Long-term impact on cardiopulmonary function and quality of life among patients recovered from SARS-CoV-2 infection in a 6-month follow-up period in Lima, Peru: FUNCTION cohort study protocol

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ABSTRACT

Introduction  The sequelae of COVID-19 have been described as a multisystemic condition, with a great impact on the cardiovascular and pulmonary systems with abnormalities in pulmonary function tests, such as lower diffusing capacity of the lung for carbon monoxide (Dlco) levels and pathological patterns in spirometry; persistence of radiological lesions; cardiac involvement such as myocarditis and pericarditis; and an increase in mental disorders such as anxiety and depression. Several factors, such as infection severity during the acute phase as well as vaccination status, have shown some variable effects on these post-COVID-19 conditions, mainly at a clinical level such as symptoms persistence. Longitudinal assessments and reversibility of changes across the spectrum of disease severity are required to understand the long-term impact of COVID-19.

Methods and analysis  A prospective cohort study aims to assess the impact of SARS-CoV-2 infection on cardiopulmonary function and quality of life after the acute phase of the disease over a 6-month follow-up period. Sample size was calculated to recruit 200 participants with confirmatory COVID-19 tests who will be subsequently classified according to infection severity. Four follow-up visits at baseline, month 1, month 3 and month 6 after discharge from the acute phase of the infection will be scheduled as well as procedures such as spirometry, DLco test, 6-minute walk test, chest CT scan, echocardiogram, ECG, N-terminal pro-B-type natriuretic peptide measurement and RAND-36 scale. Primary outcomes are defined as abnormal pulmonary function test considered as DLco <80%, abnormal cardiovascular function considered as left ventricular ejection fraction <50% and abnormal quality of life considered as a <40 score for each sphere in the RAND-36-Item Short Form Health Survey.

Ethics and dissemination  The study protocol was approved by the Institutional Ethics Committee of the Universidad Peruana Cayetano Heredia (SIDISI 203725) and the Ethics Committee of the Hospital Cayetano Heredia (042-2021). Protocol details were uploaded in ClinicalTrials.gov. Findings will be disseminated through peer-reviewed journals, scientific conferences and open-access social media platforms.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The study results will be generalisable at least to study populations similar to ours: inpatients and outpatients who have tested positive for SARS-CoV-2 infection in middle-income countries and have had asymptomatic, mild, moderate, severe or critical COVID-19. Additionally, we have increased generalisability by including non-vaccinated and vaccinated individuals into our study.
⇒ Diffusing capacity of the lung for carbon monoxide tests are adjusted to the patient’s haemoglobin value; nevertheless, carboxyhaemoglobin adjustment is not performed in this study.
⇒ This study seeks to assess the long-term complications of the broad spectrum of clinical presentation of COVID-19, classifying them under the severity of symptoms and clinical signs they presented in the acute phase of the disease.
⇒ This study performs a multidisciplinary assessment of the patient’s health status after COVID-19, which includes measuring cardiovascular, pulmonary and quality of life indicators.
⇒ Peru is one of the hardest-hit countries by the COVID-19 pandemic with the highest mortality rate per capita in the world; yet, there is a lack of research studies in the country that aim to identify the long-term complication of this disease. This study is one of the few in the region that seeks to generate evidence that could help the understanding and management of post-COVID-19 sequelae.
INTRODUCTION

Post-acute COVID-19 syndrome or long-COVID-19 involves health issues that persist for at least more than 4 weeks after being infected with COVID-19, although a homogeneous definition is urgently required.\cite{1, 2} The sequelae of COVID-19 have been shown to be multisystemic and most significantly affecting the respiratory and cardiovascular system leading to a broad clinical spectrum with several unspecific symptoms such as fatigue, cardiovascular system leading to a broad clinical spectrum with several unspecific symptoms such as fatigue, dyspnoea, cough, headache, dizziness and depression.\cite{3-7} Hence, some studies aim to congregate these cluster manifestations into clinical phenotypes to better understand the underlying pathophysiological mechanisms of the disease and its health implications.\cite{8, 9} Radiological and clinical resolution of long-COVID-19 has been obtained in most of the patients in follow-up studies but its persistence up to 2 years after the acute infection has also been described, which could be considered an underestimated disabling condition that potentially burdens the daily activities.\cite{10, 11}

COVID-19 is well-known for rapidly causing respiratory failure also known as acute respiratory distress syndrome (ARDS). The pathophysiology of the lung damage produced by the coronavirus includes alveolar epithelial and endothelial cell destruction, capillary damage, followed by fibroproliferation.\cite{12} These changes lead to tissue remodelling, which produces lung fibrosis.\cite{12} Additionally, autopsies of patients with COVID-19 have shown destruction in alveolar structure leading to pulmonary interstitial disease.\cite{13} Evaluation of respiratory function in individuals post-infection by COVID-19 has shown most significantly altered diffusion capacity of the lungs for carbon monoxide (DLco), in addition to restrictive patterns and obstructive patterns in spirometry.\cite{14}

Cardiovascular complications of COVID-19 have also been described.\cite{15} During the acute infection, immune activation can trigger an inflammatory response that may lead to altered peripheral resistance, microvascular dysfunction, plaque instability and endothelial injury.\cite{16} Common symptoms such as palpitations and chest pain have been reported and less commonly cardiovascular incidents.\cite{17} Nevertheless, Xie et al provide evidence that people with COVID-19 exhibited increased risks beyond the first 30 days of infection and incident cardiovascular disease up until 12 months, which includes cerebrovascular disorders, dysrhythmias, inflammatory heart disease, ischaemic heart disease, heart failure, thromboembolic disease and other cardiac disorders.\cite{18} Additionally, myocarditis, pericarditis and pericarditis have also been diagnosed as late as 11 weeks after COVID-19 symptoms onset.\cite{19}

COVID-19 has substantially impacted quality of life. Huang et al described that 6 months after acute infection, COVID-19 survivors suffered with fatigue, muscle weakness, insomnia, anxiety and depression.\cite{20} A study in the UK that investigated quality of life using RAND-36-Item Short Form Health Survey (SF-36) reported that the administration of the questionnaire 8–12 weeks to individuals with mild, moderate and severe acute COVID-19 shows lower scores in all domains compared with age-matched results.\cite{21} Accordingly, physical scores were significantly lower in individuals with severe disease.\cite{21} In Spain, a study showed that at 6 months of follow-up to COVID-19 hospitalised patients showed a 67% decrease in quality of life.\cite{22} Additionally, another study in China found that 23% of previously hospitalised patients with COVID-19 suffered from anxiety and depression.\cite{23} In Peru, even though there are cumulative 10896 confirmed cases of SARS-CoV-2 infection per 100 000 people during the time of writing the manuscript of the protocol (June 2022), there are no studies that describe such effect in the population.\cite{23}

Risk of developing sequelae has been seen both in older individuals admitted to hospitals because of COVID-19 and those with no pre-existing conditions not admitted to hospitals for COVID-19.\cite{24} A comprehensive assessment of post-acute COVID-19 sequelae across the spectrum of care including non-hospitalised, hospitalised and intensive care-admitted patients is lacking in Peru.

METHODS AND ANALYSIS

Overall purpose

The purpose of the study is to longitudinally evaluate the impact of SARS-CoV-2 infection on pulmonary and cardiovascular function and quality of life of those who recovered from the acute phase of the infection with four prospective follow-up visits over a period of 6 months after discharge. Two major protocol amendments were approved after grant allocation: (1) limiting participants’ inclusion to those with reverse transcription (RT)-PCR or antigen test—after universal access to local antigen test; and (2) inclusion of vaccinated population into the study.

Objectives

► To assess the long-term impact on pulmonary and cardiovascular function and quality of life stratified by severity after the acute phase of SARS-CoV-2 infection over 6-month follow-up after discharge in Lima, Peru.
► To explore the long-term impact on pulmonary and cardiovascular function and quality of life stratified by vaccination status after the acute phase of SARS-CoV-2 infection over 6-month follow-up after discharge in Lima, Peru.
► To evaluate the reversibility of changes on radiological lesions and pulmonary and cardiovascular function and quality of life at baseline, 1, 3 and 6 months after discharge in Lima, Peru.
► To identify risk factors associated with abnormal and prolonged pulmonary and cardiovascular function and poor quality of life after the acute phase of SARS-CoV-2 infection over a 6-month follow-up after discharge in Lima, Peru.
Study design
This will be an observational prospective cohort study with four structured visits over a period of 6 months of follow-up after discharge conducted between 12 August 2021 and 3 November 2022. The four studies will be divided as follows: (1) baseline visit within the next 7 days after discharge; (2) month 1 visit ±5 days after discharge; (3) month 3 visit ±7 days after discharge and (4) month 6 visit ±10 days after discharge. Imaging studies and function tests will be performed in each visit and an extensive clinical assessment will be carried out by specialists.

Study population
Lima is the fourth most populated South American city with nearly 9.5 million inhabitants and 3697 inhabitants/km². Since the first case of SARS-CoV-2 infection has been reported in March 2020, Lima permanently remained at the top of the most affected cities nationwide. Health coverage exclusively by the Ministry of Health (MoH) accounts for 28.8% in Lima, while 32.1% and 9.7% are covered exclusively by EsSalud and private companies, respectively, according to 2017 national census.35 The MoH health coverage is structured into four health directorates (Integrated Health Network Directorate; DIRIS for its acronym in Spanish): north (n=3 097 193), south (n=2 378 223), central (n=26 070 377) and east (n=1 709 382).36-39

Cayetano Heredia Hospital will be the main site where the hospitalised patients will be screened, although individuals admitted to different public or private institutions in Lima could also be included. Cayetano Heredia Hospital is a tertiary-level referral hospital situated in DIRIS Lima Norte; given the pandemic situation, it was transformed to a COVID-19 hospital increasing the number of beds from 468 to 572.30 Ambulatory individuals will be screened from the healthcare centres from DIRIS. All the activities will be carried out at Cayetano Heredia Clinic, which is located in front of Cayetano Heredia Hospital.

Study population will be divided into five categories based on severity during acute SARS-CoV-2 infection. These categories will be defined following the WHO case definition used at the time of the protocol writing (2020) and adjusted to clinical judgement of the study team based on the cut-off value of peripheral oxygen saturation (SpO₂) as a principal indicator of mortality according to Mejia et al.11 The definitions are as follows:
1. Group A (asymptomatic infection): absence of clinical manifestation during the acute phase of SARS-CoV-2 infection until discharge (end of quarantine).
2. Group B (symptomatic infection):
   a. Group B1—mild: clinical manifestation of SARS-CoV-2 infection without severity signs (respiratory rate <30/min; SpO₂ >94%) and no radiological evidence of pneumonia.
   b. Group B2—moderate: clinical manifestation of SARS-CoV-2 infection without severity signs (respiratory rate <30/min; SpO₂ >94%) and radiological evidence of pneumonia.
   c. Group B3—severe: clinical manifestation of SARS-CoV-2 infection with severity signs (respiratory rate >30/min; SpO₂ <94%) and radiological evidence of pneumonia.
   d. Group B4—critical: clinical manifestation of SARS-CoV-2 infection with severity signs (respiratory rate >30/min; SpO₂ <94%) and at least one of the following:
      - ARDS: defined under Berlin Criteria mostly defined by the ratio of arterial oxygen partial pressure to fractional inspired oxygen (PaO₂/FiO₂) <200.
      - Shock: patients diagnosed with sepsis who, despite adequate fluid resuscitation, require vasopressors to maintain a mean arterial pressure ≥65mm Hg and lactate values >2mmol/L (>18mg/dL).
      - Organ dysfunction that requires intensive care unit (ICU) admission.

Our population will be restricted to individuals who tested positive for SARS-CoV-2 infection, via a positive antigen or RT-PCR test. The result will be considered valid if the time of report is within 14 days of the positive test result. Discharge will be considered for (1) ambulatory individuals: at the last day of quarantine; (2) hospitalised individuals: hospital discharge or at the end of quarantine, whichever occurs first.

Vaccination status will be defined as (1) non-vaccinated individuals who have not received any or only a single dose of the SARS-CoV-2 vaccine, in a scheme which requires at least two doses; (2) vaccinated individuals who have received at least two doses of SARS-CoV-2 vaccine or a single dose, in case of the laboratories which consider complete vaccination with a single dose. Individuals with a single dose of a two-dose vaccine, plus infection with COVID-19, will be considered as non-vaccinated. We will consider the vaccination status at the moment of the diagnosis of SARS-CoV-2 infection and will promote vaccination to all individuals throughout the study.

For analysis purposes (see the Sample size calculation section), our control group will be considered as groups A and B1, because of the complexity of evaluating non-infected individuals during a pandemic: uncertainty regarding government restrictions, multiple time-consuming assessments and exposure of individuals to COVID-19 hospital settings in the context of a collapsed public health system in the country with the highest mortality rate worldwide.

Eligibility criteria
Inclusion criteria
Individuals must meet all of the following criteria (either for group A or B) in order to participate in the study:
► Individuals of any sex over 18 years of age.
► A positive COVID-19 test result by nasopharyngeal swab using either RT-PCR or antigen techniques.
within the 14 days prior to enrolment. A laboratory-based confirmatory report is required.

- Recruitment no longer than 7 days after discharge.
- Group A: individuals who have not reported signs or symptoms suggestive of COVID-19 infection.
- Group B: individuals who have reported signs or symptoms suggestive of COVID-19 infection.

Exclusion criteria
An individual meeting any of the following criteria at the time of enrolment will be excluded from study participation:

- A negative COVID-19 test result by nasopharyngeal swab using either RT-PCR or antigen techniques.
- Known inability to maintain adherence to the study (e.g., incarceration, migration, travel).
- Active pregnancy confirmed by either qualitative urine or quantitative beta-hCG blood test.

Study procedures
Screening and enrolment
For passive recruitment, the study’s staff will present the study to the health centre authorities to distribute flyers and banners for visual dissemination of the study to patients attending COVID-19 testing areas. The dissemination material will contain the aim of the study, summarised eligibility criteria and contact information in case the individual is willing to participate. The study will also be advertised through social media in order for potential participants to contact the study team, who will evaluate the patient’s eligibility criteria.

Active recruitment will be done by reaching out to individuals to inform them about the study, and if they are interested in participating, personal contact information will be asked and the study’s staff will contact them after their positive test result. They will also be asked about the willingness of other SARS-CoV-2-positive family members to participate. The staff will also visit the Hospital Cayetano Heredia COVID-19 hospitalisation rooms and will interview individuals who are close to being discharged, and will assess their eligibility to participate in the study.

Interested individuals will be provided with information regarding the risks and benefits of the study procedures as well as a detailed schedule of visits and the importance of adherence. The participants will undergo the informed consent process and sign the consent form to be included in the study prior to any data collection or procedures.

Study data
Demographic, medical history and clinical evaluation
Participants will be interviewed during the baseline visit to collect the demographic information including: age, sex, education level, current and previous occupation, household characteristics (number of individuals and beds inside their house, house material, cooking methods and combustible material used for cooking, water supply), active and passive smoking habits, current or prior tuberculosis infection, occupational exposures and long-term use of medications.

Medical history will include data through participants’ interview about comorbidities during the baseline visit, focused but not limited to those described as risk factors for COVID-19: hypertension, type 2 diabetes, coronary disease, chronic obstructive pulmonary disease, autoimmune disease, neoplasia, dyslipidaemia, chronic kidney disease, stroke, dementia, epilepsy, cirrhosis, chemotherapy treatment, radiotherapy and dialysis.

Clinical data collection will be ambidirectional: retrospective information will be collected from medical records for B3 and B4 participants, which will include: hospitalisation service (emergency, hospitalisation and ICU services), date of admission and date of discharge; complications; type of oxygen support (nasal cannula, mask, high-flow cannula, mechanical ventilation and tracheostomy), including date of onset and end date. Prospective information will be assessed during study follow-up (month 1, month 3, month 6) by trained study physicians, including a general practitioner, cardiologist and pneumologist in all groups and will include signs and symptoms of COVID-19, including date of onset, date of diagnosis, type of COVID-19 test, treatment received; current symptoms after being discharged; physical examination including anthropometric measurements; reinfections or readmissions to emergency department or hospitalisation; vaccination data, including type of vaccine, number of doses, date of inoculation, adverse events; current treatments and enrolment in physical therapy or a rehabilitation programme.

Pulmonary evaluation
Pulmonary function tests will include spirometry and DLco at each visit performed by the study’s pulmonologist in a temperature-controlled room. Spirometry parameters will be assessed both with and without bronchodilator. It will be recorded as numbers and predicted % and will include: forced vital capacity (FVC), forced expiratory volume in 1 s (FEV₁), FEV₁/FVC, peak expiratory flow and forced expiratory flow 25–75. DLco testing will be recorded as numbers and predicted % for DLco, adjusted DLco and DLco divided by the alveolar volume. Final recordings will be adjusted for recent haemoglobin values taken at both baseline or from medical records and month 6 visits.

Functional exercise capacity will be assessed using the 6-minute walk test following the American Thoracic Society guidelines for performed-based tolerance evaluation at each scheduled visit (baseline, month 1, month 3, month 6). Variables will include heart rate, oxygen saturation, blood pressure, BORG score and distance reached.

A chest CT scan will be performed at baseline and month 6 visits. Women of childbearing age will require a negative urine pregnancy test before radiation exposure. Electronic images will be stored into the study’s database and two expert radiologists will interpret the images, blinded by severity of infection and vaccination status.
Discrepancies will be consulted to a third study member, either a radiologist or a pulmonologist.

Finally, a pulmonary-specific clinical assessment (thorax inspection and percussion, lung auscultation, elicitation of cough, dyspnoea, cyanosis and thoracic pain and its characteristics) will be conducted alongside the interpretation of the examinations on each scheduled visit (baseline, month 1, month 3, month 6). All the findings will be explained to the participants.

Cardiovascular evaluation

A transthoracic echocardiogram will be performed at each visit by the study’s cardiologist and will include the following variables: M mode (measures of the ventricle’s diameters at systole and diastole, interventricular septum, posterior wall, left atrium, aorta and the aorta’s opening, ejection fraction and fractional shortening); bidimensional study (cardiac position, cardiac valves, cardiac chambers, segment contraction, septum and pericardium); and Doppler study (e wave, a wave and e/a proportion for mitral valve, maximum velocity and gradient for aortic, tricuspid and pulmonary valves). Images will be saved and entered into the study’s database.

ECGs will be taken at each visit by trained staff. Twelve-lead recordings will be interpreted, scanned and entered into the study’s database. Variables include: rhythm, atrial and ventricular frequency, P wave, PR interval, QRS complex, QT segment, ST segment and T wave. Abnormalities will be further characterised.

A blood sample will be drawn at baseline and month 6 visits to measure N-terminal pro-B type natriuretic peptide (NT-ProBNP) in order to evaluate for heart failure. This will require a 4 mL blood sample in an SST-covered tube. A 125 pg/mL cut-off value will be used for laboratory reports.

Finally, a cardiological-specific clinical assessment (cardiac auscultation, characterisation of palpitations, dyspnoea, thoracic pain) will be conducted alongside the interpretation of the examinations on each scheduled visit (baseline, month 1, month 3, month 6) and findings will be explained to the participants.

Quality of life assessment

The SF-36 will be used to measure the quality of life at each study visit. The tool includes questions categorised into eight spheres: physical functioning, bodily pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, emotional well-being, social functioning, energy/fatigue and general health perceptions. Missing values will not be considered into the score. The final 0–100 scoring will follow the guideline based on the Spanish version of SF-36.

Sample handling

A total of three blood samples will be drawn at baseline and month 6 visits. Three millilitres of venous blood will be collected in EDTA tubes to calculate haemoglobin values using the sodium lauryl sulphate (SLS) method. Four millilitres of venous blood will be collected in serum separating-tubes (SST) to calculate NT-ProBNP values using electrochemiluminescence immunoassay (ECLIA) technique and the remaining serum will be stored in a 1.8 mL cryovial at −70°C. Three millilitres of venous blood will be collected in EDTA tubes to separate plasma by centrifugation method and stored in a 1.8 mL cryovial at −70°C. Plasma and serum will only be stored if it was previously authorised by the participant in the informed consent.

Scheduling visits

Study visits will be scheduled by phone with participants. The dates should meet the time frame allowed for each visit: (1) baseline visit within the next 7 days after discharge; (2) month 1 visit ±5 days after discharge; (3) month 3 visit ±7 days after discharge and (4) month 6 visit ±10 days after discharge. Reminders 48 and 24 hours prior to the visit will be sent by phone to ensure adherence. Participants will be transported to the site and returned to their house by cars rented by the study to reduce the risk of COVID-19 exposure by public transport.

Missed visits, loss to follow-up and study withdrawal

In case the participant misses a scheduled visit, the following actions will be immediately carried out: (1) trying to contact the participant by phone, (2) counselling and reinforcing the importance of study adherence, (3) in case of no response, up to three additional attempts will be done within the time frame allowed by protocol (see the Scheduling visits section), and (4) if prior measures fail, a missed visit will be reported. However, subsequent scheduled visits will be allowed.

If the study participant misses the baseline visit after the same measures described above are tried out, a loss to follow-up will be reported. Subsequent scheduled visits will not be allowed due to lack of baseline information.

If a participant requests to exit the study, a withdrawal will be reported. All data collected at that time will remain stored into the database.

A maximum of 40% of loss to follow-up plus withdrawal from visits have been considered for analysis purposes.

Study visits

Study visits will be scheduled as follows:

- Baseline visit within the next 7 days after discharge.
- Month 1 visit ±5 days after discharge.
- Month 3 visit ±7 days after discharge.
- Month 6 visit ±10 days after discharge.

To see the activities that will be performed in each visit, please refer to the chronogram in the online supplemental material (see section 5.4).

Data and sample management

Data management

All study records and forms will be de-identified and replaced by codes to keep sensitive information confidential. Paper-based records and forms will be stored in a locked closet and entered into a password-protected
database (REDCap) with limited access to the study team. Storage of the study data for the next 20 years will be requested during informed consent, which will allow further analysis and substudies.

Blood sample management
Serum back-up samples will be stored at −20°C, once immediately processed, and will be transferred monthly at a temperature of −70°C at the site’s biobank, while plasma back-up samples will be stored at −70°C immediately after processing them. Samples will be coded following the same subject codes. Storage of the study samples for the next 20 years will be requested during informed consent, which will allow further analysis and substudies.

Statistical considerations
Hypothesis
To assess the impact of SARS-CoV-2 infection on cardiorespiratory function and quality of life stratified by severity:

- Null hypothesis: hospitalised individuals do not have a worse long-term impact on cardiorespiratory function and quality of life than non-hospitalised individuals over 6-month follow-up after discharge.
- Alternative hypothesis: hospitalised individuals do have a worse long-term impact on cardiorespiratory function and quality of life than non-hospitalised individuals over 6-month follow-up after discharge.

To explore the impact of SARS-CoV-2 infection on cardiorespiratory function and quality of life stratified by vaccination status:

- Null hypothesis: individuals not vaccinated against SARS-CoV-2 infection do not have a worse long-term impact on cardiorespiratory function and quality of life than those vaccinated over 6-month follow-up after discharge.
- Alternative hypothesis: individuals not vaccinated against SARS-CoV-2 infection do have a worse long-term impact on cardiorespiratory function and quality of life than those vaccinated over 6-month follow-up after discharge.

To assess the reversibility of pulmonary lesions on radiological assessments, cardiopulmonary function and quality of life at baseline and months 1, 3 and 6 after discharge from SARS-CoV-2 infection, stratified by severity and vaccination status:

- Null hypothesis: hospitalised individuals and individuals not vaccinated against SARS-CoV-2 infection do not have slower times to recovery from radiological changes, cardiopulmonary function and quality of life over 6-month follow-up after discharge from SARS-CoV-2 infection.
- Alternative hypothesis: hospitalised individuals and individuals not vaccinated against SARS-CoV-2 infection do have slower times to recovery from radiological changes, cardiopulmonary function and quality of life over 6-month follow-up after discharge from SARS-CoV-2 infection.

Outcome definitions

### Abnormal pulmonary function
DLco <80% will be considered an abnormal pulmonary function test. The dependent variable will be categorised as a dichotomous variable for further analysis.

### Abnormal cardiovascular function
Left ventricular ejection fraction (LVEF) <50% will be considered an abnormal cardiovascular function test. The dependent variable will be categorised as a dichotomous variable for further analysis.

### Abnormal quality of life
SF-36 score <40 for each sphere will be considered an abnormal quality of life. The dependent variable will be categorised as a dichotomous variable for further analysis.

Sample size calculation
The five groups already described were regrouped into four categories:

- Subgroup 1: group A (asymptomatic)+group B1 (mild)—control group.
- Subgroup 3: group B3 (severe).
- Subgroup 4: group B4 (critical).

Sample size should be calculated based on each of the primary endpoints for pulmonary (DLco<80%) and cardiovascular function (LVEF<50%). However, given the lack of studies about cardiovascular function in the convalescent phase of SARS-CoV-2 infection at the moment this protocol was written (2020), the sample size will be estimated based only on the pulmonary endpoint.

Mo et al. reported a prevalence of DLco<80% at 30 days after discharge of 84% among severely infected individuals and 42% among those categorised as moderate. Few studies have been published to firmly support the prevalence of abnormal DLco in the convalescent phase of the infection for which we decided to be more conservative and adjust the prevalence to 80% and 46% for severely ill and moderately ill, respectively. Therefore, to detect the difference between the two prevalence values (80%–46%=34%) at 80% power and 95% CI, considering 40% of the total sample size as lost to follow-up, we estimated 50 participants by group, which was finally extrapolated for each of the four subgroups. A total sample size of 200 participants was calculated.

Analysis
The study data will be entered in the REDCap platform as previously described (see the Data management section) and the analysis will be conducted using R software and STATA V.17 (Stata Corp, College Station, Texas, USA).

Exploratory analysis will consist of descriptive statistics; categorical variables will be described as absolute and relative frequencies and percentages, and numerical variables as means with SDs or medians with IQRs according to their normal or non-normal distribution, respectively, on histograms. Dependent variables will
be selected based on each research question as follows: 
(a) abnormal DLco for pulmonary function; (b) abnormal LVEF for cardiovascular function and (c) abnormal RAND-36 score for quality of life. Comparisons between independent variables and each of the outcome variables will be analysed using the $X^2$ test or Fisher’s exact test if both variables are categorical, and Student’s t-test or analysis of variance tests for numerical variables, with two and greater than two variables, respectively, following parametric distribution or Mann-Whitney U test and Kruskal-Wallis if the variables follow a non-parametric distribution. Alpha level of significance of 5% ($p<0.05$) will be considered.

Multivariable analysis will be conducted based on each research question and study objective as follows:

Assess the impact of SARS-CoV-2 infection on pulmonary and cardiovascular function and quality of life at discharge and 1, 3 and 6 months later.

Multivariable Cox regression model to quantify the risks of each outcome and crude and adjusted HRs will be reported. Interactions will be assessed and models will be compared using likelihood ratio test. Primary exposures will be severity and vaccination status. Linear trends for severity and number of doses of SARS-CoV-2 vaccine will also be explored.

Assess the reversibility of the radiological lesions, impact of SARS-CoV-2 infection on pulmonary and cardiovascular function and quality of life at discharge and during follow-up 1, 3 and 6 months after discharge, according to severity of infection and vaccination status.

We will perform a survival analysis to estimate the time until the event according to the different exposures (severity and vaccination status). Time of onset will be considered since the baseline visit, which is the beginning of the follow-up period, and the event will be considered the regression of the abnormality for each outcome variable. Kaplan-Meier curves will be first obtained. Comparisons between the two survival curves will be performed using log-rank test or Gehan-Wilcoxon if early differences are seen. A p value of <0.05 will be considered statistically significant. Those significant variables will be fitted into the multivariable Cox regression model to quantify the risks of each outcome, and crude and adjusted HRs will be reported. Interactions will be assessed and models will be compared using likelihood ratio test. Primary exposures will be severity and vaccination status. Linear trends for severity and number of doses of SARS-CoV-2 vaccine will also be explored.

Identify risk factors associated with abnormal pulmonary and cardiovascular function and quality of life after 6 months of being discharged from SARS-CoV-2 infection.

After conducting a univariate analysis, we will run a bivariate explanatory logistic regression model to explore associations between the outcome and exposures describing it as crude ORs. Those statistically significant ($p<0.05$) will be considered for further exploration of confounders against the main exposure and adjusted ORs will be reported. Those results with associations with alpha level of 5% ($p<0.05$) will be considered into the full final logistic regression model and parsimony principle will be followed. Interactions will be explored and models will be compared by likelihood ratio tests until the final logistic regression model. Plausible confounders that will be expectedly considered into the model are: age, sex, body mass index, smoking, chronic obstructive pulmonary disease and hypertension.

**Patient and public involvement**

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

**ETHICS AND DISSEMINATION**

The study protocol was evaluated and approved by the Institutional Ethics Committee of the Universidad Peruana Cayetano Heredia (SIDIS 203725) and the Hospital Cayetano Heredia (042-2021). Invitations to participate in the study were authorised to be disseminated to all healthcare centres of the DIRIS Lima Norte, Lima Centro and Lima Sur from the MoH as well as COVID-19 testing centres of EsSalud. Dissemination of the study via social media was granted by the Institutional Ethics Committee of the Universidad Peruana Cayetano Heredia. All participants will go through the informed consent process and if they agree to participate, they will be asked to sign a written informed consent form and will receive a copy of it, before providing data, biological samples and before procedures were performed. Participants will be anonymised with a code (study identification) after signing the informed consent form, and all the study forms and samples will be managed using this code prior to database entry and analysis. All study staff will require to complete an International Conference on Harmonization - Good Clinical Practice (ICH-GCP) training certification.

The study protocol was registered with ClinicalTrials.gov website (NCT05386485).

**Dissemination plan**

**Substudies**

Substudies from secondary database analysis and new research proposals using the study data/samples will be necessarily evaluated by the Institutional Ethics Committee of the Universidad Peruana Cayetano Heredia and the principal investigators for their approvals.

**Dissemination strategies**

Results of the study will be disseminated in at least three different ways:

**Publications**

Manuscripts will be published in peer-reviewed international medical journals. Authorship and contributions will follow the definitions of the International Committee of Medical Journal Editors to ensure the
credits and responsibilities of each study member on the results.
A detailed final report will be delivered to the health authorities for further evaluation of the public health policy implications of disease control and prevention strategies.

Conferences and presentations
Local or international meetings and conferences, either for poster or oral presentations, will be selected for presenting interim analysis as well as final pooled results.

Social media
Scientific dissemination and results of the study will be available on open-access social media platforms after preprint submission to the journal. Illustrative videos will be created for a straightforward communication to stakeholders and communities. A legend in Peruvian sign language will be included as a commitment to the inclusion of minorities in critical public health topics.

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