Protocol

Protocol for: Hampshire A, Azor A, Atchison C, et al. Cognition and memory after Covid-19 in a large community sample. N Engl J Med 2024;390:806-818. DOI: 10.1056/NEJMoa2311330

This trial protocol has been provided by the authors to give readers additional information about the work.

Supplemental appendix

This supplement contains the following items:

1. Original protocol
2. Final protocol
3. Summary of changes to protocol
4. Original statistical analysis plan
5. Final statistical analysis plan
6. Summary of changes to statistical analysis plan

1. Original protocol

Imperial College London

Research Governance and Integrity Team

Protocol for

REACT-LC

REal-time Assessment of Community Transmission – Long COVID

Version 1.1

MAIN SPONSOR: Imperial College London
FUNDERS:UKRI/NIHR
STUDY COORDINATION CENTRE: xxx

IRAS Project ID: 298404
REC reference: xxx

Protocol authorised by:

Name & Role	**Date**	**Signature**

Study Management Group

Chief Investigator: Professor Paul Elliott

Co-investigators: Professor Helen Ward, Professor Graham Cooke, Professor Sir Mark Caulfield

Statistician: Professor Marc Chadeau-hyam

Study Management: Kimberly Bennett

Study Coordination Centre

For general queries, supply of study documentation, and collection of data, please contact:

Study Coordinator: Kimberly Bennett
Address: 1st Floor, Medical School, St Mary's Campus
E-mail: k.bennet@imperial.ac.uk

Clinical Queries

Clinical queries should be directed to Professor Graham Cooke who will direct the query to the appropriate person

Address: Infectious Diseases Section, Winston Churchill Wing, St Mary's Campus
E-mail: g.cooke@imperial.ac.uk

Sponsor

Imperial College London/ is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Research Governance and Integrity at:

Research Governance and Integrity Team
Imperial College London and Imperial College Healthcare NHS Trust
Room 215, Level 2, Medical School Building
Norfolk Place
London, W2 1PG
Tel: **0207 594 1862**

Funder

The study is funded by UKRI and NIHR Reference 28863: Research into the longer term effects of COVID-19 in non-hospitalised individuals.

This protocol describes the REACT Long COVID (REACT-LC) study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the UK Policy Frame Work for Health and Social Care Research. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

Table of Contents

GLOSSARY OF ABBREVIATIONS

KEYWORDS

COVID-19
SARS CoV-2
Long COVID
Whole genome sequencing
proteomics
metabolomics
inflammation
multi-omics
immunoassays
auto-antibodies

STUDY SUMMARY

TITLE REACT-Long COVID (REACT-LC) Programme

DESIGN Descriptive study of self-reported symptoms and experience of people testing positive in the REACT1 and REACT2 studies, with a nested study of clinical phenotype with multi-omics analyses and genetic sequencing on participants to identify links with the susceptibility and severity of their COVID illness.

AIMS The REACT-Long COVID (REACT-LC) programme aims to characterise the genetic, biological, social and environmental signatures and pathways, and their inter-relationships, that underpin progression to Long COVID, and to understand the natural history and long-term sequelae post-SARS-CoV-2 infection.

OUTCOME MEASURES

1. Detailed description of the prevalence, symptoms and experience of people with Long COVID

2. Identified genomic, biological pathway and -omic differences between participants who suffer from Long Covid and those who do not, amongst those who have not been hospitalised.

POPULATION Target minimum 120,000 participants for the regular questionnaires from which 8,000 will attend a clinical assessment centre and then 2,000 will attend a follow-up assessment.

ELIGIBILITY Adults who have taken part in the ICL REACT Study.

DURATION 3 years

1. INTRODUCTION

1.1. BACKGROUND

The UK has experienced one of the largest epidemics of COVID-19 in Europe. As a new disease, the natural history beyond the immediate illness and the possible long-term sequalae are largely unknown. As well as the risk of hospitalisation and death from COVID-19 it is clear that some people who develop symptoms have a prolonged and debilitating illness that may continue for weeks or months (so-called Long COVID or post-COVID syndrome). Pathogenesis of persistent symptoms from COVID-19 is poorly understood and represents a major knowledge gap if effective treatments and management strategies are to be developed.

Our initial estimates from REACT suggest nearly 25% of individuals with evidence of prior infection experience one or more symptoms 12 weeks after their initial illness. With 3.4 million individuals thought to have been infected during the first peak in England alone, and significant transmission since, there may be long-term major challenges to healthcare services even with rapid scale up of effective vaccination. Long COVID may require new treatment approaches, and better diagnostic and prognostic signatures will be vital for more effective management.

This study is closely related to the DHSC/ICL REACT-1, REACT-2 and REACT-GE Studies, all of which have been granted REC approval:

- REACT-1 – IRAS Number 283787
- REACT-2 – IRAS Number 283805
- REACT-GE – IRAS Number 291775.

1.2. RATIONALE FOR CURRENT STUDY

The REACT programme is uniquely placed to identify individuals with persistent symptoms who have not been hospitalised, with approximately 20,000 such people who will have been identified in home testing to January 2021.
Our aim is to characterise the genetic, biological, social and environmental signatures and pathways, and their interrelationships, that underpin progression to Long COVID, and to understand the natural history and long-term sequelae post SARS-CoV-2 infection. We will translate this knowledge to identify new treatment targets and strategies for the management of individuals with Long COVID.

2. STUDY OBJECTIVES

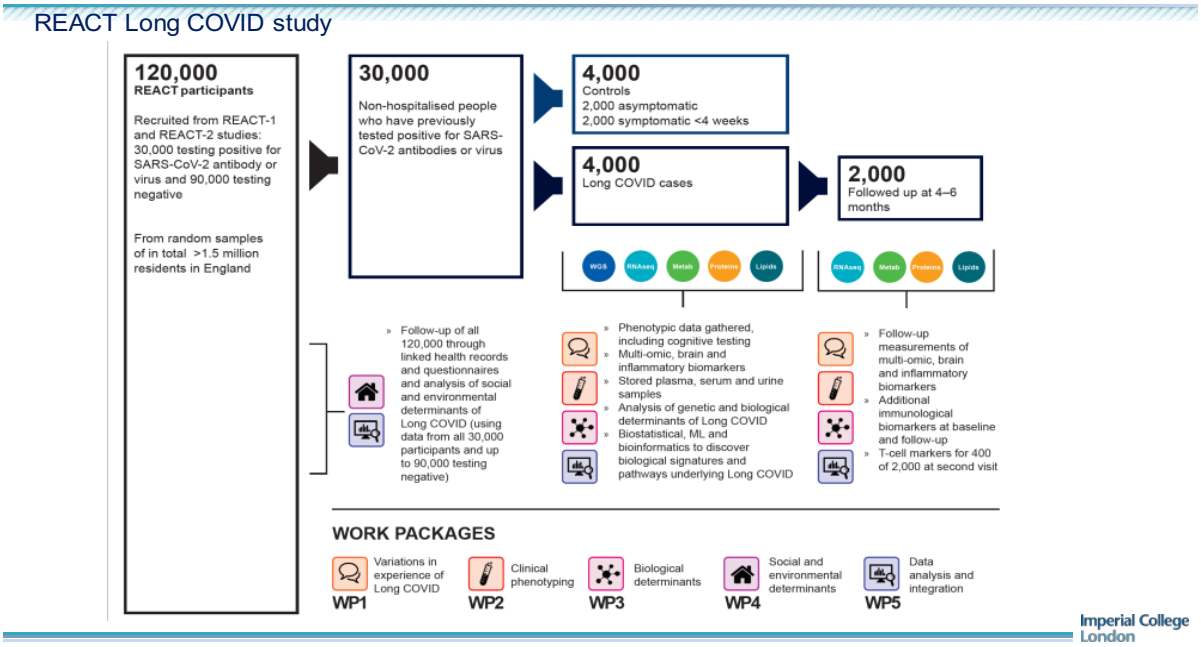
We have five primary objectives, delivered through integrated work packages (WPs):
1. Undertake public and patient involvement and engagement through our patient panels and VOICE to understand the experience of Long COVID and to help refine case definitions and outcome measures (WP1)
2. Establish a series of assessment centres for phenotyping participants through questionnaires, clinical assessment and biological sampling in 4,000 Long COVID cases and 4,000 controls (symptomatic <4 weeks, asymptomatic) identified through

the REACT and REACT-GE programme, with follow-up of 2,000 individuals for repeated measurements and samples after 4-6 months (WP2)
3. Obtain extensive multi-omics (WGS, transcriptomics, proteomics, metabolomics, lipidomics), inflammatory, immunological and brain biomarkers data, and clinical biochemistry, from the biological samples collected at the initial and follow-up visits (WP3).
4. Utilise the data on 30,000 test-positive and up to 90,000 test-negative individuals to characterise social and environmental determinants and, through questionnaires and data linkage, the natural history and longer-term sequelae of SARS-CoV-2 infection and Long COVID (WP4).
5. Undertake a comprehensive set of statistical analyses and machine learning (ML) approaches to identify genetic, biological, social and environmental determinants of Long COVID, and their interactions, and to assess the long-term health outcomes (WP5)

3. STUDY DESIGN

The REal-time Assessment of Community Transmission (REACT) study, funded by DHSC, is sampling random cross-sections of the population in England to quantify community prevalence of virus by RT-PCR (REACT-1) and of IgG anti-SARS-CoV-2 antibody based on a self-administered lateral flow immunoassay (LFIA) test (REACT-2). We collect detailed demographic and symptomatic information on participants. Each round of data collection has included between 100,000 and 170,000 participants. To date nine rounds of REACT-1 and five rounds of REACT-2 have been completed and over 2 million people have taken part, 90% of whom have consented to be re-contacted and 85% to data linkage; further rounds of both studies in January 2021 will provide an additional source of cases. In all >30,000 individuals will have tested positive for either virus or for antibody using the LFIA and given permission to be followed up for further research and through linkage to routine health records. Survey instruments are available on the study website (https://www.imperial.ac.uk/medicine/research-and-impact/groups/react-study/).

In collaboration with Genomics England, funded by UKRI and NIHR, we are conducting a multi-omics study (REACT-GE) of 8,000 non-hospitalised REACT participants (with or without symptoms) to characterise genetic and downstream biological pathways responsible for individual differences in response to SARS-CoV-2 infection. They attend an assessment centre for clinical measurements (blood pressure, pulse rate, weight waist circumference), blood draw and symptoms, health and lifestyle questionnaire.

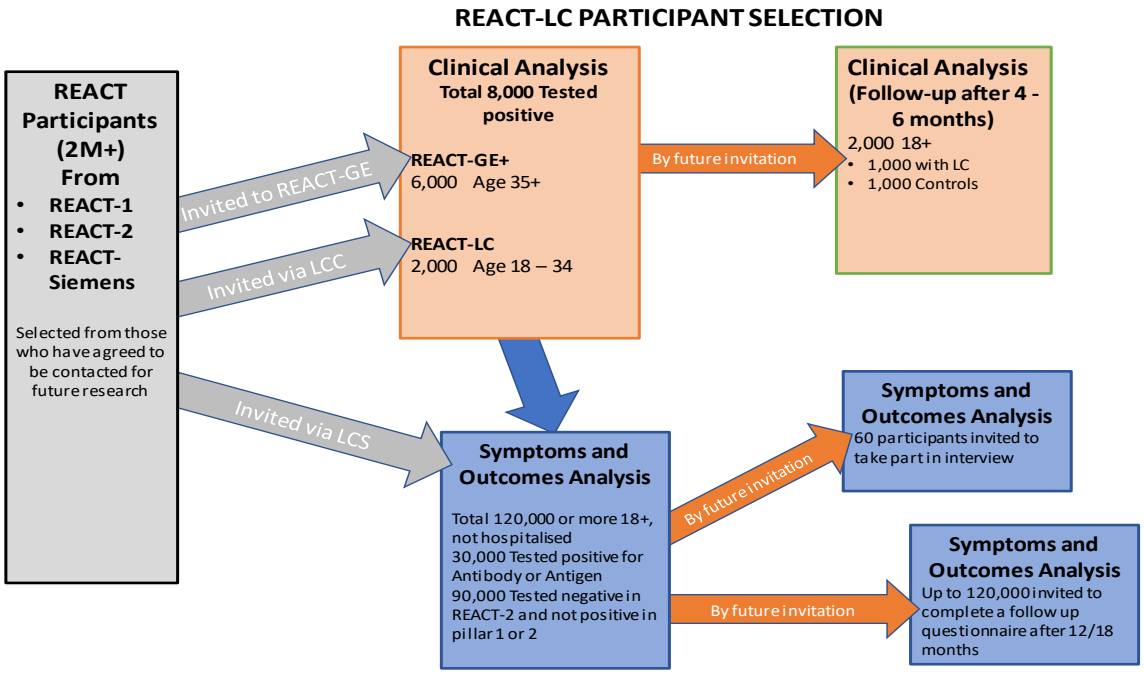


The research is to be delivered through five integrated work packages (WPs). WP1 will describe variations in experience of Long COVID and develop patient reported outcomes (PROMS) in consultation with expert collaborators and through our patient and public partners. We will use online focus groups, discussion forums, individual interviews, and surveys on the VOICE Global platform, and recruit a panel of people with Long COVID to provide input on their symptoms and experience. In WP2 we will carry out detailed clinical phenotyping on 8,000 people (4,000 with Long COVID); 2,000 will have repeat measures at 4-6 months including 400 for T-cell function. The WP2 samples will be used in WP3 which includes multi-omic analysis, brain and inflammatory biomarkers. WP4 will use data from surveys sent to 30,000 test-positive and 90,000 test-negative on RT PCR/lateral flow in REACT, plus linked health data, to explore the social and environmental determinants of Long COVID and its long-term sequelae. WP5 is the data analysis and integration to identify genetic, biological, social and environmental determinants of Long COVID. We aim to identify key biomarkers and biological pathways underlying Long COVID and possible drug targets, as well as inequalities and social determinants of variations in outcome.

The work packages are run as two parallel streams:

- Symptoms and Outcomes WP1 and WP4 (REACT-LCS)
- Clinical Phenotyping and Biological Samples WP2 and WP3 (REACT-LCC).

These streams come together for Data Analysis and Integration WP5.



Sample design is summarised in the flow chart.

We will follow 120,000 people (~30,000 testing positive for virus or antibody in the REACT programme and at least 90,000 testing negative), via regular questionnaires and health record linkage, in a nested case-cohort design. This will provide a longitudinal assessment of the long-term sequelae of SARS-CoV-2 infection and Long COVID, identified as a critical research need (see WP1 and WP4). The research will be informed by public involvement and community engagement throughout to maximise participation through design of recruitment materials and survey instruments, and to co-develop patient reported outcome measures.

Through WP2 we propose to augment the multi-omics REACT-GE sample with an additional 2,000 people, 1,000 with Long COVID (symptoms >12 weeks) and 1,000 controls (symptoms <4 weeks or asymptomatic), drawn from the REACT participant database for ages 18-34 years, as this age-group is excluded from REACT-GE. This will provide a sample for multi-omics in REACT-LC of 8,000 individuals aged 18+ years, half with prolonged symptoms and half controls, 6,000 of whom are being recruited through REACT-GE which will collect the additional phenotypes required by REACT-LC.

We include a second assessment centre attendance for a sub-sample of 2,000 of the 4,000 people with prolonged symptoms recruited into REACT-LC. From these individuals we will obtain the full set of measures and samples and include additional analyses for inflammatory markers, autoantibodies and brain biomarkers at both the first and second visits. For a further subsample of 400 of these 2,000 individuals, we will assess T cell function from whole blood samples obtained at the second visit (WP3). Other subsets of participants will be studied in more depth as the results of

the initial work guides further research. Data analysis will identify key sets of biomarkers and biological pathways underlying Long COVID, and possible drug targets (WP5).To identify people with persistent symptoms who have not been hospitalised, we will use a sampling frame generated through repeated random population surveys of SARS-CoV-2 prevalence in the community, the REACT programme, which includes >2 million individuals with documented SARS-CoV-2 status (RT PCR or lateral flow test), including >30,000 with positive tests, 90% of whom have consented to be re-contacted and 85% to data linkage.

Invitation process
As with REACT-GE, for REACT-LC all the potential participants have already taken part in a related study, either Airwave or REACT-1 or -2 and so are likely to be more willing to participate. We propose to use the same core invitation strategy consisting of a total of up to four invitations as follows:

- Stage 1: Email
- Stage 2: Email
- Stage 3: SMS
- Stage 4: Letter.

Timings and methods will be varied in the light of experience as the invitation process is developed to cover the geographic spread and the later availability of potential participants from the later rounds of the REACT studies.

Where email addresses are not recorded then up to three letters may be sent by post.

Work Package 1 - Variations in experience of Long COVID

From symptomatic data in REACT-2, we estimate ~25% of people testing positive on the LFIA report long-term symptoms (>12 weeks) among whom two symptom clusters have emerged: one where anosmia and ageusia predominate, the other involving fatigue, breathlessness, muscle pains etc. We seek to develop these further in an initial rapid assessment of experience of Long COVID through a co-designed symptom survey.

We will use a combination of online focus groups, discussion forums, individual involvement interviews, and surveys on the VOICE-Global platform to inform the amended symptom and experience survey, which will also include standard measures of quality of life, anxiety, depression, sleep quality, cognition, physical activity, and socioeconomic measures.

From the more than 2 million REACT study participants REACT-LC study will aim to recruit at least 30,000 participants who have tested positive for the COVID-19 virus or antibody and at least 90,000 who have not. This will provide a target cohort of 120,000 or more people who will be invited to complete the symptom survey.

Potential participants will be invited at the start of the study by email or letter using the same process as for REACT-GE and WP2. All 120,000 participants will complete a short questionnaire at the time of registration which will also include limited essential information. They will be asked to consent online prior to being directed to the main questionnaire. After completing the main questionnaire, they may be invited to complete the 24-hour food intake questionnaire, INTAKE24.

A sub-sample of the symptom survey respondents will be invited to take part in an ongoing panel of people with Long COVID as part of a follow up study; those who agree will be asked to update their symptoms monthly using an app which we will develop with public and patient partners.

We will purposively sample from REACT cases to include a target of 60 people with different symptoms, duration and sociodemographic characteristics for an in-depth interview study. Respondents to the symptom survey who are living with Long Covid, will be given the opportunity to express a willingness to be contacted about a follow up interview. Researchers from the study team will then contact the selected participants to arrange a date and time for the interview. To reduce the risk of viral transmission we propose to conduct interviews online using Zoom or MS Teams. The interviews will be recorded locally utilising the Zoom recording tool and will be transferred and stored on DIDE servers. For members of the public who do not have digital access to tools like Zoom, we will conduct and record interviews over the phone. Interviews will be transcribed verbatim, and pseudonymised, either by a member of the research team or a qualified professional using the services of UKTranscription.com, a specialist audio transcription service. There will be a confidentiality agreement in place between Imperial College London and UK Transcription, and transfer of audio and text files will be done via a secure portal.

The interview will be semi-structured using a Topic Guide. The guide will provide a general structure to the interview with prompts and probes to be used only where necessary to elucidate the participant's responses and provide greater detail . The interviews will broadly cover the patient's Long Covid journey including:

- Personal background (including medical history/health background)
- Experience of Covid-19
- Development of Long Covid
- Impact of illness on work, family, income, social life
- Services and support accessed (including patient groups)
- Experience of recovery
- Views on Long Covid more generally e.g. the role of the LC patient community, government response, challenges to support

Analysis of the quantitative and qualitative data from both the symptom survey and the interviews will be carried out in close collaboration with our advisory group of patients and public, who will help us with interpretation and dissemination of results.

The findings of WP1 will help us later develop patient reported outcomes (PROMS) in consultation with expert collaborators and through our patient and public partners.

All 120,000 respondents will be asked to carry out a follow up questionnaire after 12/18 months. This questionnaire will be based on the main questionnaire already completed amended in the light of the analyses carried out.

Work Package 2 – Clinical Phenotyping

Sample Collection Strategy
In order to achieve the best quality samples these should be centrifuged if possible within an hour of collection, with 30 minutes tolerance (i.e.90 minutes).

The project will set up assessment centres where blood can be collected and processed with 60 minutes. Where a local laboratory able to provide these services cannot be identified then we will centrifuge the relevant tubes ourselves using our own sealed-cup centrifuges and appropriate COVID safe procedures. The samples will be transferred in a suitably temperature controlled environment to appointed laboratories daily where they will be processed and aliquoted in a timely manner. The samples will then be frozen and distributed to the various analysis laboratories as appropriate.

We will set up approximately six centres which will be located so as to maximise the likely number of participants within travelling distance but minimise the sample transport distance/cost. We do not plan to visit participants at their home to collect samples as the extended time to spinning the sample would reduce the integrity of the samples. However, this may prove necessary for some severely Long COVID affected participants.

Samples, phenotypes and information to be collected
All participants will complete a short questionnaire at the time of registration which will also include limited essential information.

They will be asked to complete a fuller 20-25 minute questionnaire, preferably before attending the appointment.

At the appointment a signed consent will be obtained electronically. Systems used to capture the consent and phenotypes will be based on those developed by ICL and used on Airwave and other REACT studies. Some additional information may be captured such as contraindications and contextual data (pregnancy, menstruation) where relevant.

Samples of urine and up to 50ml of blood will be taken in up to six tubes from each Participant. The target is to spin the tubes within 60 minutes of needle, with a maximum of 90 minutes.

Other phenotypes to be taken are:

- Blood pressure
- Weight
- Waist circumference
- Height
- Grip strength
- Lung function assessed by Spirometry and/or exercise with pulse oximeter

The SOP will detail the procedure to be followed by centre staff in case of an abnormal BP reading.

After the appointment the participants will be reminded to complete the 15-20 minute questionnaire, if not already done, and invited to complete a 24 hour food intake questionnaire. They will be provided with the links to complete them on line later.

All assessment centres will be COVID-19 secure. They will only be attended by health staff and study participants. We will adhere to social distancing, hand washing and mask wearing according to government guidance. We provide hand sanitiser. Our staff will wear appropriate PPE. A regular cleaning schedule will be in place and each test station will be cleaned between participants. We will limit touch points, implement one way systems where appropriate and limit the number of people in a centre at any one time.

Work Package 3 – Biological determinants

Multi-omic analysis will be performed on 8,000 individuals (6,000 through REACT-GE) and 2,000 second visit samples (except for WGS) along with other biological determinants (2,000 samples at each of first and second visits).

WGS and transcriptomics: We will deliver short-read 30x germline genomes from all 8,000 REACT-LC participants using Genomics England's ISO accredited WGS Illumina pipeline. The use of Tempus tubes will produce high quality RNA template for 4,000 Illumina TruSeq Stranded Total RNA sequencing.

Proteomics: A high-throughput, rapid mass spectrometry (MS)-based proteomic screen (>2000s proteins) will be undertaken on 4,000 samples.

Metabolomics and lipidomics: Broad spectrum untargeted (discovery) metabolomics will be carried out using ultra-performance liquid chromatography MS (UPLC-MS) in the Imperial Phenome Centre.

For the targeted inflammatory lipidomics Leiden University will use ultrasensitive LC-MS/MS (>200 metabolites) to provide downstream data on regulation of cell proliferation and apoptosis via quantification of lipid signalling molecules including sphingolipid messengers, covering >40 eicosanoids (e.g. prostaglandins, leukotrienes, thromboxanes).

Immunological studies: From the second visit (subset N=400), whole blood samples (from different symptom clusters) will be analysed by flow cytometry for:

i) activation of SARS-CoV-2 specific T cells, identified by tetramer staining and expression of activation markers,
ii) activation of other T cell populations potentially representing non-specific immune activation,
iii) activation of myeloid cells, particularly those populations associated with pathology in acute disease,
iv) Research into the longer term effects of COVID-19 in non-hospitalised individuals frequency of myeloid and lymphoid cell populations.

Brain injury and neurodegeneration biomarkers: Biomarkers will be assayed on the fully automated UK DRI-supported UCL DFBL Quanterix Simoa HD-X platform employing a 4plex E assay for ultrasensitive detection of plasma NfL and GFAP peptides. A separate Simoa assay will be performed for pTau181 (4,000 samples).

Autoantibodies: The Wraith laboratory will initially analyse a cross-section of 200 of the 2,000 subset with additional inflammatory and brain injury markers, to indicate which individual patterns correlate with immune pathology. We will then measure selective autoantibodies to both confirm the association and test the longevity of autoantibody seropositivity across the 2,000 samples at two time-points. We will screen for a comprehensive panel of autoantibodies related to systemic autoimmune conditions, organ-specific autoantibodies and neuroimmunological markers for central and peripheral nervous system disorders.

Work Package 4 – Social and environmental determinants of Long COVID and outcome Analysis

To describe the natural history of Long COVID and SARS-CoV-2 infection and their sequelae, we will construct a sub-cohort comprising the ~30,000 people testing positive for the virus or antibody in REACT with consent to recontact and data linkage, along with 90,000 test-negative people (case-cohort design). We will link routine primary and secondary care health data to results from the questionnaire (developed in WP1). This will enable us to quantify medium and long-term physical, mental and cognitive health outcomes of SARS-CoV-2 infection, including Long COVID. We will investigate potential social and environmental determinants to identify whether and how certain population sub-groups may be more vulnerable to Long COVID. The large scale of the study provides unprecedented opportunity to quantify the longer-term health risks following SARS-CoV-2 infection and Long COVID.

Work Package 5 – Data analysis and integration

Epidemiological analyses: First, we will undertake cluster analysis of the symptoms reported on questionnaire to refine the case definition of Long COVID and qualitative approaches to analyse the interview responses in WP1. We will use

multiple and penalised regression to identify the demographic, social, environmental, and behavioural determinants of risk of Long COVID and survival extensions of these models to quantify effects of Long COVID and SARS-CoV-2 infection on a range of health outcomes among the case-cohort sample of 120,000 people.

Multi-omic analyses: For the nested subsample of 8,000 individuals with multiple omics data including 2,000 with repeated biological measurements, we will use penalised regression guided by stability approaches, as well as non-linear alternatives (e.g. random forests), to identify multi-omic signatures of Long COVID. To address the dimensional challenge of multi-omic integration, we will stringently prune the data and identify sets of (possibly multi-) omics measurements that co-vary and contribute to population clustering. Summary latent variables of features included in each cluster will subsequently be used as predictors in place of the original covariates. Correlation structure among disease-relevant (combinations of) omics measurements will be visualised using partial correlation networks and will inform on potential mechanisms involved. We will use data-driven versatile dimensionality reduction methods (e.g. factor analysis-based methods [41]) and similarity networks to obtain clusters of participants with similar -omics profiles. These will identify biologically-driven subtypes of Long COVID which we will then correlate with e.g. clinical and demographic data to further characterise the clusters.

Disease trajectory: To understand the natural history of persistent symptoms and omic disturbances following SARS-CoV-2 infection, we will utilise individual-based multi-state models, whereby disease progression is modelled from asymptomatic to symptomatic and Long COVID to recovery or deterioration states. We will use a Bayesian approach to estimate the transition probabilities defining the model and simulate individual trajectories to characterise the dynamics of Long COVID progression or recovery and the contributions of selected molecular, biological, social and environmental factors.

Bioinformatic and pathway analyses: We will identify the biochemical pathways that are perturbed in Long COVID using a computational network biology approach. Through differential expression/abundance analysis we will map the expression of genes, and levels of proteins, lipids and metabolites onto the different pathways. We will assess potential drug targets that affect these dysregulated pathways e.g. by use of Molecule Activity Predictor (MAP) functionality in Ingenuity Pathway Analysis. Key molecules/pathways could then be tested in appropriate patient-cell-based or in vitro model based systems already in use in our labs. Relevant cell types for functional testing of candidate targets will be identified based on known gene expression/function of the candidate molecules/pathways.

Sample size and power: For WGS and sample size of 8,000, with genome-wide significance level of 5e-8, we could detect a relative risk of 1.11 with 80% power for SNPs with a disease allele frequency of 0.5 (1.24 for disease allele frequency of 0.05, and 1.40 for 0.95). For all omics other than WGS we could detect with 80% power odds ratios (OR) > 1.24 per omic feature (multiple-testing adjusted) comparing participants with/without Long COVID. Comparing two distinct clusters of participants with Long COVID (n=4,000), we could detect with 80% power OR>1.31 per omic

feature. For agnostic analyses focusing on 2,000 Long COVID cases followed-up in time we could detect with 80% power OR>1.24 per feature, and for the targeted investigation in the followed-up samples of 5, 10, 100 signals identified at first visit, we could detect OR >1.15, 1.16, 1.20 respectively. All ORs are expressed as risk change per unit increase in the standardised omics measurement.

Data Management
A research database will be established at Imperial College London where the data will be held securely on secure servers in an ISO27001 environment managed by the School of Public Health. Study participants will be assigned a study ID and the data will be stripped of identifying information for the statistical analyses; only one or two named and designated individuals will have access to the identifying information. Individuals will not be identifiable in any publications or reports arising from the study. A parallel database will also be established at Genomics England taking advantage of their secure data management system.

Data held with Genomics England will be held in the National Genomic Research Library. The Library is a comprehensive resource that allows researchers to access samples, genomic data, and other associated health data in a de-identified format. It contains the data of tens of thousands of NHS patients which researchers use to make discoveries and help develop new treatments and medicine. Access to the Library is governed by an Access Review Committee.

Feedback Reporting of Individual Results to Participants
A feedback by email of baseline clinical measurements including limited blood test results (cholesterol and diabetes) is given to the participants. If any of the results are of clinical significance, participants are advised to consult their GP for follow up or further investigation. We do not directly inform participants' GPs of their results. Participants are not notified of any further findings that may result from future assays, analyses or other procedures that are not provided for in their feedback letter.

3.1. STUDY OUTCOME MEASURES

Primary and Secondary outcomes are:

1. Symptoms and prevalence for Long COVID
2. Identified genomic, biological pathway and -omic differences between participants who suffer from Long Covid and those who do not, amongst those who have not been hospitalised.

4. PARTICIPANT ENTRY

4.1. PRE-REGISTRATION EVALUATIONS

Participants will be selected from individuals who have taken part in one of the REACT or Airwave Studies and have agreed to be contacted for further studies. They must be aged 18+. Although most Participants will be recruited from the Participants of the various REACT studies already carried out we may add further individuals who are of particular interest to the study, who have expressed an interest to the research team in taking part and meet the selection criteria. This may

include such individuals as members of the Public Advisory Groups or others who contact us because they have had Long COVID.

4.2. INCLUSION CRITERIA

For WP1(120,000): Participants will be aged 18+ and will have taken part in a REACT study.

For WP2 (8,000): Participants will have had or reported having had a positive test for COVID-19 and had mild to moderate symptoms or were asymptomatic and not hospitalised for COVID-19.

Data from the 6,000 Participants of the REACT-GE study who were included in the extended phenotype collection all of whom were aged >=35.

An additional 2,000 Participants will be recruited using the same process and phenotypes as for the REACT-GE extended phenotype study except that they will be aged over 18 and < 35. Of these a target of 1,000 will be selected to have reported having Long COVID and 1,000 controls who have not. It is intended to recruit these from as close to London as possible for logistical reasons.

From these 8,000 participants a number will be selected and invited to take part in a follow up visit for 2,000 to repeat the phenotype measurements and sample collection, and to answer a further questionnaire.

It is recognised that some of the Participants with more severe Long COVID symptoms may need additional assistance for them to be able to attend one of the assessments centres and this will be provided where possible in order to facilitate their participation.

4.3. EXCLUSION CRITERIA

Aged under 18. Hospitalised by COVID-19 infection.

4.4. WITHDRAWAL CRITERIA

Participants are free to withdraw at any time without having to provide a reason. Participants wishing to withdraw should contact the participant helpline by telephone, email or in writing. Participants who withdraw will be removed from the database, and their data will not be used again in the future. However, their data will be retained as part of any analysis that has been conducted prior to their request for withdrawal.

5. ADVERSE EVENTS

5.1. DEFINITIONS

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any untoward and unexpected medical occurrence or effect that:

- **Results in death**
- **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*

- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

5.2. REPORTING PROCEDURES

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

5.3.1 Non serious AEs

All such events, whether expected or not, should be recorded.

5.3.2 Serious AEs

An SAE form should be completed and emailed to the Chief Investigator within 24 hours.

All SAEs should be reported to the **South Central - Berkshire B Research Ethics Committee** where in the opinion of the Chief Investigator, the event was:

- 'related', ie resulted from the administration of any of the research procedures; and
- 'unexpected', ie an event that is not listed in the protocol as an expected occurrence

There are no invasive procedures other than collection of a blood sample, which may result in bruising.

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all related and unexpected SAEs.

Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

Contact details for reporting SAEs
RGIT@imperial.ac.uk
CI: p.elliott@imperial.ac.uk
Please send SAE forms to: Study Coordinator: k.bennet@imperial.ac.uk
Tel: +44 20 7594 3328 (Mon to Fri 09.00 – 17.00)

6. ASSESSMENT AND FOLLOW-UP

Feedback is provided to the participants who have attended an assessment centre, by email, of baseline clinical measurements including limited blood test results (cholesterol and diabetes). If any of the results are of clinical significance, participants are advised to consult their GP for follow up or further investigation. We do not directly inform participants' GPs of their results. Participants are not notified of any further findings that may result from future assays, analyses or other procedures that are not provided for in their feedback letter.

7. STATISTICS AND DATA ANALYSIS

These aspects are covered under Work Package 5 in Section 3 – Study Design above.

Data and all appropriate documentation will be stored for a minimum of 20 years after the completion of the study, including the follow-up period.

8. REGULATORY ISSUES

8.1. ETHICS APPROVAL

The Study Coordination Centre has obtained approval from the **South Central - Berkshire B Research Ethics Committee**. The study must also receive confirmation of capacity and capability from each participating NHS Trust before accepting participants into the study or any research activity is carried out. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

8.2. CONSENT

Consent to enter the study will be sought from each participant after a full explanation has been given, an information leaflet offered and time allowed for consideration. This will be done before the participant attends the assessment centre. Signed participant consent will be obtained at the start of the assessment centre appointment on an electronic tablet using an ICL system based on systems used on the Airwave and other REACT studies. The right of the participant to refuse to participate without giving reasons will be respected. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

8.3. CONFIDENTIALITY

The Principal Investigator will preserve the confidentiality of participants taking part in the study and fulfil transparency requirements under the General Data Protection Regulation for health and care research. Data and all appropriate documentation will be stored for 20 years after the completion of the study.

The data will be held securely and processed in a Secure Enclave. This is an isolated environment within the College for the processing of health related personal data. It provides a framework that satisfies Information Governance *requirements* that come from several sources such as:

- Legislation (e.g. GDPR)
- Regulatory bodies
- Data Providers (e.g. NHS Digital)
- Imperial College (e.g. ICT).

The Secure Enclaves are compliant with the requirements of major data providers (e.g. ONS, NHS Digital and NHS Trusts), as well as flexible to incorporate additional requirements a group may be subject to. The enclaves are ISO27001 certified.

A similar secure system is in place at Genomics England where a copy of the data will also be held.

The data will be pseudonymised and used for statistical analysis as outlined elsewhere in this protocol. Where consent is given we may link data from health records to the survey data for up to 20 years. Imperial College London with Genomics England are responsible for the data analysis Imperial College London will hold personal data for up to 20 years, but this will be held securely and separated from the survey and health data.

8.4. INDEMNITY

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

8.5. SPONSOR

Imperial College London will act as the main Sponsor for this study.

8.6. FUNDING

UK Research and Innovation / National Institute for Health Research are funding this study.

It is not planned to pay any participant for taking part in the study. Participants cost of attending may be reimbursed.

8.7. AUDITS

The study may be subject to audit by Imperial College London/ Imperial College Healthcare NHS Trust under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy Frame Work for Health and Social Care Research.

9. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through Imperial College London.

10. PUBLICATION POLICY

The Investigators and representatives of the collaborating organisations will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge the study funding as detailed in Section 8.6 above. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

11. REFERENCES

[List of useful and relevant references for the study]

2. Final protocol

Imperial College London

Research Governance and Integrity Team

Protocol for
REACT-LC

REal-time Assessment of Community Transmission – Long COVID

Version 1.6

MAIN SPONSOR: Imperial College London
FUNDERS:UKRI/NIHR
STUDY COORDINATION CENTRE: Imperial College London, St Mary's Campus

IRAS Project ID: 298404
REC reference: 21/SC/0134

Protocol authorised by:

Name & Role	Date	Signature

Study Management Group

Chief Investigator: Professor Paul Elliott

Co-investigators: Professor Helen Ward, Professor Graham Cooke, Professor Sir Mark Caulfield, Dr Christina Atchison

Statistician: Professor Marc Chadeau-hyam

Study Management: Graham Blakoe

Study Coordination Centre

For general queries, supply of study documentation, and collection of data, please contact:

Study Coordinator: Graham Blakoe
Address: St Mary's Campus
E-mail: graham.blakoe@imperial.ac.uk

Clinical Queries

Clinical queries should be directed to Professor Graham Cooke who will direct the query to the appropriate person

Address: Infectious Diseases Section, Winston Churchill Wing, St Mary's Campus
E-mail: g.cooke@imperial.ac.uk

Sponsor

Imperial College London/ is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Research Governance and Integrity at:

Research Governance and Integrity Team
Imperial College London and Imperial College Healthcare NHS Trust
Room 215, Level 2, Medical School Building
Norfolk Place
London, W2 1PG
Tel: **0207 594 1862**

Funder

The study is funded by UKRI and NIHR Reference 28863: Research into the longer term effects of COVID-19 in non-hospitalised individuals.

This protocol describes the REACT Long COVID (REACT-LC) study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the UK Policy Frame Work for Health and Social Care Research. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

Table of Contents

GLOSSARY OF ABBREVIATIONS

KEYWORDS

COVID-19
SARS CoV-2
Long COVID
Whole genome sequencing
proteomics
metabolomics
inflammation
multi-omics
immunoassays
auto-antibodies

STUDY SUMMARY

TITLE REACT-Long COVID (REACT-LC) Programme

DESIGN Descriptive study of self-reported symptoms and experience of people testing positive in the REACT1 and REACT2 studies, with a nested study of clinical phenotype with multi-omics analyses and genetic sequencing on participants to identify links with the susceptibility and severity of their COVID illness.

AIMS The REACT-Long COVID (REACT-LC) programme aims to characterise the genetic, biological, social and environmental signatures and pathways, and their inter-relationships, that underpin progression to Long COVID, and to understand the natural history and long-term sequelae post-SARS-CoV-2 infection.

OUTCOME MEASURES

1. Detailed description of the prevalence, symptoms and experience of people with Long COVID

2. Identified genomic, biological pathway and -omic differences between participants who suffer from Long Covid and those who do not, amongst those who have not been hospitalised.

POPULATION Target minimum 120,000 participants for the regular questionnaires from which 8,000 will attend a clinical assessment centre and then 2,000 will attend a follow-up assessment.

ELIGIBILITY Adults who have taken part in the ICL REACT Study.

DURATION 3 years

1. INTRODUCTION

1.1. BACKGROUND

The UK has experienced one of the largest epidemics of COVID-19 in Europe. As a new disease, the natural history beyond the immediate illness and the possible long-term sequalae are largely unknown. As well as the risk of hospitalisation and death from COVID-19 it is clear that some people who develop symptoms have a prolonged and debilitating illness that may continue for weeks or months (so-called Long COVID or post-COVID syndrome). Pathogenesis of persistent symptoms from COVID-19 is poorly understood and represents a major knowledge gap if effective treatments and management strategies are to be developed.

Our initial estimates from REACT suggest nearly 25% of individuals with evidence of prior infection experience one or more symptoms 12 weeks after their initial illness. With 3.4 million individuals thought to have been infected during the first peak in England alone, and significant transmission since, there may be long-term major challenges to healthcare services even with rapid scale up of effective vaccination. Long COVID may require new treatment approaches, and better diagnostic and prognostic signatures will be vital for more effective management.

This study is closely related to the DHSC/ICL REACT-1, REACT-2 and REACT-GE Studies and ICL-led large scale studies using online cognitive assessment, all of which have been granted REC approval:

- REACT-1 – IRAS Number 283787
- REACT-2 – IRAS Number 283805
- REACT-GE – IRAS Number 291775.
- REC 17IC4009 - which is for ICL studies using large scale online cognitive assessment

1.2. RATIONALE FOR CURRENT STUDY

The REACT programme is uniquely placed to identify individuals with persistent symptoms who have not been hospitalised, with approximately 20,000 such people who will have been identified in home testing to January 2021.

Our aim is to characterise the genetic, biological, social and environmental signatures and pathways, and their interrelationships, that underpin progression to Long COVID, and to understand the natural history and long-term sequelae post SARS-CoV-2 infection. We will translate this knowledge to identify new treatment targets and strategies for the management of individuals with Long COVID.

2. STUDY OBJECTIVES

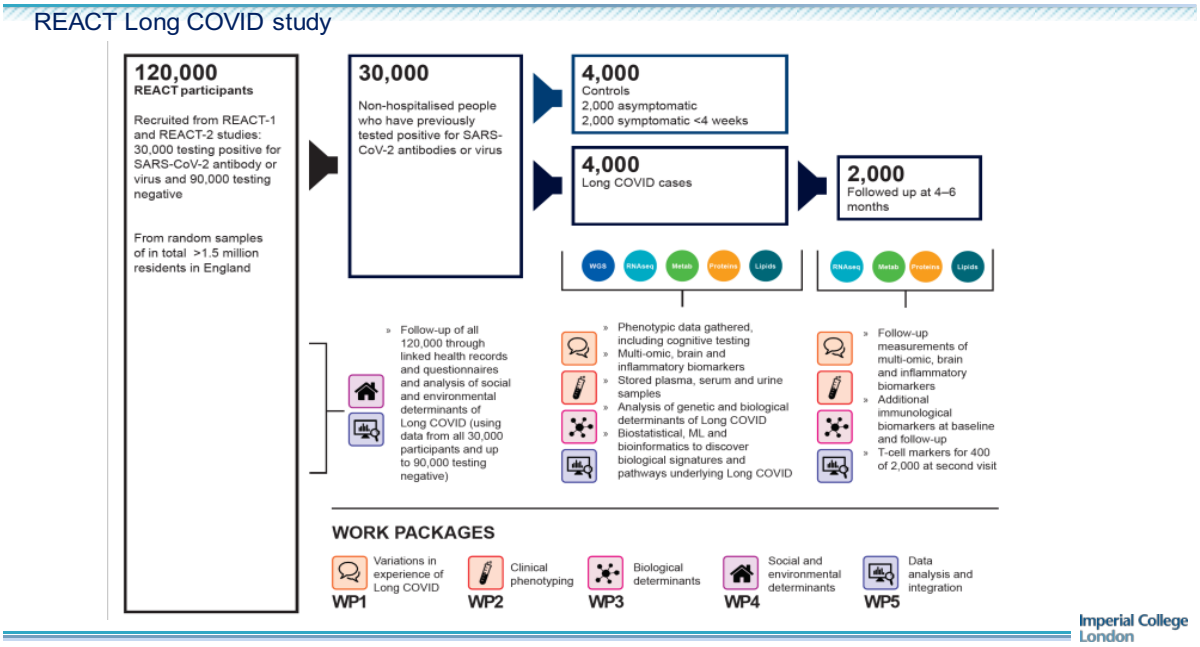
We have five primary objectives, delivered through integrated work packages (WPs):
1. Undertake public and patient involvement and engagement through our patient panels and VOICE to understand the experience of Long COVID and to help refine

case definitions and outcome measures (WP1)
2. Establish a series of assessment centres for phenotyping participants through questionnaires, clinical assessment and biological sampling in 4,000 Long COVID cases and 4,000 controls (symptomatic <4 weeks, asymptomatic) identified through the REACT and REACT-GE programme, with follow-up of 2,000 individuals for repeated measurements and samples after 4-6 months (WP2)
3. Obtain extensive multi-omics (WGS, transcriptomics, proteomics, metabolomics, lipidomics), inflammatory, immunological and brain biomarkers data, and clinical biochemistry, from the biological samples collected at the initial and follow-up visits (WP3).
4. Utilise the data on at least 40,000 test-positive and up to 80,000 test-negative individuals to characterise social and environmental determinants and, through questionnaires and data linkage, the natural history and longer-term sequelae of SARS-CoV-2 infection and Long COVID (WP4), including the impact on cognitive function
5. Undertake a comprehensive set of statistical analyses and machine learning (ML) approaches to identify genetic, biological, social and environmental determinants of Long COVID, and their interactions, and to assess the long-term health outcomes (WP5)

3. STUDY DESIGN

The REal-time Assessment of Community Transmission (REACT) study, funded by DHSC, is sampling random cross-sections of the population in England to quantify community prevalence of virus by RT-PCR (REACT-1) and of IgG anti-SARS-CoV-2 antibody based on a self-administered lateral flow immunoassay (LFIA) test (REACT-2). We collect detailed demographic and symptomatic information on participants. Each round of data collection has included between 100,000 and 170,000 participants. To date nine rounds of REACT-1 and five rounds of REACT-2 have been completed and over 2 million people have taken part, 90% of whom have consented to be re-contacted and 85% to data linkage; further rounds of both studies in January 2021 will provide an additional source of cases. In all >30,000 individuals will have tested positive for either virus or for antibody using the LFIA and given permission to be followed up for further research and through linkage to routine health records. Survey instruments are available on the study website (https://www.imperial.ac.uk/medicine/research-and-impact/groups/react-study/).

In collaboration with Genomics England, funded by UKRI and NIHR, we are conducting a multi-omics study (REACT-GE) of 8,000 non-hospitalised REACT participants (with or without symptoms) to characterise genetic and downstream biological pathways responsible for individual differences in response to SARS-CoV-2 infection. They attend an assessment centre for clinical measurements (blood pressure, pulse rate, weight waist circumference), blood draw and symptoms, health and lifestyle questionnaire.



The research is to be delivered through five integrated work packages (WPs). WP1 will describe variations in experience of Long COVID and develop patient reported outcomes (PROMS) in consultation with expert collaborators and through our patient and public partners. We will use online focus groups, discussion forums, individual interviews, and surveys on the VOICE Global platform, and recruit a panel of people with Long COVID to provide input on their symptoms and experience. In WP2 we will carry out detailed clinical phenotyping on 8,000 people (4,000 with Long COVID); 2,000 will have repeat measures at 4-6 months including 400 for T-cell function. The WP2 samples will be used in WP3 which includes multi-omic analysis, brain and inflammatory biomarkers. WP4 will use data from surveys completed by at least 40,000 test-positive (approximately 10,000 with persistent symptoms beyond 12 weeks) and up to 80,000 test-negative on RT PCR/lateral flow in REACT, plus linked health data, to explore the social and environmental determinants of Long COVID and its long-term sequelae. Test-positive individuals will be identified through positive RT PCR/lateral flow results from REACT or self reported and also through linkage to Pillar 2 data (if participants gave consent to data linkage as part of REACT). Test-positive individuals with persistent symptoms (>12 weeks) after SARS-CoV-2 will be identified through positive RT PCR/lateral flow results from REACT or self-reported, and self-reported symptoms lasting >12 weeks.

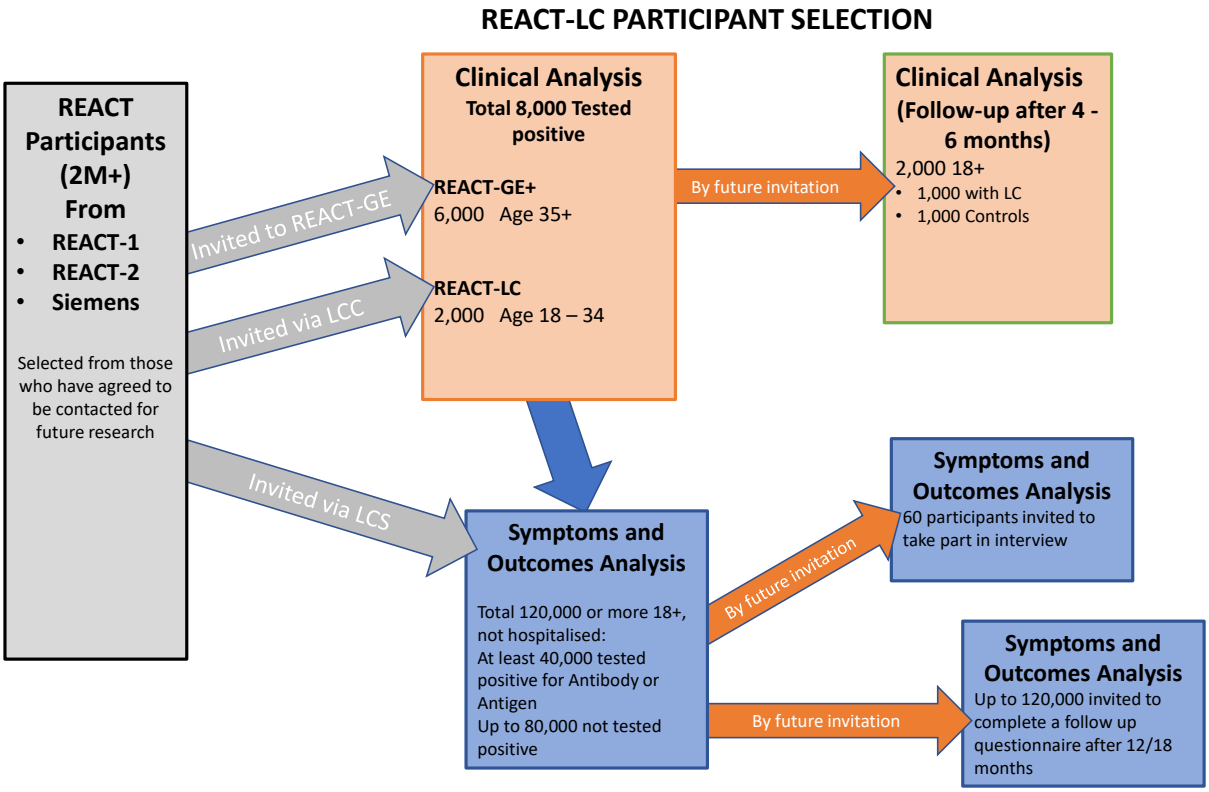
WP5 is the data analysis and integration to identify genetic, biological, social and environmental determinants of Long COVID. We aim to identify key biomarkers and biological pathways underlying Long COVID and possible drug targets, as well as inequalities and social determinants of variations in outcome.

The work packages are run as two parallel streams:

- Symptoms and Outcomes WP1 and WP4 (REACT-LCS)

- Clinical Phenotyping and Biological Samples WP2 and WP3 (REACT-LCC).

These streams come together for Data Analysis and Integration WP5.



Sample design is summarised in the flow chart.

We will follow at least 120,000 people (at least 40,000 testing positive for virus or antibody in the REACT programme (~10,000 with Long COVID (symptoms >12 weeks)) and up to 80,000 testing negative), via regular questionnaires, cognitive assessments and health record linkage, in a nested case-cohort design. This will provide a longitudinal assessment of the long-term sequelae of SARS-CoV-2 infection and Long COVID, identified as a critical research need (see WP1 and WP4). The research will be informed by public involvement and community engagement throughout to maximise participation through design of recruitment materials and survey instruments, and to co-develop patient reported outcome measures.

Through WP2 we propose to augment the multi-omics REACT-GE sample with an additional 2,000 people, 1,000 with Long COVID (symptoms >12 weeks) and 1,000 controls (symptoms <4 weeks or asymptomatic), drawn from the REACT participant database for ages 18-34 years, as this age-group is excluded from REACT-GE. This will provide a sample for multi-omics in REACT-LC of 8,000 individuals aged 18+ years, half with prolonged symptoms and half controls, 6,000 of whom are being recruited through REACT-GE which will collect the additional phenotypes required by REACT-LC.

We include a second assessment centre attendance for a sub-sample of 2,000 of the 4,000 people with prolonged symptoms recruited into REACT-LC. From these individuals we will obtain the full set of measures and samples and include additional analyses for inflammatory markers, autoantibodies and brain biomarkers at both the first and second visits. For a further subsample of 400 of these 2,000 individuals, we will assess T cell function from whole blood samples obtained at the second visit (WP3). Other subsets of participants will be studied in more depth as the results of the initial work guides further research. Data analysis will identify key sets of biomarkers and biological pathways underlying Long COVID, and possible drug targets (WP5).To identify people with persistent symptoms who have not been hospitalised, we will use a sampling frame generated through repeated random population surveys of SARS-CoV-2 prevalence in the community, the REACT programme, which includes >2 million individuals with documented SARS-CoV-2 status (RT PCR or lateral flow test), including >30,000 with positive tests, 90% of whom have consented to be re-contacted and 85% to data linkage.

Because of the importance of the data on the 2,000 participants who have attended a second clinic visit then, if we require additional samples or to confirm information, we may invite a number of them for a further clinical assessment. If just samples are required, and it is more convenient for the participant then this could be by a home visit. These will be repeats of previous measurements, no new measurements or sample types will be taken.

Invitation process

As with REACT-GE, for REACT-LC all the potential participants have already taken part in a related study, either Airwave or REACT-1 or -2 and so are likely to be more willing to participate. We propose to use the same core invitation strategy consisting of a total of up to four invitations as follows:

- Stage 1: Email
- Stage 2: Email
- Stage 3: Email or SMS reminder
- Stage 4: Email, SMS or letter

Timings and methods will be varied in the light of experience as the invitation process is developed to cover the geographic spread and the later availability of potential participants from the later rounds of the REACT studies.

Work Package 1 - Variations in experience of Long COVID

From symptomatic data in REACT-2, we estimate ~25% of people testing positive on the LFIA report long-term symptoms (>12 weeks) among whom two symptom clusters have emerged: one where anosmia and ageusia predominate, the other involving fatigue, breathlessness, muscle pains etc. We seek to develop these further in an initial rapid assessment of experience of Long COVID through a co-designed symptom survey.

We will use a combination of online focus groups, discussion forums, individual involvement interviews, and surveys on the VOICE-Global platform to inform the amended symptom and experience survey, which will also include standard measures of quality of life, anxiety, depression, sleep quality, cognition, physical activity, and socioeconomic measures.

Survey

From the more than 2 million REACT study participants REACT-LC study will aim to recruit at least 40,000 participants who have tested positive for the COVID-19 virus or antibody and up to 80,000 who have not. This will provide a target cohort of120,000 or more people complete the symptom survey. Assuming ~25% of people testing positive report long-term symptoms (>12 weeks) we hope to recruit at least 10,000 individuals with Long COVID. We will oversample people reporting symptom duration beyond 12 and then 4 weeks in REACT-1 and REACT-2 to increase our sample of individuals with Long COVID.

Potential participants will be invited at the start of the study by email using the same process as for REACT-GE and WP2. All participants will complete a questionnaire at the time of registration which will also include limited essential information. They will be asked to consent online prior to being directed to the main questionnaire. After completing the main questionnaire, they may be invited to take part in a 20 minute online cognitive assessment. In addition, at the end of the main questionnaire, participants will be asked whether or not they would be happy to be contacted to take part in an interview to better understand the experiences of people with persistent symptoms of COVID-19. They will also be invited to complete a follow-up survey(s) 12/18 months later, but they can say no.

A sub-sample of the symptom survey respondents will be invited to take part in an ongoing panel of people with Long COVID as part of a follow up study; those who agree will be asked to update their symptoms monthly using an app which we will develop with public and patient partners.

The question items for the REACT-LC main survey have been taken from REACT-1 and a set of validated questionnaires:

- REACT-1 subset of questions including:
  - o demographic
  - o physical symptoms
  - o pre-existing health conditions
  - o History of COVID-19 infection
  - o COVID-19 vaccinations

- EUROQL - quality of life/functioning – EQ-5D (EQ-5D (euroqol.org))
- The MRC Dyspnoea Scale (MRC Dyspnoea Scale – UKRI)
- Dyspnea-12 (D-12) Questionnaire
- MOS Sleep Scale
- The DePaul Post-Exertional Malaise Questionnaire (DPEMQ)
- Patient Health Questionnaire (PHQ-9)

Pilot stage: prior to recruiting all the participants to the main 120,000 survey we will test our processes and questionnaire through a pilot. In the pilot phase, will include 10,000 total invitations to be sent using the methods outlined for the main survey. The findings from the pilot study will determine the final sampling approach for the main survey.

Cognitive Assessment

For those consenting to take part in the online cognitive assessment, they will be directed to the assessment via a link which they can do directly after the survey or at a time convenient for them. The assessment will comprise a sequence of nine tests from the Imperial College London Brain Sciences Department broader library of validated cognitive tests that is available on their server system based on prior data showing that they can be used to measure distinct aspects of human cognition, spanning planning/reasoning, working memory, attention and emotion processing abilities, in a manner that is sensitive to population variables of interest whilst being robust against the type of device that a person is tested on.

On completing the assessment, participants will be provided with the option of receiving a summary report of their performance relative to all other people who have undertaken each of the tests, which will highlight the cognitive tests that they performed relatively highest on. This report is used as a way to motivate people to take part in the study by finding out what their cognitive strengths were. The cognitive tests included in this study can be viewed at https://gbit.cognitron.co.uk. The cognitive tests have been previously approved by the Imperial College Research Ethics Committee (17IC4009).for use in other studies looking at the impact of COVID-19 on cognitive function.

Interviews

REACT-LC participants with persistent symptoms who attend the clinical assessment centres for WP2 will be invited to complete a short form to express a willingness to be contacted about an interview. Those completing the main survey will also be asked if they are willing to be contacted. Researchers from the study team will then contact selected participants to arrange a date and time for the interview. To reduce the risk of viral transmission we propose to conduct interviews online using Zoom or MS Teams. The interviews will be recorded using a digital recorder or locally utilising the Zoom recording tool and will be transferred and stored on DIDE servers. For members of the public who do not have digital access to tools like Zoom, we will conduct and record interviews over the phone. Interviews will be transcribed verbatim, and pseudonymised, either by a member of the research team or a qualified professional using the services of UKTranscription.com, a specialist audio transcription service. There will be a confidentiality agreement in place between Imperial College London and UK Transcription, and transfer of audio and text files will be done via a secure portal.

The interview will be semi-structured using a Topic Guide. The guide will provide a general structure to the interview with prompts and probes to be used only where necessary to elucidate the participant's responses and provide greater detail. The interviews will broadly cover the patient's Long Covid journey including:

- Personal background (including medical history/health background)
- Experience of Covid-19
- Development of Long Covid
- Impact of illness on work, family, income, social life
- Services and support accessed (including patient groups)
- Experience of recovery
- Views on Long Covid more generally e.g. the role of the LC patient community, government response, challenges to support

At the end of the interview participants will be asked about whether they wish to take part in future follow up qualitative research related to this study.

Analysis of the quantitative and qualitative data from both the symptom survey and the interviews will be carried out in close collaboration with our advisory group of patients and public, who will help us with interpretation and dissemination of results.

The findings of WP1 will help us later develop patient reported outcomes (PROMS) in consultation with expert collaborators and through our patient and public partners.

All respondents will be asked to carry out a follow up questionnaire after 12/18 months. This questionnaire will be based on the main questionnaire already completed amended in the light of the analyses carried out.

Work Package 2 – Clinical Phenotyping

Sample Collection Strategy
In order to achieve the best quality samples these should be centrifuged if possible within an hour of collection, with 30 minutes tolerance (i.e.90 minutes).

The project will set up assessment centres where blood can be collected and processed with 60 minutes. Where a local laboratory able to provide these services cannot be identified then we will centrifuge the relevant tubes ourselves using our own sealed-cup centrifuges and appropriate COVID safe procedures. The samples will be transferred in a suitably temperature controlled environment to appointed laboratories daily where they will be processed and aliquoted in a timely manner. The samples will then be frozen and distributed to the various analysis laboratories as appropriate.

We will set up approximately six centres which will be located so as to maximise the likely number of participants within travelling distance but minimise the sample transport distance/cost. We do not plan to visit participants at their home to collect

samples as the extended time to spinning the sample would reduce the integrity of the samples. However, this may prove necessary for some severely Long COVID affected participants.

Samples, phenotypes and information to be collected
All participants will complete a short questionnaire at the time of registration which will also include limited essential information.

They will be asked to complete a fuller 20-25 minute questionnaire, preferably before attending the appointment.

At the appointment a signed consent will be obtained electronically. Systems used to capture the consent and phenotypes will be based on those developed by ICL and used on Airwave and other REACT studies. Some additional information may be captured such as contraindications and contextual data (pregnancy, menstruation) where relevant.

Samples of urine and up to 50ml of blood will be taken in up to six tubes from each Participant. The target is to spin the tubes within 60 minutes of needle, with a maximum of 90 minutes.

Other phenotypes to be taken are:

- Blood pressure
- Weight
- Waist circumference
- Height
- Grip strength
- Lung function assessed by Spirometry and/or exercise with pulse oximeter

The SOP will detail the procedure to be followed by centre staff in case of an abnormal BP reading.

After the appointment the participants will be reminded to complete the 15-20 minute questionnaire, if not already done, and invited to complete a 24 hour food intake questionnaire. They will be provided with the links to complete them on line later.

All assessment centres will be COVID-19 secure. They will only be attended by health staff and study participants. We will adhere to social distancing, hand washing and mask wearing according to government guidance. We provide hand sanitiser. Our staff will wear appropriate PPE. A regular cleaning schedule will be in place and each test station will be cleaned between participants. We will limit touch points, implement one way systems where appropriate and limit the number of people in a centre at any one time.

Work Package 3 – Biological determinants

Multi-omic analysis will be performed on 8,000 individuals (6,000 through REACT-GE) and 2,000 second visit samples (except for WGS) along with other biological determinants (2,000 samples at each of first and second visits).

WGS and transcriptomics: We will deliver short-read 30x germline genomes from all 8,000 REACT-LC participants using Genomics England's ISO accredited WGS Illumina pipeline. The use of Tempus tubes will produce high quality RNA template for 4,000 Illumina TruSeq Stranded Total RNA sequencing.

Proteomics: A high-throughput, rapid mass spectrometry (MS)-based proteomic screen (>2000s proteins) will be undertaken on 4,000 samples.

Metabolomics and lipidomics: Broad spectrum untargeted (discovery) metabolomics will be carried out using ultra-performance liquid chromatography MS (UPLC-MS) in the Imperial Phenome Centre.

For the targeted inflammatory lipidomics Leiden University will use ultrasensitive LC-MS/MS (>200 metabolites) to provide downstream data on regulation of cell proliferation and apoptosis via quantification of lipid signalling molecules including sphingolipid messengers, covering >40 eicosanoids (e.g. prostaglandins, leukotrienes, thromboxanes).

Immunological studies: From the second visit (subset N=400), whole blood samples (from different symptom clusters) will be analysed by flow cytometry for:

i) activation of SARS-CoV-2 specific T cells, identified by tetramer staining and expression of activation markers,
ii) activation of other T cell populations potentially representing non-specific immune activation,
iii) activation of myeloid cells, particularly those populations associated with pathology in acute disease,
iv) Research into the longer term effects of COVID-19 in non-hospitalised individuals frequency of myeloid and lymphoid cell populations.

Brain injury and neurodegeneration biomarkers: Biomarkers will be assayed on the fully automated UK DRI-supported UCL DFBL Quanterix Simoa HD-X platform employing a 4plex E assay for ultrasensitive detection of plasma NfL and GFAP peptides. A separate Simoa assay will be performed for pTau181 (4,000 samples).

Autoantibodies: The Wraith laboratory will initially analyse a cross-section of 200 of the 2,000 subset with additional inflammatory and brain injury markers, to indicate which individual patterns correlate with immune pathology. We will then measure selective autoantibodies to both confirm the association and test the longevity of autoantibody seropositivity across the 2,000 samples at two time-points. We will screen for a comprehensive panel of autoantibodies related to systemic autoimmune conditions, organ-specific autoantibodies and neuroimmunological markers for central and peripheral nervous system disorders.

Work Package 4 – Social and environmental determinants of Long COVID and outcome Analysis

To describe the natural history of Long COVID and SARS-CoV-2 infection and their sequelae, we will construct a sub-cohort comprising at least 40,000 people testing positive for the virus or antibody in REACT with consent to recontact and data linkage, along with up to 80,000 test-negative people (case-cohort design). Assuming ~25% of people testing positive report long-term symptoms (>12 weeks) we hope to recruit at least 10,000 individuals with Long COVID. We will oversample people reporting symptom duration beyond 12 and then 4 weeks in REACT-1 and REACT-2 to increase our sample of individuals with Long COVID. We will link routine primary and secondary care health data to results from the questionnaire (developed in WP1). This will enable us to quantify medium and long-term physical, mental and cognitive health outcomes of SARS-CoV-2 infection, including Long COVID. We will investigate potential social and environmental determinants to identify whether and how certain population sub-groups may be more vulnerable to Long COVID. The large scale of the study provides unprecedented opportunity to quantify the longer-term health risks following SARS-CoV-2 infection and Long COVID.

Work Package 5 – Data analysis and integration

Epidemiological analyses: First, we will undertake cluster analysis of the symptoms reported on questionnaire to refine the case definition of Long COVID and qualitative approaches to analyse the interview responses in WP1. We will use multiple and penalised regression to identify the demographic, social, environmental, and behavioural determinants of risk of Long COVID and survival extensions of these models to quantify effects of Long COVID and SARS-CoV-2 infection on a range of health outcomes among the case-cohort sample of at least 120,000 people.

Multi-omic analyses: For the nested subsample of 8,000 individuals with multiple omics data including 2,000 with repeated biological measurements, we will use penalised regression guided by stability approaches, as well as non-linear alternatives (e.g. random forests), to identify multi-omic signatures of Long COVID. To address the dimensional challenge of multi-omic integration, we will stringently prune the data and identify sets of (possibly multi-) omics measurements that co-vary and contribute to population clustering. Summary latent variables of features included in each cluster will subsequently be used as predictors in place of the original covariates. Correlation structure among disease-relevant (combinations of) omics measurements will be visualised using partial correlation networks and will inform on potential mechanisms involved. We will use data-driven versatile dimensionality reduction methods (e.g. factor analysis-based methods [41]) and similarity networks to obtain clusters of participants with similar -omics profiles. These will identify biologically-driven subtypes of Long COVID which we will then correlate with e.g. clinical and demographic data to further characterise the clusters.

Disease trajectory: To understand the natural history of persistent symptoms and omic disturbances following SARS-CoV-2 infection, we will utilise individual-based multi-state models, whereby disease progression is modelled from asymptomatic to

symptomatic and Long COVID to recovery or deterioration states. We will use a Bayesian approach to estimate the transition probabilities defining the model and simulate individual trajectories to characterise the dynamics of Long COVID progression or recovery and the contributions of selected molecular, biological, social and environmental factors.

Bioinformatic and pathway analyses: We will identify the biochemical pathways that are perturbed in Long COVID using a computational network biology approach. Through differential expression/abundance analysis we will map the expression of genes, and levels of proteins, lipids and metabolites onto the different pathways. We will assess potential drug targets that affect these dysregulated pathways e.g. by use of Molecule Activity Predictor (MAP) functionality in Ingenuity Pathway Analysis. Key molecules/pathways could then be tested in appropriate patient-cell-based or in vitro model based systems already in use in our labs. Relevant cell types for functional testing of candidate targets will be identified based on known gene expression/function of the candidate molecules/pathways.

Sample size and power: For WGS and sample size of 8,000, with genome-wide significance level of 5e-8, we could detect a relative risk of 1.11 with 80% power for SNPs with a disease allele frequency of 0.5 (1.24 for disease allele frequency of 0.05, and 1.40 for 0.95). For all omics other than WGS we could detect with 80% power odds ratios (OR) > 1.24 per omic feature (multiple-testing adjusted) comparing participants with/without Long COVID. Comparing two distinct clusters of participants with Long COVID (n=4,000), we could detect with 80% power OR>1.31 per omic feature. For agnostic analyses focusing on 2,000 Long COVID cases followed-up in time we could detect with 80% power OR>1.24 per feature, and for the targeted investigation in the followed-up samples of 5, 10, 100 signals identified at first visit, we could detect OR >1.15, 1.16, 1.20 respectively. All ORs are expressed as risk change per unit increase in the standardised omics measurement.

Data Management

A research database will be established at Imperial College London where the data will be held securely on secure servers in an ISO27001 environment managed by the School of Public Health. Study participants will be assigned a study ID and the data will be stripped of identifying information for the statistical analyses; only one or two named and designated individuals will have access to the identifying information. Individuals will not be identifiable in any publications or reports arising from the study. A parallel database will also be established at Genomics England taking advantage of their secure data management system.

Data held with Genomics England will be held in the National Genomic Research Library. The Library is a comprehensive resource that allows researchers to access samples, genomic data, and other associated health data in a de-identified format. It contains the data of tens of thousands of NHS patients which researchers use to make discoveries and help develop new treatments and medicine. Access to the Library is governed by an Access Review Committee.

Feedback Reporting of Individual Results to Participants

A feedback by email of baseline clinical measurements including limited blood test results (cholesterol and diabetes) is given to the participants. If any of the results are of clinical significance, participants are advised to consult their GP for follow up or further investigation. We do not directly inform participants' GPs of their results. Participants are not notified of any further findings that may result from future assays, analyses or other procedures that are not provided for in their feedback letter.

3.1. STUDY OUTCOME MEASURES

Primary and Secondary outcomes are:

1. Symptoms and prevalence for Long COVID
2. Identified genomic, biological pathway and -omic differences between participants who suffer from Long Covid and those who do not, amongst those who have not been hospitalised.

4. PARTICIPANT ENTRY

4.1. PRE-REGISTRATION EVALUATIONS

Participants will be selected from individuals who have taken part in one of the REACT or Airwave Studies and have agreed to be contacted for further studies. They must be aged 18+. Although most Participants will be recruited from the Participants of the various REACT studies already carried out we may add further individuals who are of particular interest to the study, who have expressed an interest to the research team in taking part and meet the selection criteria. This may include such individuals as members of the Public Advisory Groups or others who contact us because they have had Long COVID.

4.2. INCLUSION CRITERIA

For WP1(at least 120,000): Participants will be aged 18+ and will have taken part in a REACT study.

For WP2 (8,000): Participants will have had or reported having had a positive test for COVID-19 and had mild to moderate symptoms or were asymptomatic and not hospitalised for COVID-19.

Data from the 6,000 Participants of the REACT-GE study who were included in the extended phenotype collection all of whom were aged >=35.

An additional 2,000 Participants will be recruited using the same process and phenotypes as for the REACT-GE extended phenotype study except that they will be aged over 18 and < 35. Of these a target of 1,000 will be selected to have reported having Long COVID and 1,000 controls who have not. It is intended to recruit these from as close to London as possible for logistical reasons.

From these 8,000 participants a number will be selected and invited to take part in a follow up visit for 2,000 to repeat the phenotype measurements and sample collection, and to answer a further questionnaire.

It is recognised that some of the Participants with more severe Long COVID symptoms may need additional assistance for them to be able to attend one of the assessments centres and this will be provided where possible in order to facilitate their participation.

4.3. EXCLUSION CRITERIA

Aged under 18. Hospitalised by COVID-19 infection.

4.4. WITHDRAWAL CRITERIA

Participants are free to withdraw at any time without having to provide a reason. Participants wishing to withdraw should contact the participant helpline by telephone, email or in writing. Participants who withdraw will be removed from the database, and their data will not be used again in the future. However, their data will be retained as part of any analysis that has been conducted prior to their request for withdrawal.

5. ADVERSE EVENTS

5.1. DEFINITIONS

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any untoward and unexpected medical occurrence or effect that:

- **Results in death**
- **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*
- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

5.2. REPORTING PROCEDURES

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

5.3.1 Non serious AEs

All such events, whether expected or not, should be recorded.

5.3.2 Serious AEs

An SAE form should be completed and emailed to the Chief Investigator within 24 hours.

All SAEs should be reported to the **South Central - Berkshire B Research Ethics Committee** where in the opinion of the Chief Investigator, the event was:

- 'related', ie resulted from the administration of any of the research procedures; and
- 'unexpected', ie an event that is not listed in the protocol as an expected occurrence

There are no invasive procedures other than collection of a blood sample, which may result in bruising.

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all related and unexpected SAEs.

Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

Contact details for reporting SAEs
RGIT@imperial.ac.uk
CI: p.elliott@imperial.ac.uk
Please send SAE forms to: Study Coordinator: k.bennet@imperial.ac.uk
Tel: +44 20 7594 3328 (Mon to Fri 09.00 – 17.00)

6. ASSESSMENT AND FOLLOW-UP

Feedback is provided to the participants who have attended an assessment centre, by email, of baseline clinical measurements including limited blood test results (cholesterol and diabetes). If any of the results are of clinical significance, participants are advised to consult their GP for follow up or further investigation. We do not directly inform participants' GPs of their results. Participants are not notified of any further findings that may result from future assays, analyses or other procedures that are not provided for in their feedback letter.

7. STATISTICS AND DATA ANALYSIS

These aspects are covered under Work Package 5 in Section 3 – Study Design above.

Data and all appropriate documentation will be stored for a minimum of 20 years after the completion of the study, including the follow-up period.

8. REGULATORY ISSUES

8.1. ETHICS APPROVAL

The Study Coordination Centre has obtained approval from the **South Central - Berkshire B Research Ethics Committee**. The study must also receive confirmation of capacity and capability from each participating NHS Trust before accepting participants into the study or any research activity is carried out. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

8.2. CONSENT

Consent to enter the study will be sought from each participant after a full explanation has been given, an information leaflet offered and time allowed for consideration. This will be done before the participant attends the assessment centre. Signed participant consent will be obtained at the start of the assessment centre appointment on an electronic tablet using an ICL system based on systems used on the Airwave and other REACT studies. The right of the participant to refuse to participate without giving reasons will be respected. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

8.3. CONFIDENTIALITY

The Principal Investigator will preserve the confidentiality of participants taking part in the study and fulfil transparency requirements under the General Data Protection Regulation for health and care research. Data and all appropriate documentation will be stored for 20 years after the completion of the study.

The data will be held securely and processed in a Secure Enclave. This is an isolated environment within the College for the processing of health related personal data. It provides a framework that satisfies Information Governance *requirements* that come from several sources such as:

- Legislation (e.g. GDPR)
- Regulatory bodies
- Data Providers (e.g. NHS Digital)
- Imperial College (e.g. ICT).

The Secure Enclaves are compliant with the requirements of major data providers (e.g. ONS, NHS Digital and NHS Trusts), as well as flexible to incorporate additional requirements a group may be subject to. The enclaves are ISO27001 certified.

A similar secure system is in place at Genomics England where a copy of the data will also be held.

The data will be pseudonymised and used for statistical analysis as outlined elsewhere in this protocol. Where consent is given we may link data from health records to the survey data for up to 20 years. Imperial College London with Genomics England are responsible for the data analysis Imperial College London will hold personal data for up to 20 years, but this will be held securely and separated from the survey and health data.

8.4. INDEMNITY

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

8.5. SPONSOR

Imperial College London will act as the main Sponsor for this study.

8.6. FUNDING

UK Research and Innovation / National Institute for Health Research are funding this study.

It is not planned to pay any participant for taking part in the study. Participants cost of attending may be reimbursed.

8.7. AUDITS

The study may be subject to audit by Imperial College London/ Imperial College Healthcare NHS Trust under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy Frame Work for Health and Social Care Research.

9. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through Imperial College London.

10. PUBLICATION POLICY

The Investigators and representatives of the collaborating organisations will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge the study funding as detailed in Section 8.6 above. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

11. REFERENCES

[List of useful and relevant references for the study]

3. Summary of changes to protocol

First REC approved version of the Protocol: IRAS 298404 REACT-LC Protocol version 1.1 20210409

REC Amendment 01: 5 May 2021
This amendment implements four changes:

1. Deletion of the Privacy Policies as suggested at the REC Meeting
2. An amendment to enable interviews to take place at the assessment centres where practical
3. Updates to the Interview Topic Guide to include input from the Public Advisory Group
4. Minor clarification of wording of PIS

IRAS 298404 REACT-LC Protocol version 1.2 20210425

REC Amendment 02: 3 June 2021
The REACT-LC Study was set up to include the Participants aged under 35 who had been excluded from the REACT-GE Study but carries out the same clinical sampling and phenotypes. In order to optimise the use of Assessment Centres and to make recruitment easier it has been agreed that Participants aged 35 and over could also be brought into the REACT-LC Study.
IRAS 298404 REACT-LC Protocol v1.3_050422A1

REC Amendment 03: 24 September 2021
The REACT-LC Study Clinical Stream (LCC) has now recruited over 10,000 participants for their first visit to the Assessment Centre for the phenotypes and blood samples and over 80% have completed the main questionnaire. The participants were either recruited as part of the REACT-GE study (Aged 35 and over) or recruited directly into the REACT-LC study (aged under 35). We are now entering the Follow-up stage where we invite them back for a repeat visit and questionnaires. Our target is to recruit at least 2,000 follow-up participants. We have updated the documents to reflect the follow up stage of the study.
No change to Protocol

REC Amendment 04: None submitted

REC Amendment 05: 31 January 2022
This amendment is to submit an updated version of the topic guide, PIS and consent form for REACT-LC Q60. Documents to be reviewed: IRAS 298404 REACT-LC Main Qual_Topic Guide_V2.1 TRACKED_310121, IRAS 298404 REACT-LC Mail Qual_ PIS_V1_3 after NW review TRACKED_280121 and IRAS 298404 REACT-LC consent-form-v1.3 after NW review (002) TRACKED_280122
No change to Protocol

REC Amendment 06: 7 April 2022

The main survey for the REACT-Long COVID study is about to start. This involves inviting previous REACT Study 1 & 2 participants to take part in a survey and cognitive assessment. The key documents were submitted to REC and approved during the original submission. However, in the light of experience and after public consultation these have been slightly amended.
IRAS 298404 REACT-LC Protocol v1.4_060422c
Notes on Amendment 6
The main survey for the REACT-Long COVID study is about to start. This involves inviting previous REACT Study 1 & 2 participants to take part in a survey and cognitive assessment. The key documents were submitted to REC and approved during the original submission. However, in the light of experience and public involvement these have been slightly amended.
The protocol has been amended to incorporate the invitation to take part in the cognitive assessment, to slightly increase the target number of participants taking part. The selection criteria specifically include the use of the Pillar 2 linked health data to enable the best selection of participants who have not had COVID-19. More detail is added on the content of the main questionnaire which has been aligned more closely with the REACT 1 survey and other standard surveys to improve cross study analysis. We are also adding a small pilot to assess the processes and responses prior to sending the main batches of invitations.
The Participant Information Sheet has been amended based on significant input from the PPIE group and has been updated to reflect the changes in protocol. Whilst most of the text remains the same as the original version the presentation has been considerably improved.
A new set of FAQs has been prepared, focussed on this section of the study.

REC Amendment 07: 20 July 2022
As a result of running the pilot for the Work Package 1 survey we have amended the survey to shorten both the questionnaire and the cognitive function assessment, and further align the questionnaire more closely with those being used by other studies eg PHOSP. Based on the pilot response rates and latest analysis of the potential participants we have revised the likely number and characteristics of the respondents. Also, we have included in the protocol the possibility of inviting some of the consented participants to a further clinic or home visit to take replacement samples or measurements where these are missing or unusable and their information would be particularly valuable.
IRAS 298404 REACT-LC Protocol v1.6c_150722
Notes on Amendment 7
As a result of running the pilot of the large-scale survey in Work Package 1 we have revised the survey to:

- Shorten both the survey and the cognitive function assessment
- Align the questions in the questionnaire more closely with those being used by other studies.

We have removed some questions in the survey entirely and, in some cases, replaced some with either standard questions (eg EuroQL) or ones being used by other approved studies (eg PHOSP). Run time for the Cognitive Assessment is now 18 minutes, plus the instruction time. The motor control assessment has been removed.
The following documents gave been updated to reflect these changes:

- Survey

- Participant Information Sheet
- Protocol
- Invitation emails and reminders for the survey and cognitive test. A slightly different version has been created specifically for participants who have attended REACT-GE/LC assessment centre.

The Protocol has also been updated to incorporate the following:

- Based on the response rate which is indicated we expect to achieve around a total of at least 120K participants taking part which should partially compensate for the reduced numbers of responses from participants who have not had any COVID infection. However, the number of non-positive tested participants is still likely to be reduced.
- To include the possibility of inviting already consented clinical participants to a further appointment or home visit for replacement samples or measurements to be taken where these are missing or are not useable and the information would be particularly valuable to the study. These will be repeats of previous measurements, no new measurements or sample types will be taken.

4. Original statistical analysis plan

Statistical Analysis Plan (04/09/23)

The REACT-LC study ethical protocol is provided in a separate document. This document specifies the detailed plan for analysing the objective cognitive assessment data collected as part of REACT-LC and was prepared after completion of the analyses. All analyses were conducted using MATLAB Version R2022b.

Analysis Overview

1. Study aims
2. Endpoints
3. Selection criteria & sampling
4. Preprocessing
5. Time since illness analysis
6. Global cognitive differences analysis
7. Sensitivity of different cognitive domains
8. Symptom correlates of global cognitive ability
9. Objective cognitive correlates of subjective cognitive problems

1 – Study Aims

The aim of REACT-LC was to study how differences in COVID-19 factors, e.g., virus variant, illness severity and duration, vaccination status, and acute symptoms, including any interactions of such factors with pre-existing medical conditions and demographic factors, associate with persistent symptoms. This was to be achieved at large population scale within a subset of the REal-time Assessment of Community Transmission (REACT) study in England

community cohort to enable the detailed modelling of these factors and their interactions whilst accounting for confounding sociodemographic factors. The particular focus of the submitted article was to understand the basis of persistent cognitive symptoms such as ‘brain fog’ and ‘memory problems’, which are commonly reported by people who have recovered from COVID-19 and amongst people with ongoing ‘Long COVID’, by applying in this cohort a suite of computerised assessment tasks that objectively measure distinct aspects of cognitive functioning and that have previously shown sensitivity to COVID-19 in smaller scale studies.

2 – Endpoints

The primary endpoint of our analyses was a global cognitive measure (G), which is a composite score formed by taking the first factor from an exploratory factor analysis of the eight main summary scores that are output by the eight cognitive tasks that comprise the computerised assessment battery. The task designs are detailed in supplementary appendix. G was extracted as a single factor for complete datasets (i.e., where participants finished all eight tasks of the assessment) without imputation and without applying rotation (i.e., as there was only one axis in the model). G was calculated after applying linear models to adjust the raw task scores to account for association with demographic factors and pre-existing conditions, with a rank inverse transform applied to ensure a normal distribution of scores with mean=0 and standard deviation (SD) = 1 (details below).

Secondary endpoints of the study were the eight main summary scores that are outputted by the cognitive tasks. These were analysed after normative modelling and rank transformation as detailed below.

Exploratory endpoints were the additional, secondary scores that are outputted by the tasks, typically comprising mean response times and contrasts between specific task conditions, also after normative modelling and rank transformation to normality.

3 – Selection Criteria & Sampling

3.1 Sampling

The REACT programme tracked SARS-CoV-2 infection (REACT-1, 19 rounds of data collection between May 1, 2020 and March 31, 2022)[1] and antibody prevalence (REACT-2, 6 rounds between June 20, 2020 and May 25, 2021)[2] based on a series of random samples from the population in England, using the National Health Service (NHS) general practitioner register of patients (near-universal coverage) as the sampling frame. Of the 3,554,932 participants in the REACT programme, 3,099,386 were adults aged ≥18 years, of whom 2,494,309 (80.5%) consented to recontact and data linkage to their NHS health records. From these 2,494,309 adults, we invited a sample of 800,000 (32.1%) to take part in a follow-up survey of health and wellbeing[3]. They comprised: (i) all REACT-1 or REACT-2 participants with self-reported test confirmed or suspected COVID-19 and persistent symptoms ≥12 weeks (n=52,501), (ii) all REACT-1 participants who tested positive for SARS-CoV-2 (n=13,482), (iii) all REACT-2 participants who were positive for SARS-CoV-2 IgG antibodies prior to vaccination using an at-home Lateral Flow Immunoassay (LFIA) test (n=85,757), (iv) a random sample of all remaining adults (n=648,260). Invitations were emailed between August 1 and December 30, 2022. Study registration was via an online portal. Participants completed an online questionnaire (**Table S1**) collecting information on sociodemographics; COVID-19 history; frequency, severity, duration of symptoms; SARS-CoV-2 test results (Polymerase Chain Reaction (PCR) for presence of virus or LFIA for antibodies); physical and mental health. We asked about 29 symptoms both current and past or ongoing following COVID-19 including: (i) loss or change of sense of smell or taste, (ii) coryzal symptoms, (iii) gastrointestinal symptoms, (iv) fatigue, (v) respiratory or cardiac symptoms, and (vi) neurological/cognitive symptoms. On completion of the questionnaire, participants were invited to undertake the ~20-minute cognitive assessment.

3.2 – Selection criteria

Participants were included in the reported analyses if (a) they completed all the cognitive tasks and (b) their survey data were available linked via a participant code. Supplemental sensitivity analyses were also conducted on individual task scores (secondary endpoints) for all participants who had completed that specific task. This was done to evaluate any sensitivity of the results to completion bias. For consistency, survey data were provided for analysis in format as reported previously (https://doi.org/10.1101/2023.04.24.23289043) with minimal further processing. The exceptions were for hospitalization status, where for ease of evaluating effect sizes, individuals were sorted into exclusive categories, such that the more severe category was selected (i.e., intensive care > hospital stay > visited A&E). Similarly, for number of vaccinations, participants were assigned categories as either 0, 1 or >1.

4 – Preprocessing

4.1 - Cognitive Task Data

Each Cognitron task outputs a primary score, which is typically related to performance accuracy, and secondary scores, which typically relate to response times or finer grained error types. Each type of task score was sorted into a vector of participants for preprocessing. Where multiple task versions were used, preprocessing was conducted separately for each version and scores concatenated after normative modelling, e.g., to account for any variability in difficulty of stimuli/problems.

Score vectors were rank transformed onto a normal distribution with mean 0 and standard deviation 1. A linear model was then fitted that explained the transformed score vector based on a set of population variables that could putatively associate with cognitive performance in the general population plus a constant term. These included:

- Age as a categorical variable in 5-year bins, which can account for non-linear age-performance associations,
- Biological sex,
- Ethnicity,
- Index of Multiple Deprivation,
- Education level,
- Pre-existing conditions.

The residuals of the linear modelling, that is, capturing variance unexplained by these population variables, were taken forward for analysis.

Notably, our analyses had very high statistical power due to participant numbers, which means that very small associations can have significant p values. Therefore, when these pre-processed cognitive scores were used as predicted variables in the analyses described below, we evaluated effect sizes in relation to Cohen's notion as expanded by Sawilowsky, where 0.1SD=very small, 0.2SD=small, 0.5SD=medium, 0.8SD=large, 1.2SD=very large and 2.0SD=huge.

4.2 – Primary Endpoint

The global composite G was calculated using the exploratory factor analysis function in MATLAB for all participants who had completed the entire assessment battery. Specifically, a factor model was calculated across the pre-processed primary cognitive scores as described above, with model order set to one factor. The resultant vector of factor scores was rank inverse transformed onto a normal distribution with 0 mean and standard deviation 1.

5 – Time Since Illness Analysis

5.1 – Analysis Without Stratification

All participants who (a) had been recorded as having just one episode of COVID-19 illness, (b) had completed the full assessment battery and therefore a G score could be calculated, and (c) had an illness onset estimate were included in the analysis of time since illness. Participants who had more than one illness episode were not included as they would have multiple time since illness estimates. Time was calculated from estimated illness onset to the date at which the individual started the survey.

To account for any non-linear relationships between time and G, participants were sorted into consecutive 100-day long time-bin groups according to time since illness onset. A linear model was fitted explaining G from these groups as a categorical variable plus a constant term. Participants within <12 weeks since infection, where illness duration remained ambiguous, therefore fell within the first time-bin in this analysis.

5.2 – Stratification by Variant

Virus variant was defined as the dominant variant at the time of infection; therefore, time and variant are not independent. Consequently, a stratified approach was applied to evaluate whether time associated with cognitive differences while controlling for the dominant variant epoch, that is, by determine whether time since illness accounted for differences in G amongst people who were estimated to have the same virus variants (Wildtype, Alpha, Delta or Omicron). The linear model from 5.1 was refitted to these data using the same 100-day time-bins.

6 – Global Cognitive Differences Analysis

6.1 – Modelling Main Effects

A linear model was fitted to G to identify which general features of acute and chronic COVID-19 illness severity features associate with differences in global cognition. This analysis included the factor ‘duration’, which was calculated based on the episode with longest duration and had the following levels.

1. No COVID: no known history of SARS-CoV-2 infection,
2. Asymptomatic: SARS-CoV-2 infection without symptoms,
3. Resolved short COVID <4 weeks: symptoms resolved within 4 weeks,
4. Resolved short COVID ≥4 to <12 weeks: symptoms resolved within 4-12 weeks,
5. Resolved persistent symptoms post-COVID: symptoms lasted ≥12 weeks,
6. Ongoing persistent symptoms post-COVID: symptoms ≥12 weeks and ongoing.

An additional ‘unknown’ group comprising individuals <12 weeks since infection at the time of the survey was excluded from the linear model as their illness duration was ambiguous. The unknown group were all from the Omicron dominant variant epoch.

Other COVID-19 factors were virus variant (Wild Type, Alpha, Delta, Omicron), Hospital Status (A&E, Admitted, Intensive Care), Vaccine Doses (0,1, >1) and a factor accounting for any effects of having multiple infections.

For completeness, demographics and pre-existing condition features were included in this analysis as nuisance variables, although we expect these to have little relationship in the analysis of main effects as they should be accounted for in the models applied during preprocessing as described above.

6.2 – Identifying Significant Interactions

A stepwise approach was used to evaluate whether there were interactions that further accounted for differences in global cognitive score G. For example, although the normative

model accounted for age, age * acute illness features might interact to further predict G if older adults suffered greater cognitive consequences of COVID-19 even when main effects of the acute illness features were accounted for. A stepwise approach was chosen because there are many possible interaction terms and including all of them together would overfit the model. The stepwise approach employed was flexible, which unlike fixed stepwise regression avoids bias in estimates dependent on the order in which terms are evaluated. All possible 2-way interaction terms were evaluated. To determine the stability of the resultant model across selection criteria, the stepwise process was run twice, using inclusion/exclusion of terms based on either F statistics (add if $p<0.001$ remove if $p>0.1$ for the change in the sum of squared errors) or the Bayesian Information Criteria (add if <0 remove if >0.01).

6.3 – Stratification by Variant

Stratified analysis was applied to further examine whether associations as identified in 6.2 of features of acute and chronic illness duration and global cognitive performance G were comparable and statistically significant across virus variants. Specifically, linear models with features as identified in 6.2 but excluding virus variant were fitted to G scores for all cases who were from the same dominant variant epoch during their worst illness episode. The No COVID group was also included as a common reference in these four models. The ‘unknown’ duration group (<12 weeks post infection) was excluded.

7 – Sensitivity of Different Cognitive Domains

7.1 – Linear Modelling

We evaluated which aspects of cognition were most closely associated with COVID-19 illness in more detail. Linear models were fitted to predict each of the main scores from the cognitive tasks. Each model included the set of features identified in the stepwise regression in 6.2. This analysis was first run with all participants who had completed all the cognitive assessment

tasks and for whom illness duration was known. This was then repeated as a sensitivity analysis for all participants who completed at least one task to determine the sensitivity of the results to biases associated with probability of completing all tasks.

8 – Symptom Correlates of Global Cognitive Ability

8.1 – Ongoing Symptoms

Independent samples t-tests were used to explore which if any ongoing symptoms predicted differences in cognition in participants who (a) had confirmed COVID-19 and (b) had ongoing persistent symptoms >=12 weeks at the time of completing the survey. Symptoms were analysed individually. For each analysis, participants were divided into groups according to whether they had reporting having that symptom in response to the question “Indicate which persistent symptoms (lasting more than 12 weeks) you think may be linked to you having had COVID-19?” t-tests then evaluated whether the groups differed in global cognitive score G. Bonferroni adjustment for multiple comparisons was applied across the set of symptoms analysed.

8.2 – Resolved Symptoms

Independent samples t-tests were used to identify which if any acute illness symptoms predicted differences in cognitive performance in all participants who had an illness duration label (i.e., $\geq$12 weeks post infection). Symptoms were analysed individually. For each analysis, participants were divided into groups according to whether they indicated having had that symptom in response to the question “Which of the following symptoms were part of your COVID-19 illness?”. t-tests evaluated whether these groups differed in global cognitive score G. Bonferroni adjustment for multiple comparisons was applied across the set of symptoms analysed.

9 – Objective Cognitive Correlates of Subjective Cognitive Problems

Independent samples t-tests were used to identify which of the primary and secondary cognitive scores related to self-reported subjective cognitive symptoms from within the two-week period prior to completing the survey. Subjective symptoms included “difficulty thinking or concentrating (brain fog)” and “poor memory”. These symptoms did not have to relate to COVID-19; therefore, they were also reported for people in the resolved and No COVID groups. Participants were sorted into three categories for this analysis. (a) All participants who reported having ongoing persistent COVID-19. (b) All participants with confirmed COVID-19 who reported that their symptoms had resolved. (c) All participants from the No COVID group. For each symptom and category, participants were divided into groups according to whether they reported having experienced that symptom within the past two weeks. t-tests then evaluated whether the groups differed in their primary or secondary cognitive scores. Bonferroni correction for multiple comparisons was applied across the set of symptoms for each of the three categories. Pearson’s correlations were applied between the categories across the resultant estimates to determine whether their patterns of subjective cognitive symptoms vs. objective cognitive score associations were similar.

References

1. Elliott P, Whitaker M, Tang D, et al. Design and Implementation of a National SARS-CoV-2 Monitoring Program in England: REACT-1 Study. Am J Public Health 2023;113:545-54.

2. Ward H AC, Whitaker M, Davies B, Ashby D, Darzi A, Chadeau-Hyam M, Riley S, Donnelly CA, Barclay W, Cooke GS, Elliott P. Design and implementation of a national

program to monitor the prevalence of SARS-CoV-2 IgG antibodies in England using self-testing: REACT-2 Study. American Journal of Public Health In Press.

3. Atchison C J, Davies B, Cooper E, et al. LONG-TERM PHYSICAL AND MENTAL HEALTH IMPACT OF COVID-19 ON ADULTS IN ENGLAND: FOLLOW UP OF A LARGE RANDOM COMMUNITY SAMPLE. medRxiv 2023.

5. Final statistical analysis plan

Statistical Analysis Plan V3 (30/12/23)

The REACT-LC study ethical protocol is provided in a separate document. This document specifies the detailed plan for analyzing the objective cognitive assessment data collected as part of REACT-LC and was prepared after completion of the analyses. It was updated relative to version 1 to include additional analyses that were run in response to peer and statistical review. All analyses were conducted using MATLAB Version R2022b.

Analysis Overview

1. Study aims
2. Endpoints
3. Selection criteria & sampling
4. Preprocessing
5. Regression analysis testing of the primary hypothesis
6. Propensity score matching testing of the primary hypothesis
7. Sensitivity testing using stratification
8. Sensitivity of different cognitive domains
9. Symptom correlates of global cognitive ability
10. Objective cognitive correlates of brain fog and poor memory

1 – Study Aims

The aim of REACT-LC was to study how differences in COVID-19 factors, e.g., virus variant, illness severity and duration, vaccination status, and acute symptoms, including any interactions of such factors with pre-existing medical conditions and demographic factors, associate with persistent

symptoms. This was to be achieved at large population scale within a subset of the REal-time Assessment of Community Transmission (REACT) study in England community cohort to enable the detailed modelling of these factors and their interactions whilst accounting for confounding sociodemographic factors. The particular focus of the submitted article was to understand the basis of persistent cognitive symptoms such as 'brain fog' and 'memory problems', which are commonly reported by people who have recovered from COVID-19 and amongst people with ongoing 'Long COVID', by applying in this cohort a suite of computerized assessment tasks that objectively measure distinct aspects of cognitive functioning and that have previously shown sensitivity to COVID-19 in smaller scale studies.

2 – Endpoints

The primary endpoint of our analyses was a global cognitive measure (G), which is a composite score formed by taking the first factor from an exploratory factor analysis of the eight main summary scores that are output by the eight cognitive tasks that comprise the computerized assessment battery. The task designs are detailed in supplementary appendix. G was extracted as a single factor for complete datasets (i.e., where people finished all eight tasks of the assessment) without imputation and without applying rotation (i.e., as there was only one axis in the model). G was calculated after applying linear models to adjust the raw task scores to account for association with demographic factors and pre-existing conditions, with a rank inverse transform applied to ensure a normal distribution of scores with mean=0 and standard deviation (SD) = 1 (details below).

Secondary endpoints of the study were the eight main summary scores that are outputted by the cognitive tasks. These were analyzed after normative modelling and rank transformation as detailed below.

Exploratory endpoints were the additional, secondary scores that are outputted by the tasks, typically comprising mean response times and contrasts between specific task conditions, also after normative modelling and rank transformation to normality.

3 – Selection Criteria & Sampling

3.1 Sampling

The REACT program tracked SARS-CoV-2 infection (REACT-1, 19 rounds of data collection between May 1, 2020 and March 31, 2022)[1] and antibody prevalence (REACT-2, 6 rounds between June 20, 2020 and May 25, 2021)[2] based on a series of random samples from the population in England, using the National Health Service (NHS) general practitioner register of patients (near-universal coverage) as the sampling frame. Of the 3,554,932 people in the REACT program, 3,099,386 were adults aged ≥18 years, of whom 2,494,309 (80.5%) consented to recontact and data linkage to their NHS health records. From these 2,494,309 adults, we invited a sample of 800,000 (32.1%) to take part in a follow-up survey of health and wellbeing[3]. They comprised: (i) all REACT-1 or REACT-2 people with self-reported test confirmed or suspected COVID-19 and persistent symptoms ≥12 weeks (n=52,501), (ii) all REACT-1 people who tested positive for SARS-CoV-2 (n=13,482), (iii) all REACT-2 people who were positive for SARS-CoV-2 IgG antibodies prior to vaccination using an at-home Lateral Flow Immunoassay (LFIA) test (n=85,757), (iv) a random sample of all remaining adults (n=648,260). Invitations were emailed between August 1 and December 30, 2022. Study registration was via an online portal. People completed an online questionnaire (**Table S1**) collecting information on sociodemographics; COVID-19 history; frequency, severity, duration of symptoms; SARS-CoV-2 test results (Polymerase Chain Reaction (PCR) for presence of virus or LFIA for antibodies); physical and mental health. We asked about 29 symptoms both current and past or ongoing following COVID-19 including: (i) loss or change of sense of smell or taste, (ii) coryzal symptoms, (iii) gastrointestinal symptoms, (iv) fatigue, (v) respiratory or cardiac symptoms, and (vi)

neurological/cognitive symptoms. On completion of the questionnaire, people were invited to undertake the ~20-minute cognitive assessment.

3.2 – Selection criteria

People were included in the reported analyses if (a) they completed all the cognitive tasks and (b) their survey data were available linked via a participant code. The assessment software returns scores on completion of a task. It only presents people with the next task in the battery if the previous one was completed. Therefore, all people who completed the tasks had completed summary score data and were included in the analyses. Supplemental sensitivity analyses were also conducted on individual task scores (secondary endpoints) for all people who had completed that specific task. This was done to evaluate any sensitivity of the results to completion bias. For consistency, survey data were provided for analysis in format as reported previously (https://doi.org/10.1101/2023.04.24.23289043) with minimal further processing. The exceptions were for hospital attendance, where for ease of evaluating effect sizes, people were sorted into exclusive categories, such that the more severe category was selected (i.e., intensive care unit > hospital admitted > visited A&E > non-hospital based medical assistance). Similarly, for number of vaccinations, people were assigned categories as either 0, 1 or >1.

4 – Preprocessing

4.1 - Cognitive Task Data

Each Cognitron task outputs a primary score, which is typically related to performance accuracy, and secondary scores, which typically relate to response times or finer grained error types. Each type of task score was sorted into a vector of people for preprocessing. Where multiple task versions were used, preprocessing was conducted separately for each version and scores concatenated after normative modelling, e.g., to account for any variability in difficulty of

stimuli/problems.

To ensure normality and mitigate outliers, score vectors were rank transformed onto a normal distribution with mean 0 and standard deviation 1. A linear model was then fitted that explained the transformed score vector based on a set of population variables that could putatively associate with cognitive performance in the general population plus a constant term. These included:

- Age as a categorical variable in 5-year bins, which can account for non-linear age-performance associations,
- Biological sex,
- Ethnicity,
- Index of Multiple Deprivation,
- Education level,
- Pre-existing conditions as binary variables.

The residuals of the linear modelling, that is, capturing variance unexplained by these population variables, were taken forward for analysis.

4.2 – Primary Endpoint

The global composite G was calculated using the exploratory factor analysis function in MATLAB for all people who had completed the entire assessment battery. Specifically, a factor model was calculated across the pre-processed primary cognitive scores as described above, with model order set to one factor. The resultant vector of factor scores was rank inverse transformed onto a normal distribution with 0 mean and standard deviation 1.

4.3 – Vaccination Status

We used vaccination status labels provided from a previous study focused on self-reported

outcomes[3]. There, participants were labelled as (a) unvaccinated, (b) one dose, (c) two or more doses. We accounted for a lag period of 14 days for the vaccine dose to be effective and defined "valid" vaccination status as the number of doses having been administered 14 days or more days prior to test date. In case of multiple infections, we considered the date of the infection which yielded the most prolonged manifestation. Dates were taken from linked healthcare records. Where dates were not available from records, self-report was cross referenced to confirm lack of vaccination. Of these, 0.04% self-reported vaccination dates for the 1st vaccine dose and 0.31% self-reported vaccination dates for subsequent doses were input based on self-report. Where vaccination dates from self-reported data and from linked data were available, these matched at 81.9% when allowing a 7-day margin of error.

5 - Regression analysis testing of the primary hypothesis

5.1 – Plotting global cognitive score G in relation to infection date

All people who (a) had been recorded as having just one episode of COVID-19 illness, (b) had completed the full assessment battery and therefore a G score could be calculated, and (c) had an illness onset estimate were included when plotting cognitive scores in relation to infection date. Time was calculated as estimated illness onset minus 01/01/2020.To account for any non-linear relationships between infection time and G, people were sorted into consecutive 100-day long time-bin groups. A linear model was fitted explaining G from these groups as a categorical variable plus a constant term. Covariates that were proxies for and likely mediates of COVID-19 illness severity varied across time. Therefore, we also plotted G across the time-bins after regressing out illness duration and hospital attendance, and virus variant period and vaccination status (see definitions below), for the single infection sub-group.

5.2 - Main regression analysis testing primary hypothesis

5.2.1 – Modelling Main Effects

A linear model was fitted to G to identify which general covariates of acute and chronic COVID-19 illness severity associate with differences in global cognition. This analysis included the factor 'duration', which was calculated based on the episode with longest duration and had the following levels.

1. No COVID: no known history of SARS-CoV-2 infection,
2. Asymptomatic: SARS-CoV-2 infection without symptoms,
3. Resolved short COVID <4 weeks: symptoms resolved within 4 weeks,
4. Resolved short COVID ≥4 to <12 weeks: symptoms resolved within 4-12 weeks,
5. Resolved persistent symptoms post-COVID: symptoms lasted ≥12 weeks,
6. Ongoing persistent symptoms post-COVID: symptoms ≥12 weeks and ongoing.

An additional 'unknown' group comprising people <12 weeks since infection at the time of the survey was excluded from the linear model as their illness duration was ambiguous. The unknown group were all from the Omicron dominant variant epoch.

Other COVID-19 factors were virus variant period (defined as the period where Wildtype, Alpha, Delta or Omicron was the most prevalent strain in England), Hospital Attendance (A&E, Admitted, Intensive Care), Vaccine Doses (0,1, >1) and a factor accounting for any effects of having multiple infections.

For completeness, demographics and pre-existing conditions were included in this analysis as nuisance variables, although we expected these to have little relationship in the analysis of main effects as they should be accounted for in the normative models applied during preprocessing as described above.

5.2.2 – Identifying Significant Interactions

A stepwise approach was used to evaluate whether there were interactions that further accounted for differences in global cognitive score G. For example, although the normative model accounted for age, age * acute illness covariates might interact to further predict G if older adults suffered greater cognitive consequences of COVID-19 even when main effects of the acute illness covariate were accounted for. A stepwise approach was chosen because there are many possible interaction terms and including all of them together would overfit the model. The stepwise approach employed was flexible, which unlike fixed stepwise regression avoids bias in estimates dependent on the order in which terms are evaluated. All possible 2-way interaction terms were evaluated.

We use the MATLAB stepwise function, which by default applies different thresholds for adding and removing covariates from the model. The rationale for having two thresholds is based on the principle of parsimony or model simplicity; the goal is to build a model that is as simple as possible (with fewer covariates) but still captures the essential relationships in the data. If there is only one threshold, it can lead to either too many variables being added (making the model overly complex) or too few variables being retained (potentially missing important predictors).

More specifically:

- Adding Threshold: The criterion for deciding whether a new covariate should be added to the model is typically more stringent to avoid overfitting - i.e., avoiding allowing the model to become too complex and start capturing noise in the data rather than the true underlying trends.
- Removing Threshold: The removal threshold helps in simplifying / reducing overfitting but is deliberately less strict to avoid losing potentially useful information. That is, once a covariate is included in the model, there's a tendency to keep it unless there's strong evidence that it no longer contributes significantly.

To determine the stability of the resultant model across selection criteria, the stepwise process was run twice, using inclusion/exclusion of terms based on either F statistics (add if $p<0.001$

remove if p>0.1 for the change in the sum of squared errors) or the Bayesian Information Criteria (add if <0 remove if >0.01). These are MATLAB's default adding and removing thresholds.

.

5.3 3. Interpretation of effect sizes

Notably, our analyses had very high statistical power due to participant numbers, which means that very small associations can have significant p values. Therefore, when these pre-processed cognitive scores were used as predicted variables in the analyses described below, we evaluated effect sizes in relation to Cohen's notion as expanded by Sawilowsky[4], where 0.1SD=very small, 0.2SD=small, 0.5SD=medium, 0.8SD=large, 1.2SD=very large and 2.0SD=huge. Comparison was made to IQ points by converting to the conventional 15-point SD scale. Standard deviation cut-offs also were applied to quantify number of people falling within ranges that may be considered clinically relevant. We defined G<-1SD to indicate below average cognitive ability, G<-2SD to indicate moderate cognitive impairment and G<-3 to indicate profound cognitive impairment.

6. Propensity score matched testing of the primary hypothesis

Residual biasing can affect model estimates. Therefore, propensity score matching (PSM) was performed to align groups in terms of their sociodemographic compositions. PSM analyses all report parameter estimates with 95% confidence intervals to enable comparison to the above regression model estimates.

More specifically, we used PSM re-estimate mean differences between factor levels while controlling for potentially confounding variables. For any given contrast, people were sorted into the two categories to be contrasted. A binomial logistic regression model was fitted predicting those categories from the potentially confounding variables. Each participant was allocated a propensity score based on the fitted logistic regression model. They were then matched between groups according to the propensity score within fixed probability ranges. The goodness of the probability matching was evaluated as the mean difference of the probability score in standard deviation units. The width of the probability range was treated as a hyperparameter, being adjusted until a good match (defined as <0.1SD difference in propensity scores) or smaller was achieved while retaining as many observations as possible. Mean differences in cognitive score with 95% confidence intervals were calculated between the propensity matched groups. The following propensity matching analyses were performed.

- People from the no-COVID group were matched independently to each cell within the illness-duration * variant period factorial, with demographic factors (age in 5-year bins, sex, education level, social deprivation, ethnicity), number of pre-existing conditions and subjective cognitive symptoms included as predictors in the propensity model.
- The above model was rerun including presence of subjective cognitive symptoms (self-reported Brain Fog or Problems with Memory in the past two weeks) as a predictor in the propensity model.
- People from the no-COVID group were matched independently to each of the hospital groups, with demographic factors and pre-existing conditions included in the propensity model.
- People who did not receive medical support during their longest COVID-19 illness were matched independently to each of the hospital groups, with demographic factors and pre-existing conditions included in the propensity model.
- People from the no-COVID group were matched to people who had not attended the hospital during their longest COVID-19 illness but who had sought medical assistance

outside of the hospital system (e.g., called 111, had a GP appointment or gone to a walk-in center), with demographic factors, pre-existing conditions, variant period, vaccination status and illness-duration in the propensity model.

- People who did not receive medical support during their longest COVID-19 illness were matched to people who had not attended the hospital during their longest COVID-19 illness but who had sought medical assistance outside of the hospital system, with demographic factors, pre-existing conditions, variant period, vaccination status and illness-duration in the propensity model.
- People who had one or more than one vaccination prior to their longest COVID-19 illness were matched independently to people who were not vaccinated prior to their longest COVID-19 illness, with demographic factors, variant period and pre-existing conditions in the propensity model.
- People who had two valid AstraZeneca vaccinations prior to their longest COVID-19 illness were matched to those who had two valid Pfizer vaccinations, with demographic factors, variant period and pre-existing conditions in the propensity model.
- People who had multiple COVID-19 infections were matched with those who had a single COVID-19 infection, with demographic factors and pre-existing conditions and vaccination status in the propensity model.
- The above analysis was rerun including variant, illness duration, vaccination status and hospital attendance at longest infection in the propensity model.

7. Sensitivity testing using stratification

Sampling and completion biases can distort model estimates. Therefore, the following sensitivity analyses were performed to evaluate the robustness of the main results in terms of pattern and magnitude of estimates when excluding different sub-sets of the data. Sensitivity analyses all report parameter estimates with 95% confidence intervals to enable comparison to the main regression model estimates.

7.1 Confounding of main effects by vaccination status

The regression model with parameters as identified in the stepwise process described in 5.2.2. was rerun excluding people who had one or more valid COVID-19 vaccinations prior to their longest illness. It was then rerun including either only those people who did or those who did not report experiencing Brain Fog or Poor Memory within the past two weeks to control for subjective cognitive problems.

7.2 – Stratification by Variant

Stratified analysis was applied to examine whether associations as identified in 5.2.2 of covariates of acute and chronic illness duration and global cognitive performance G were comparable and statistically significant across virus variants. Specifically, linear models with covariates as identified in 5.2.2 but excluding virus variant were fitted to G scores for all cases who were from the same dominant variant epoch during their longest illness episode. The No-COVID group was also included as a common reference in these four models. The 'unknown' duration group (<12 weeks post infection) was excluded.

7.3 – Impact of missing education data

A subset of people did not report their education level, which could affect model estimates due to being used in the normative model. Therefore, stratified analysis was applied to examine whether

associations as identified in 5.2.2 of covariates of acute and chronic illness duration and global cognitive performance G were comparable and statistically significant when analyzing only people for whom education level information was available.

8 – Sensitivity of Different Cognitive Domains

8.1 – Linear Modelling

We evaluated which aspects of cognition were most closely associated with COVID-19 illness in more detail. Linear models were fitted to predict each of the main scores from the cognitive tasks. Each model included the set of covariates identified in the stepwise regression in 5.2.2 This analysis was first run with all people who had completed all the cognitive assessment tasks and for whom illness duration was known. This was then repeated as a sensitivity analysis for all people who completed at least one task to determine the sensitivity of the results to biases associated with probability of completing all tasks.

9 – Symptom Correlates of Global Cognitive Ability

9.1 – Ongoing Symptoms

We explored which if any ongoing symptoms predicted differences in cognition in people who (a) had confirmed COVID-19 and (b) had ongoing persistent symptoms >=12 weeks at the time of completing the survey. Symptoms were analyzed individually. For each analysis, people were divided into groups according to whether they had reporting having that symptom in response to the question “Indicate which persistent symptoms (lasting more than 12 weeks) you think may be linked to you having had COVID-19?” Mean differences with 90% C.Is were used to evaluated whether the groups differed in global cognitive score G.

9.2 – Resolved Symptoms

We identified which if any acute illness symptoms predicted differences in cognitive performance in all people who had an illness duration label (i.e., ≥12 weeks post infection). Symptoms were analyzed individually. For each analysis, people were divided into groups according to whether they indicated having had that symptom in response to the question "Which of the following symptoms were part of your COVID-19 illness?". Mean differences with 90% C.I.s were applied between groups with vs. without each symptom.

10 – Objective cognitive correlates of brain fog and poor memory

We examined which of the primary and secondary cognitive scores related to self-reported subjective cognitive symptoms from within the two-week period prior to completing the survey. Subjective symptoms included "difficulty thinking or concentrating (brain fog)" and "poor memory". These symptoms did not have to relate to COVID-19; therefore, they were also reported for people in the resolved and No COVID groups. People were sorted into three categories for this analysis. (a) All people who reported having ongoing persistent COVID-19. (b) All people with confirmed COVID-19 who reported that their symptoms had resolved. (c) All people from the No COVID group. For each symptom and category, people were divided into groups according to whether they reported having experienced that symptom within the past two weeks. Mean differences with 95% confidence intervals were calculated to evaluate whether the groups differed in their primary or secondary cognitive scores. Pearson's correlations were applied between the categories across the resultant estimates to quantify the degree to which their patterns of subjective cognitive symptoms vs. objective cognitive score associations were similar.

References

1. Elliott P, Whitaker M, Tang D, et al. Design and Implementation of a National SARS-CoV-2 Monitoring Program in England: REACT-1 Study. Am J Public Health 2023;113:545-54.
2. Ward H AC, Whitaker M, Davies B, Ashby D, Darzi A, Chadeau-Hyam M, Riley S, Donnelly CA, Barclay W, Cooke GS, Elliott P. Design and implementation of a national program to monitor the prevalence of SARS-CoV-2 IgG antibodies in England using self-testing: REACT-2 Study. American Journal of Public Health In Press.
3. Atchison CJ, Davies B, Cooper E, et al. Long-term health impacts of COVID-19 among 242,712 adults in England. Nat Commun 2023;14:6588.
4. Sawilowsky S. New Effect Size Rules of Thumb. Journal of modern applied statistical methods 2009.

6. Summary of changes to statistical analysis plan

We updated the statistical analysis plan to reflect changes implemented when addressing two rounds of reviewer and editor comments. The following changes were made.

Round 1.

- Inclusion of details of the propensity score matching analyses, which were added on request to further corroborate the results of the original main regression analysis.
- Inclusion of details of sensitivity analyses where participants subsets were excluded to examine the robustness of the findings, e.g., across vaccinated and unvaccinated participants and between dominant variants periods.
- Explicitly stating where analyses tested the primary or secondary hypotheses.
- Removal of multiple comparisons correction and replacement with point estimates with 95% confidence intervals for all secondary analyses.
- Addition of specification of how effects were to be interpreted in terms of standard effect size ranges and standard deviation performance cut-offs.
- Addition of new analyses focused on vaccine number of doses and type.

Round 2.

- Addition of comparison of effect sizes to standard 15-point Intelligence Quotient scale effect sizes, on reviewer request.
- Removal of participants with more than one infection from the analysis of global cognitive score (G) in relation to infection date.
- Changing of U.K. to U.S. English spelling