhD

Clinical Adjudication Committee

David Corcoran MBChB/PhD, Ninian N. Lang MBChB/PhD, Queen Elizabeth University Hospital, and Vera Lennie MBChB University Hospital Ayr

Coordinators: Andrew Morrow MBChB and Anna Kamdar BMedSci

Steering Committee

College of Medical, Veterinary and Life Sciences, University of Glasgow
Iain B. McInnes MBChB/PhD, Stefan Siebert MBChB/PhD, and Neil Basu MBChB/PhD

Conflict of interest: C.B. is employed by the University of Glasgow which holds consultancy and research agreements with Abbott Vascular, AstraZeneca, Boehringer Ingelheim, Corovantis, GSK, HeartFlow, Menarini, Novartis, and Siemens Healthcare. All other authors have no conflicts to declare.

Author contributions: C.B. designed the study and wrote the first draft of the manuscript with K.M. The co-authors reviewed the manuscript drafts. Each author has either individually contributed to 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.

References

- Li B, Yang J, Zhao F, Zhi L, Wang X, Liu L, Bi Z, Zhao Y. Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China. *Clin Res Cardiol* 2020;**109**: 531–538.
- Inciardi RM, Lupi L, Zaccone G, Italia L, Raffo M, Tomasoni D, Cani DS, Cerini M, Farina D, Gavazzi E, Maroldi R, Adamo M, Ammirati E, Sinagra G, Lombardi CM, Metra M. Cardiac involvement in a patient with coronavirus disease 2019 (COVID-19). *JAMA Cardiol* 2020;doi: 10.1001/jamacardio.2020.1096.
- Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, Wang H, Wan J, Wang X, Lu Z. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). *JAMA Cardiol* 2020;doi: 10.1001/jamacardio.2020.1017.
- Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, Gong W, Liu X, Liang J, Zhao Q, Huang H, Yang B, Huang C. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. *JAMA Cardiol* 2020;doi: 10.1001/jamacardio.2020.0950.
- Hu H, Ma F, Wei X, Fang Y. Coronavirus fulminant myocarditis treated with glucocorticoid and human immunoglobulin. *Eur Heart J* 2020;**41**;doi.org/10.1093/eurheartj/ehaa190.
- Madjid M, Safavi-Naeini P, Solomon SD, Vardeny O. Potential effects of coronaviruses on the cardiovascular system: a review. *JAMA Cardiol* 2020;doi: 10.1001/jamacardio.2020.1286.
- Bonow RO, Fonarow GC, O'Gara PT, Yancy CW. Association of coronavirus disease 2019 (COVID-19) with myocardial injury and mortality. *JAMA Cardiol* 2020;doi: 10.1001/jamacardio.2020.1105.
- Clerkin KJ, Fried JA, Raikhelkar J, Sayer G, Griffin JM, Masoumi A, Jain SS, Burkhoff D, Kumaraiah D, Rabbani L, Schwartz A, Uriel N. Coronavirus disease 2019 (COVID-19) and cardiovascular disease. *Circulation* 2020;**141**:1648–1655.
- Driggin E, Madhavan MV, Bikdeli B, Chuich T, Laracy J, Biondi-Zoccai G, Brown TS, Nigoghossian CD, Zidar DA, Haythe J, Brodie D, Beckman JA, Kirtane AJ, Stone

- GW, Krumholz HM, Parikh SA. Cardiovascular considerations for patients, health care workers, and health systems during the COVID-19 pandemic. *J Am Coll Cardiol* 2020;**75**:2352–2371.
10. Guzik TJ, Mohiddin SA, Dimarco A, Patel V, Savvatis K, Marelli-Berg FM, Madhur MS, Tomaszewski M, Maffia P, D'Acquisto F, Nicklin SA, Marian AJ, Nosalski R, Murray EC, Guzik B, Berry C, Touyz RM, Kreutz R, Wang DW, Bhella D, Sgalliooco O, Crea F, Thomson EC, McInnes IB. COVID-19 and the cardiovascular system: implications for risk assessment, diagnosis, and treatment options. *Cardiovasc Res* 2020;doi: 10.1093/cvr/cvaa106.
 11. Varga Z, Flammer AJ, Steiger P, Haberecker M, Andermatt R, Zinkernagel AS, Mehra MR, Schuepbach RA, Ruschitzka F, Moch H. Endothelial cell infection and endothelitis in COVID-19. *Lancet* 2020;**395**:1417–1418.
 12. Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, Si H-R, Zhu Y, Li B, Huang C-L, Chen H-D, Chen J, Luo Y, Guo H, Jiang R-D, Liu M-Q, Chen Y, Shen X-R, Wang X, Zheng X-S, Zhao K, Chen Q-J, Deng F, Liu L-L, Yan B, Zhan F-X, Wang Y-Y, Xiao G-F, Shi Z-L. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020;**579**:270–273.
 13. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu N-H, Nitsche A, Müller MA, Drosten C, Pöhlmann S. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 2020;**181**:271–280.e8.
 14. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, Donovan M, Woolf B, Robison K, Jeyaseelan R, Breitbart RE, Acton S. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ Res* 2000;**87**:E1–E9.
 15. Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J Biol Chem* 2000;**275**:33238–33243.
 16. Chen L, Li X, Chen M, Feng Y, Xiong C. The ACE2 expression in human heart indicates new potential mechanism of heart injury among patients infected with SARS-CoV-2. *Cardiovasc Res* 2020;**116**:1097–1100.
 17. Crackower MA, Sarao R, Oudit GY, Yagil C, Kozieradzki I, Scanga SE, Oliveira-dos-Santos AJ, da Costa J, Zhang L, Pei Y, Scholey J, Ferrario CM, Manoukian AS, Chappell MC, Backx PH, Yagil Y, Penninger JM. Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature* 2002;**417**:822–828.
 18. Flores-Muñoz M, Smith NJ, Haggerty C, Milligan G, Nicklin SA. Angiotensin1-9 antagonises pro-hypertrophic signalling in cardiomyocytes via the angiotensin type 2 receptor. *J Physiol (Lond)* 2011;**589**:939–951.
 19. Flores-Munoz M, Work LM, Douglas K, Denby L, Dominiczak AF, Graham D, Nicklin SA. Angiotensin-(1-9) attenuates cardiac fibrosis in the stroke-prone spontaneously hypertensive rat via the angiotensin type 2 receptor. *Hypertension* 2012;**59**:300–307.
 20. Santos RAS, Silva ACS eMaric C, Silva DMR, Machado RP, Buhr I de, Heringer-Walther S, Pinheiro SVB, Lopes MT, Bader M, Mendes EP, Lemos VS, Campagnole-Santos MJ, Schultheiss H-P, Speth R, Walther T. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proc Natl Acad Sci USA* 2003;**100**:8258–8263.
 21. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, Huan Y, Yang P, Zhang Y, Deng W, Bao L, Zhang B, Liu G, Wang Z, Chappell M, Liu Y, Zheng D, Leibbrandt A, Wada T, Slutsky AS, Liu D, Qin C, Jiang C, Penninger JM. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med* 2005;**11**:875–879.
 22. Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, Yang P, Sarao R, Wada T, Leong-Poi H, Crackower MA, Fukamizu A, Hui C-C, Hein L, Uhlir S, Slutsky AS, Jiang C, Penninger JM. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* 2005;**436**:112–116.
 23. Li SS, Cheng C, Fu C, Chan Y, Lee M, Chan JW, Yiu S. Left ventricular performance in patients with severe acute respiratory syndrome: a 30-day echocardiographic follow-up study. *Circulation* 2003;**108**:1798–1803.
 24. Yu C, Wong RS, Wu EB, Kong S, Wong J, Yip GW, Soo YOY, Chiu MLS, Chan Y, Hui D, Lee N, Wu A, Leung C, Sung JJ. Cardiovascular complications of severe acute respiratory syndrome. *Postgrad Med J* 2006;**82**:140–144.
 25. Chan RWY, Chan MCW, Agnihothram S, Chan LLY, Kuok DIT, Fong JHM, Guan Y, Poon LLM, Baric RS, Nicholls JM, Peiris JSM. Tropism of and innate immune responses to the novel human betacoronavirus lineage C virus in human ex vivo respiratory organ cultures. *J Virol* 2013;**87**:6604–6614.
 26. Sardu C, Gambardella J, Morelli MB, Wang X, Marfella R, Santulli G. Hypertension, thrombosis, kidney failure, and diabetes: is COVID-19 an endothelial disease? A comprehensive evaluation of clinical and basic evidence. *J Clin Med* 2020;**9**:1417.
 27. Zou X, Chen K, Zou J, Han P, Hao J, Han Z. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. *Front Med* 2020;**14**:185–192.
 28. Cheng H, Wang Y, Wang G-Q. Organ-protective effect of angiotensin-converting enzyme 2 and its effect on the prognosis of COVID-19. *J Med Virol* 2020;**92**:726–730.
 29. Dong B, Zhang C, Feng JB, Zhao YX, Li SY, Yang YP, Dong QL, Deng BP, Zhu L, Yu QT, Liu CX, Liu B, Pan CM, Song HD, Zhang MX, Zhang Y. Overexpression of ACE2 enhances plaque stability in a rabbit model of atherosclerosis. *Arterioscler Thromb Vasc Biol* 2008;**28**:1270–1276.
 30. Burrell LM, Risvanis J, Kubota E, Dean RG, MacDonald PS, Lu S, Tikellis C, Grant SL, Lew RA, Smith AI, Cooper ME, Johnston CI. Myocardial infarction increases ACE2 expression in rat and humans. *Eur Heart J* 2005;**26**:369–375; discussion 322–324.
 31. Vaduganathan M, Vardeny O, Michel T, McMurray JVV, Pfeffer MA, Solomon SD. Renin-angiotensin-aldosterone system inhibitors in patients with Covid-19. *N Engl J Med* 2020;**382**:1653–1659.
 32. Sama IE, Ravera A, Santema BT, Goor H van, Maaten JM ter, Cleland JGF, Rienstra M, Friedrich AW, Samani NJ, Ng LL, Dickstein K, Lang CC, Filippatos G, Anker SD, Ponikowski P, Metra M, Veldhuisen DJ van, Voors AA. Circulating plasma concentrations of angiotensin-converting enzyme 2 in men and women with heart failure and effects of renin-angiotensin-aldosterone inhibitors. *Eur Heart J* 2020;**41**:1810–1817.
 33. Doyen D, Mocerri P, Ducreux D, Dellamonica J. Myocarditis in a patient with COVID-19: a cause of raised troponin and ECG changes. *Lancet* 2020;**395**:1516.
 34. Kim I-C, Kim JY, Kim HA, Han S. COVID-19-related myocarditis in a 21-year-old female patient. *Eur Heart J* 2020;**41**:1859–1859.
 35. Solano-López J, Sánchez-Recalde A, Zamorano JL. SARS-CoV-2, a novel virus with an unusual cardiac feature: inverted takotsubo syndrome. *Eur Heart J* 2020;doi: 10.1093/eurheartj/ehaa390.
 36. Sala S, Peretto G, Gramegna M, Palmisano A, Villatore A, Vignale D, De Cobelli F, Tresoldi M, Cappelletti AM, Basso C, Godino C, Esposito A. Acute myocarditis presenting as a reverse Tako-Tsubo syndrome in a patient with SARS-CoV-2 respiratory infection. *Eur Heart J* 2020;**41**:1861–1862.
 37. Wichmann D, Sperhake J-P, Lütgehetmann M, Steurer S, Edler C, Heinemann A, Heinrich F, Mushumba H, Kniep I, Schröder AS, Burdelski C, G de Heer, Nierhaus A, Frings D, Pfefferle S, Becker H, Brederke-Wiedling H, Weerth A de, Paschen H-R, Sheikhzadeh-Eggers S, Stang A, Schmiedel S, Bokemeyer C, Addo MM, Aepfelbacher M, Püschel K, Kluge S. Autopsy findings and venous thromboembolism in patients with COVID-19: a prospective cohort study. *Ann Intern Med* 2020;doi: 10.7326/M20-2003.
 38. Puelles VG, Lütgehetmann M, Lindenmeyer MT, Sperhake JP, Wong MN, Allweiss L, Chilla S, Heinemann A, Wanner N, Liu S, Braun F, Lu S, Pfefferle S, Schröder AS, Edler C, Gross O, Glatzel M, Wichmann D, Wiet H, Kluge S, Püschel K, Aepfelbacher M, Huber TB. Multiorgan and renal tropism of SARS-CoV-2. *N Engl J Med* 2020;doi: 10.1056/NEJMc2011400.
 39. Yao XH, Li TY, He ZC, Ping YF, Liu HW, Yu SC, Mou HM, Wang LH, Zhang HR, Fu WJ, Luo T, Liu F, Guo QN, Chen C, Xiao HL, Guo HT, Lin S, Xiang DF, Shi Y, Pan GQ, Li QR, Huang X, Cui Y, Liu XZ, Tang W, Pan PF, Huang XQ, Ding YQ, Bian XW. [A pathological report of three COVID-19 cases by minimal invasive autopsies]. *Zhonghua Bing Li Xue Za Zhi* 2020;**49**:411–417.
 40. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, Liu S, Zhao P, Liu H, Zhu L, Tai Y, Bai C, Gao T, Song J, Xia P, Dong J, Zhao J, Wang F-S. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 2020;**8**:420–422.
 41. Barton LM, Duval EJ, Stroberg E, Ghosh S, Mukhopadhyay S. COVID-19 autopsies, Oklahoma, USA. *Am J Clin Pathol* 2020;**153**:725–733.
 42. Fox SE, Akmatbekov A, Harbert JL, Li G, Brown JQ, Vander Heide RS. Pulmonary and cardiac pathology in Covid-19: the first autopsy series from New Orleans. *medRxiv* 2020;doi: 10.1101/2020.04.06.20050575.
 43. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, Vanstapel A, Werlein C, Stark H, Tzankov A, Li WW, Li VW, Menter SJ, Jonigk D. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N Engl J Med* 2020;doi: 10.1056/NEJMoa2015432.
 44. Guan W, Ni Z, Hu Y, Liang W, Ou C, He J, Liu L, Shan H, Lei C, Hui DSC, Du B, Li L, Zeng G, Yuen K-Y, Chen R, Tang C, Wang T, Chen P, Xiang J, Li S, Wang J, Liang Z, Peng Y, Wei L, Liu Y, Hu Y, Peng P, Wang J, Liu J, Chen Z, Li G, Zheng ZJ, Qiu SQ, Luo J, Ye CJ, Zhu SY, Zhong NS; China Medical Treatment Expert Group for Covid-19. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med* 2020;**382**:1708–1720.
 45. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, Huang H, Zhang L, Zhou X, Du C, Zhang Y, Song J, Wang S, Chao Y, Yang Z, Xu J, Zhou X, Chen D, Xiong W, Xu L, Zhou F, Jiang J, Bai C, Zheng J, Song Y. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA Intern Med* 2020;**180**:1–11.
 46. Panitchote A, Mehri O, Hastings A, Hanane T, Demirjian S, Torbic H, Mireles-Cabodevila E, Krishnan S, Duggal A. Factors associated with acute kidney injury in acute respiratory distress syndrome. *Ann Intensive Care* 2019;**9**:74.
 47. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, White HD, Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, White HD, Micklely H, Crea F, Van de Werf F, Bucciarelli-Ducci C, Katus HA, Pinto FJ, Antman EM, Hamm CW, De Caterina R, Januzzi JL, Apple FS, Alonso Garcia MA, Underwood SR, Carty JM, Lyon AR, Devereaux PJ, Zamorano JL, Lindahl B, Weintraub WS, Newby LK, Virmani R, Vranckx P, Cutlip D, Gibbons RJ, Smith SC, Atar D, Luepker RV,

- Robertson RM, Bonow RO, Steg PG, O'Gara PT, Fox KAA. Fourth universal definition of myocardial infarction (2018). *Eur Heart J* 2019;**40**:237–269.
48. McCarthy C, Murphy S, Cohen JA, Rehman S, Jones-O'Connor M, Olshan DS, Singh A, Vaduganathan M, Januzzi JL, Wasfy JH. Misclassification of myocardial injury as myocardial infarction: implications for assessing outcomes in value-based programs. *JAMA Cardiol* 2019;**4**:460–464.
 49. Ferreira VM, Schulz-Menger J, Holmvang G, Kramer CM, Carbone I, Sechtem U, Kindermann I, Gutberlet M, Cooper LT, Liu P, Friedrich MG. Cardiovascular magnetic resonance in nonischemic myocardial inflammation: expert recommendations. *J Am Coll Cardiol* 2018;**72**:3158–3176.
 50. Caforio ALP, Adler Y, Agostini C, Allanore Y, Anastasakis A, Arad M, Böhm M, Charron P, Elliott PM, Eriksson U, Felix SB, Garcia-Pavia P, Hachulla E, Heymans S, Imazio M, Klingel K, Marcolongo R, Matucci Cerinic M, Pantazis A, Plein S, Poli V, Rigopoulos A, Seferovic P, Shoenfeld Y, Zamorano JL, Linhart A. Diagnosis and management of myocardial involvement in systemic immune-mediated diseases: a position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Disease. *Eur Heart J* 2017;**38**:2649–2662.
 51. Cosyns B, Lochy S, Luchian ML, Gimelli A, Pontone G, Allard SD, Mey J de, Rosseel P, Dweck M, Petersen SE, Edvardsen T. The role of cardiovascular imaging for myocardial injury in hospitalized COVID-19 patients. *Eur Heart J Cardiovasc Imaging* 2020;**21**:709–714.
 52. Kramer CM, Barkhausen J, Bucciarelli-Ducci C, Flamm SD, Kim RJ, Nagel E. Standardized cardiovascular magnetic resonance imaging (CMR) protocols: 2020 update. *J Cardiovasc Magn Reson* 2020;**22**:17.
 53. Lanza GA, Buffon A, Sestito A, Natale L, Sgueglia GA, Galiuto L, Infusino F, Mariani L, Centola A, Crea F. Relation between stress-induced myocardial perfusion defects on cardiovascular magnetic resonance and coronary microvascular dysfunction in patients with cardiac syndrome X. *J Am Coll Cardiol* 2008;**51**:466–472.
 54. Thoracic Imaging in COVID-19 Infection. Guidance for the Reporting Radiologist British Society of Thoracic Imaging. The British Society of Thoracic Imaging. Version 2. 16th March 2020. The British Society of Thoracic Imaging. <https://www.bsti.org.uk/>.
 55. ISARIC 4C (Coronavirus Clinical Characterisation Consortium). <https://isaric4c.net/index.html> (15 May 2020)
 56. EurQuoI-5Dimensions-5Level (EQ-5D-5L). <https://euroqol.org/eq-5d-instruments/eq-5d-5l-about/>
 57. Broadbent E, Ellis CJ, Thomas J, Gamble G, Petrie KJ. Further development of an illness perception intervention for myocardial infarction patients: a randomized controlled trial. *J Psychosom Res* 2009;**67**:17–23.
 58. Löwe B, Wahl I, Rose M, Spitzer C, Glaesmer H, Wingenfeld K, Schneider A, Brähler E. A 4-item measure of depression and anxiety: validation and standardization of the Patient Health Questionnaire-4 (PHQ-4) in the general population. *J Affect Disord* 2010;**122**:86–95.
 59. Hlatky MA, Boineau RE, Higginbotham MB, Lee KL, Mark DB, Califf RM, Cobb FR, Pryor DB. A brief self-administered questionnaire to determine functional capacity (the Duke Activity Status Index). *Am J Cardiol* 1989;**64**:651–654.
 60. Lee PH, Macfarlane DJ, Lam T, Stewart SM. Validity of the international physical activity questionnaire short form (IPAQ-SF): a systematic review. *Int J Behav Nutr Phys Act* 2011;**8**:115.
 61. Liu A, Wijesurendra RS, Liu JM, Forfar JC, Channon KM, Jerosch-Herold M, Piechnik SK, Neubauer S, Kharbada RK, Ferreira VM. Diagnosis of microvascular angina using cardiac magnetic resonance. *J Am Coll Cardiol* 2018;**71**:969–979.
 62. Kotecha T, Martinez-Naharro A, Boldrini M, Knight D, Hawkins P, Kalra S, Patel D, Coghlan G, Moon J, Plein S, Lockie T, Rakhit R, Patel N, Xue H, Kellman P, Fontana M. Automated pixel-wise quantitative myocardial perfusion mapping by CMR to detect obstructive coronary artery disease and coronary microvascular dysfunction: validation against invasive coronary physiology. *JACC Cardiovasc Imaging* 2019;**12**:1958–1969.
 63. Wei J, Mehta PK, Shufelt C, Yang Y, Gill E, Kahlon R, Cook-Wiens G, Minissian M, Kar S, Thomson L, Bertram D, Merz CNB. Diastolic dysfunction measured by cardiac magnetic resonance imaging in women with signs and symptoms of ischemia but no obstructive coronary artery disease. *Int J Cardiol* 2016;**220**:775–780.
 64. Hsu L-Y, Groves DW, Aletras AH, Kellman P, Arai AE. A quantitative pixel-wise measurement of myocardial blood flow by contrast-enhanced first-pass CMR perfusion imaging: microsphere validation in dogs and feasibility study in humans. *JACC Cardiovasc Imaging* 2012;**5**:154–166.
 65. Hsu L-Y, Jacobs M, Benovoy M, Ta AD, Conn HM, Winkler S, Greve AM, Chen MY, Shanbhag SM, Bandettini WP, Arai AE. Diagnostic performance of fully automated pixel-wise quantitative myocardial perfusion imaging by cardiovascular magnetic resonance. *JACC Cardiovasc Imaging* 2018;**11**:697–707.
 66. Knott KD, Seraphim A, Augusto JB, Xue H, Chacko L, Aung N, Petersen SE, Cooper JA, Manisty C, Bhuvana AN, Kotecha T, Bourantas CV, Davies RH, Brown LAE, Plein S, Fontana M, Kellman P, Moon JC. The prognostic significance of quantitative myocardial perfusion. *Circulation* 2020;**141**:1282–1291.
 67. Ohta Y, Kitao S, Yunaga H, Fujii S, Mukai N, Yamamoto K, Ogawa T. Myocardial delayed enhancement CT for the evaluation of heart failure: comparison to MRI. *Radiology* 2018;**288**:682–691.
 68. Scully PR, Bastarrika G, Moon JC, Treibel TA. Myocardial extracellular volume quantification by cardiovascular magnetic resonance and computed tomography. *Curr Cardiol Rep* 2018;**20**:15.
 69. von Elm E, Altman, DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* 2007;**370**:1453–1457.