BMJ Open

SARS-CoV-2 humoral immune response in patients with cardiovascular risk factors: the COmmunity Cohort Study protocol

Vivienne C Bachelet ,[1] Ignacio Silva-Ayarza ,[1,2] Francisca J Lizana,[1] Patricio Gomolán,[1] Diego Silva-Villalobos ,[1] María S Navarrete [1]

To cite: Bachelet VC, Silva-Ayarza I, Lizana FJ, *et al*. SARS-CoV-2 humoral immune response in patients with cardiovascular risk factors: the COmmunity Cohort Study protocol. *BMJ Open* 2022;**12**:e061345. doi:10.1136/bmjopen-2022-061345

► Prepublication history for this paper is available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2022-061345).

Received 24 January 2022
Accepted 28 April 2022

[1]Escuela de Medicina, Universidad de Santiago de Chile, Santiago de Chile, Chile
[2]Department of Infectious Diseases, Hospital Barros Luco Trudeau, Santiago de Chile, Chile

Correspondence to
Dr Vivienne C Bachelet;
vivienne.bachelet@usach.cl

ABSTRACT

Introduction The COmmunity Cohort Study aims to determine, after natural exposure to SARS-CoV-2 or anti-SARS-CoV-2 vaccines deployed in Chile to prevent COVID-19 in the context of the current pandemic, the strength and duration of detectable neutralising antibodies in adult ambulatory primary care patients with cardiovascular risk factors.

Methods and analysis We will set up a community-based longitudinal, prospective cohort study. The study will be conducted in two public outpatient clinics located in the southern district of Santiago, Chile. We expect to begin recruitment in the second quarter of 2022. Each patient will be followed up for at least 1 year after inclusion in the cohort. The eligible population will be adult patients registered in the Cardiovascular Health Programme. Exposure in this study is defined as any event where participants have contact with SARS-CoV-2 antigens from natural exposure or vaccination. The primary outcomes are seroconversion and strength and duration of the neutralising IgG antibodies to SARS-CoV-2. Secondary outcomes are any COVID-19-related event or intercurrent morbidities or death. Data will be collected by extracting serial blood samples and administering a questionnaire at the first face-to-face contact and monthly follow-up time points. The sample size estimated for this study is 1060. We will characterise the cohort, determine the seroprevalence rate of neutralising antibodies at baseline and determine the rates of antibody decline using a longitudinal mixed-effects model.

Ethics and dissemination The Scientific Ethics Committee of the South Metropolitan Health Care Service approved the study protocol (Memorandum No 191/2021). We will present the results in two peer-reviewed publications and national and international professional and academic meetings. We will organise seminars with relevant stakeholders and hold town hall meetings with the local community. We will set up a COmmunity Cohort Study website at www.communitystudy.cl to disseminate the study purpose, research team and milestones.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study will provide longitudinal prospective humoral immune response measurements to SARS-CoV-2 and its vaccines in a medium-sized cohort of community-dwelling individuals with cardiovascular risk factors as they are exposed to the virus or the vaccines.
⇒ Neutralising antibodies will be measured with an emergency use-licensed assay with a known correlation with live virus neutralising antibody assays.
⇒ The major limitation of this study is the 1-year follow-up, which we hope to extend as we obtain further funding.
⇒ Our study has a risk of attrition bias that we will address with close follow-up of participants and strong patient and community engagement.

INTRODUCTION

Recovery from many viral infectious diseases is followed by a period of infection-induced immune protection against reinfection. This phenomenon is widely observed in many respiratory viral infections, including endemic coronaviruses, for which acquired immunity wanes over time, making individuals susceptible to reinfection.[1–3]

SARS-CoV-2 has some similarities with the other coronaviruses with pandemic behaviour that cause severe acute respiratory syndromes, such as SARS and MERS. They have a common zoonotic origin, a similar transmission route, and worse clinical outcomes in older people and individuals with underlying health conditions.[4 5] Following infection, the humoral immune response can be evaluated with total antibodies, specific antibodies (IgA, IgM and IgG) and neutralising antibodies. Long-term immunity depends on the presence of sensitised memory B cells and CD4+ and CD8+ T cells, which are much more challenging to measure given the complex laboratory methods required.[6–9]

While 95% of people infected with SARS-CoV-2 develop specific antibodies in the first weeks after infection,[10] the strength and duration of this humoral response and its

correlation with protection against the disease have yet to be established.[7 11] Thus, the anti-SARS-CoV-2 immune response cannot only be determined with broad-based serological testing—measurements of the humoral response should also include an assessment of protection against the disease.[12 13]

The relationship between antibody titres, the severity of infection, and the risk of infection and reinfection, as well as the absence of seroconversion in some individuals and the role of cellular immunity in the immune response against COVID-19, are being actively studied.[1 14–16] Some studies have shown that the greater the severity of the infection, the greater the magnitude of the humoral immune response[17 18]; that the probability of reinfection is significantly lower in seropositive individuals[19–22]; and that vaccination after SARS-CoV-2 infection increases the magnitude of this response.[19 23] Moreover, constantly emerging variants of the virus are also being looked into due to increased transmissibility and resistance to vaccine-induced humoral immunity.[24–27]

Neutralising antibodies target the receptor-binding domain of SARS-CoV-2 protein S and prevent its interaction with the host ACE 2.[9 28–30] The quantification of neutralising antibodies makes it possible to define an immune threshold above which individuals are likely to be protected while below they are likely to be susceptible.[9 30–32] The viral neutralisation test is the gold standard for measuring neutralising antibodies. This test can be done with a functional infectivity assay, such as the plaque reduction neutralisation test.[30 33] Pseudoviral vectors are being introduced for anti-S neutralising antibody assays in biosafety level 2 laboratories to avoid the live virus test that requires a level 3 facility.[9 29–31] Additionally, commercial enzyme immunoassays or chemiluminescent immunoassays have been introduced that determine the serum neutralising activity directed to the SARS-CoV-2 spike protein.[9 32 34 35]

While these tests are useful for assessing the humoral immune response to infection or vaccination, correlations of protection need to be established. Neutralising antibodies have been isolated in 99% of individuals previously infected with SARS-CoV-2,[10] and it is postulated that their presence would correlate strongly with protection against infection and reinfection in individuals with no history of COVID-19 but who have been previously vaccinated.[9 19 28 33 36–38]

The duration of immunity to SARS-CoV-2, whether acquired by disease or vaccination, is also under study. Most people have levels of IgG and neutralising antibodies that are detectable for at least 6 months after infection,[7 9 19 39 40] depending on, among other factors, age and severity of infection,[3 7 10 14 16 41] the immune status of the individual,[3 41 42] infection by emerging genetic variants,[3 9 24–26 42–50] vaccination status,[3 9 15 19 42 47] the vaccine administered,[3 37 38 41 42 48–52] and the deployed dosing schedule for primary vaccination and booster doses.[3 9 36 38 49–55]

Different publications have shown that a higher cardiovascular risk from conditions such as arterial hypertension,[4 56–58] diabetes,[57–61] dyslipidaemia,[59] heart failure[58 62 63] and coronary heart disease[56 57 62 64 65] is associated with a higher risk of severe COVID-19 and death. Smoking has also been identified as a cardiovascular risk factor associated with greater risk of COVID-19.[66 67] Other factors associated with worse prognosis are advanced age, male sex, obesity, chronic obstructive pulmonary disease and other pulmonary pathologies, chronic kidney disease, liver failure, cancer, history of solid organ and haematopoietic precursor transplantation, as well as socioeconomic status and being part of under-represented racial/ethnic groups.[59 61 68]

Since the publication of the first reported cases in Wuhan, China, in December 2019, the disease caused by SARS-CoV-2 has resulted in over 336 million cases and 5.6 million deaths worldwide,[69] making it one of the most devastating pandemics in recent times. To date, more than 9.8 billion doses of SARS-CoV-2 vaccines have been administered globally, with more than 60% of the world population inoculated with at least one dose.[70] In Chile, to date, more than 90% of the population has been vaccinated with at least two doses and 60% with booster doses.

Under the name of the 'COmmunity Cohort Study', where the 'CO' stands for COVID-19, we aim to determine, after natural exposure to SARS-CoV-2 or anti-SARS-CoV-2 vaccines deployed in Chile to prevent COVID-19 in the context of the current pandemic, the strength and duration of detectable neutralising antibodies in adult ambulatory primary care patients with cardiovascular risk factors.

METHODS AND ANALYSIS

The methods of this study were designed in concordance with the WHO Unity Studies[71] and are reported following the Strengthening the Reporting of Observational Studies in Epidemiology statement for cohort studies.[72]

Study design

We will set up a community-based longitudinal, prospective cohort study.

Setting

The study will be conducted in two public, primary care, outpatient clinics located in the municipality of Pedro Aguirre Cerda in the southern district of Santiago, the capital of Chile. We expect to begin recruitment in the second quarter of 2022 and conclude after 6 months. Both outpatient clinics provide care for the population within the family health model framework as per ministerial technical guidance.[73]

Pedro Aguirre Cerda municipality has a population of 101 174 according to the most recent national census (2017), with an average age of 38.3 years. According to the National Household Survey,[74] in 2020, 11.7% of this municipality's population was classified as poor by income and the 2020 Social Priority Index rated it as having a medium-high social priority.[75]

Participants

The eligible population will be adult patients registered in the Cardiovascular Health Programme. This programme is guided by the national Ministry of Health and seeks to reduce the incidence of cardiovascular events in the catchment population by implementing primary and secondary cardiovascular risk factor preventive actions in primary care.[76] Patients are referred to this programme if one or more of the following conditions or risk factors are present: atherosclerotic cardiovascular diseases (coronary artery disease, stroke, peripheral arterial disease, aortic atherosclerosis, kidney vascular disease, carotid artery disease), high blood pressure, type 2 diabetes mellitus, dyslipidaemia and smoking.

Participants will be recruited in each outpatient clinic as they come in for their ambulatory visits or to pick up prescriptions from the pharmacy. Clinic personnel will provide patients with information materials on the study and prompt them to contact the recruitment office of each site.

We will include patients aged 18 years or older, without serious concomitant conditions and clinically stable, with reasonable willingness to adhere to the follow-up protocol, and who voluntarily give informed consent. There will be no restrictions regarding previous symptomatic COVID-19 or vaccination status.

Each patient will be followed up for at least 1 year after inclusion in the cohort. We will seek funding during 2022 to extend the follow-up for more than 1 year.

Variables

Exposure in this study is defined as any event where participants have contact with SARS-CoV-2 antigens from natural exposure or vaccination. The primary outcomes are seroconversion and strength and duration of the neutralising IgG antibodies to SARS-CoV-2. Secondary outcomes are any COVID-19-related event or intercurrent morbidities or death.

At baseline, sociodemographic variables (age, gender, occupation, educational level, among others) and clinical data (vaccination status, number of doses received, platform and commercial name of the vaccine or vaccines, previous COVID-19 events, cardiovascular morbidity, other known risk factors) will be collected.

Any patient who presents with a COVID-19-related event during the study follow-up period will be further characterised regarding other contributing factors to contextualise the events in more detail.

Data sources and measurements

Primary data will be collected by extracting blood samples and administering a questionnaire at the first face-to-face contact with the participant and at various follow-up time points (figure 1). Every 3 months, participants will either be approached in their homes or get an appointment in their outpatient clinic for the follow-up blood sample extractions. Overall, each participant will be contacted face-to-face four times during the study period: once for the baseline measurements and three follow-up measurements to draw blood samples. Participants will be contacted monthly by study personnel by telephone

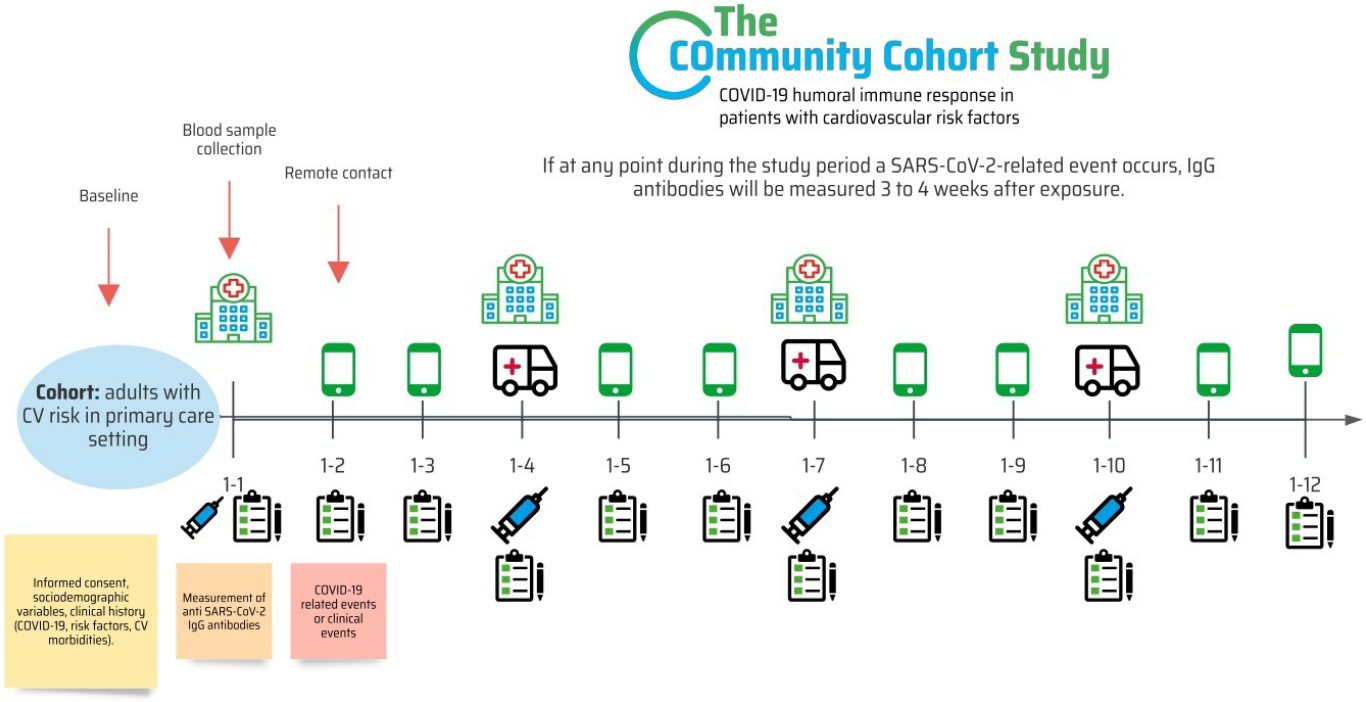

Figure 1 Frequency and modality of measurements and follow-up during the 1-year study period for the cohort. Source: prepared by the authors. CV, cardiovascular.

or WhatsApp, and their COVID-19 health status will be monitored, including any related events. Figure 1 shows the frequency and modality of contacts over the 1-year follow-up period.

Antibodies will be measured using the Atellica IM SARS-CoV-2 IgG (sCOVG) assay. If a COVID-19-related event occurs or the participant's vaccination status changes in any way, an additional measurement will be done 3–4 weeks post-exposure. A blood sample will be collected through phlebotomy from each study subject and processed by an IM Atellica Analyzer in BioNet, the reference laboratory participating in this study. The result will be informed as a data point in a measuring interval from 0.5 to 150.

The sCOVG assay is an in vitro diagnostic test that uses chemiluminescent immunoassay for the qualitative and semiquantitative detection of IgG antibodies to SARS-CoV-2 in human serum and plasma including neutralising antibodies. The results are reported as non-reactive (negative) when an index is less than 1 and reactive (positive) when above or equal to 1. Numerical results are reported for samples with values between 1 and 150 index, while results above 150 are reported as above 150 index or below 3000 index. According to Public Health England, the assay gave a specificity of 100% (95% CI: 98.9% to 100.0%) and a sensitivity of 78.3% (95% CI: 66.7% to 87.3%) ≥21 days after symptom onset.[77] The manufacturer reports a sensitivity of 96.41% (95% CI: 92.74% to 98.44%) for samples ≥21 days post-RT-PCR confirmation and a specificity of 99.64%–99.99% for apparently healthy individuals.[78] The Federal Drug Administration approved the assay in July 2021 for emergency use.[79]

Three paper-based data collection forms will be administered by study site personnel at baseline after the participant has signed the informed consent. First, a form will gather the participant's full contact details and social networks, including contact details of a close family member, neighbour or friend, to ensure contactability for the duration of the study follow-up period. This form will not be anonymised and will include the unique sequential folio number of the signed informed consent. Second, a short sociodemographic instrument with the unique folio number will be administered. Third, a clinical event form will inquire on COVID-19-related events, vaccinations—including platform and brand name, cardiovascular risk factors, and any other clinical event or morbidity deemed important to register at this stage. Site personnel will input the information each day into an electronic form. Contact details will be uploaded into a Google sheet shared and accessed only by the study site personnel and the field coordinators. Conversely, the sociodemographic and clinical data extraction forms will be inputted into REDCap for later analysis using only the unique folio number, thus precluding identification of patients by data analysts.

Participants will be followed up monthly by telephone to inquire about their COVID-19 status, including vaccines and booster shots, close contact with patients with COVID-19 and any other relevant information of this sort. This will be done using the same clinical event form administered at the initial contact. Field personnel will regularly contact participants to schedule the blood sample drawing.

Strategies to minimise bias

There are three possible sources of bias in this study: selection, measurement and attrition.

Selection bias may occur if enrolment rates are low. Stated in other words, if many eligible participants refuse to enrol in the study, the resulting cohort could be different from the population they were drawn from in substantive ways, thus potentially introducing bias in the results. To minimise selection bias, we will work closely with each site's healthcare workers, who will help us enrol participants. Likewise, we will engage local patient associations and the community to promote awareness of our study and thus facilitate willingness to participate.

We deem the risk of measurement bias to be low in our study, given that the detection of antibodies to SARS-CoV-2 in serum is an objective measurement, straightforward, automated and performed by an external laboratory. The personnel in charge of processing the samples will not know the exposure status of the participants. All samples will be anonymised. The items included in the questionnaires will be straightforward, and no other measurements will be done. All field personnel will be previously trained.

Attrition bias is always a risk in studies involving an extended follow-up of participants. An essential step to ensure adherence to the study is the informed consent process. We will recruit participants coming to their medical appointments or retrieving medication from the pharmacy unit. Recruitment will be done with sufficient time to explain the study process, clarify any doubts, communicate the importance of the research being done, all in line to ensure a willing and open mindset towards the study.

Study size

We plan to recruit a sample of 1060 patients over a period of 6 months. We did not base this number on a formal sample size calculation for two reasons. First, at the time of grant proposal writing, which still holds true, the seroprevalence rates were irregular and strongly dependent on pandemic waves and country vaccination campaign characteristics such as timing, number of doses and products being rolled out. Second, the available funds for this project were capped at a sum that made it impossible to increase the sample size further. Notwithstanding the former considerations, we believe that this sample size will allow us to estimate seroprevalence with good precision. According to the Unity Studies of the WHO,[71] a sample of 300 has an expected margin of error of 4%. Similarly, we calculate a margin of error of 1% with a sample of 1000 at an expected seroprevalence of 3% and a confidence level of 95%.

Statistical analysis

Due to the constant and dynamic shifts in the COVID-19 epidemiology, from new vaccines being introduced to booster doses of vaccines being rolled out to the emergence of new variants, it is difficult to predict any outcome; hence, planning a statistical analysis is challenging. Furthermore, the unvaccinated population (natural controls) decreases as vaccine coverage increases. Finally, a drawback for the analysis plan arises from the inadequate capacity to distinguish between vaccine-induced or infection-induced antibodies, as this depends on the type of vaccine and assay used.

Keeping in mind this context, we expect to be able to report the following results:

1. Participant flow: number of eligible patients invited to participate, number of participants effectively included in the study, number of participants who dropped out of the study prematurely (with reasons), number of participants included in the principal and secondary analyses.
2. We will do a descriptive characterisation of the baseline cohort demographics and clinical and vaccine history. The seroprevalence rate of neutralising IgG antibodies will be determined for the entire cohort initially and subsequently for subpopulations by age, vaccination status, cardiovascular risk and any other pertinent subgrouping.
3. For the principal analysis, we will provide:
   - Graphic and descriptive statistics of the observed changes in serum neutralising antibody levels for the different exposed groups, at different time points (calendar time for all participants, and time-to-event for participants who experienced an exposure event: vaccination or COVID-19).
   - Rates of antibody decline (or half-life, if possible) estimated using a longitudinal mixed-effects model. The 95% CIs and IQRs will be calculated for the estimated rates. The fixed-effects model will consider age, gender, smoke habits and cardiovascular risk factors.
4. For the secondary analysis, we will estimate:
   - Incident COVID-19 clinical events occurring during the study analysed by severity and other clinical and epidemiological features, along with neutralising antibody levels measured at 3–4 weeks after the COVID-19 event.
   - Vaccine exposure events occurring during the study will be tabulated and analysed by type of vaccine and other clinical and epidemiological features, along with neutralising antibody levels measured close to the time of vaccine administration.
   - Other post hoc analyses may be performed driven by the concurrent pandemic developments.
5. When applicable, missing data regarding key variables will be reported and handled by appropriate statistical imputation methods. Diagnostic checks of model assumptions will be done and reported as supplemental material.

We will use the R Project for Statistical Computing, particularly the package 'nlme' for modelling purposes.

Patient and public involvement

We will engage patient organisations of each primary care clinic from the start of the planning process, and we will work with them in each sensitive stage of the study. Together with the two clinic directors and staff, we will contact and engage two to four representatives from the local patient associations to participate from the beginning of the study in planning and creating the educational and explanatory materials. These representatives will be asked to advise the research team on the relevance and clarity of the messages and on the best way to approach the eligible population. We consider patients and the community as essential stakeholders in the project and will be key participants throughout the process.

Dissemination to the local community will be done through a simple, targeted communication campaign beginning before recruitment that will explain the importance of the study, the questions it seeks to answer and the applicability of the findings. This campaign aims to make users of the cardiovascular health programme aware of the study, instilling in them a desire to participate and, therefore, a willingness to enrol when the time comes. The campaign will include a talk by the study director and a co-investigator in a town hall meeting with the community of each clinic. In addition, leaflets will be distributed in the pharmacy and consultation offices. At the end of the study, we will again hold another town hall meeting with the community and the municipal authorities to present the study's main results and the likely impact on important public health decisions regarding the COVID-19 pandemic. We will use lay language for all communications with the community.

ETHICS AND DISSEMINATION

The Scientific Ethics Committee of the South Metropolitan Health Care Service approved the study protocol, as stated in Memorandum No 191/2021. The observational design entails minimal risk to participants. No direct benefits to participants are anticipated, and no incentives for participation will be provided nor reimbursement for expenses. Personal data will be handled according to Chilean laws and regulations (Act No 19 628), and data will be anonymised as described above. At enrolment, potential participants will be fully informed on all aspects of the study and will be prompted to share their full contact details for follow-up. If we raise funds for a serum bank, a separate form will be created for consent to store the retrieved samples. The form will then be submitted for specific ethics approval by the committee that granted the initial approval. Under Chilean Act No 20 120, the study must also be authorised by the directors of the study sites and the corresponding municipality. These authorisations have already been obtained.

We will present the results in two peer-reviewed publications and national and international professional and academic meetings. We will organise seminars with relevant stakeholders (health policymakers and authorities, experts, academics) and hold town hall meetings with the local community. We will set up a COmmunity Cohort Study website at www.communitystudy.cl to disseminate the study purpose, research team and milestones.

DISCUSSION

At the time of writing, the COVID-19 pandemic is in a phase of steadily increasing community transmission in Chile and the world due to the rapid dissemination of Omicron. The immunisation of populations with different types of vaccines has been ongoing for a year in some countries or at a bare minimum in other countries. Many decisions are still made based on incomplete, short-term evidence or evidence that changes over the months, which is understandable and unavoidable; however, prospective cohort studies are needed to answer questions that can only be addressed with longitudinal designs. This project focuses on an essential tool for epidemiological surveillance of COVID-19, which is the presence of anti-SARS-CoV-2 antibodies in the community.

Chile is one of the countries with the highest cumulative rate of confirmed cases of COVID-19 per 100 000 inhabitants. It is also one of the countries with the most significant vaccine coverage. As of writing, 87% of the population has been fully vaccinated, and 61% boosted. As of 5 December 2021, Chile has authorised the following vaccines: BNT162b2/Pfizer-BioNTech, CoronaVac/Sinovac, AZD1222/Oxford-AstraZeneca, Ad5-nCoV/CanSino, Ad26.COV2.S/Janssen and GAM-COVID-VAC/Gamaleya.

The COmmunity Cohort Study will allow us to:

1. Know the seroprevalence of neutralising antibodies in the same population of individuals with greater precision and at different time points.
2. Ascertain the proportion of unvaccinated and vaccinated individuals, including primary scheme and booster doses, including length of time since last exposure.
3. Estimate the seroconversion rate of initially seronegative patients at 1 year of follow-up.
4. Know the incidence of COVID-19 in this risk group, regardless of vaccination status. After establishing the baseline seroprevalence, we will prospectively capture all COVID-19 clinical events and their outcomes.
5. Better understand the strength and duration of neutralising antibodies in vaccinated individuals or those with symptomatic COVID-19.

These results will be a reliable evidence base for decision-making on various issues, such as modelling epidemic waves and needs for vaccination and testing. The results will also be relevant when considering whether to relax SARS-CoV-2 transmission control measures.

Our study has some limitations. We could not calculate a sample size based on COVID-19-related events due to the troughs and peaks of cases arising from the spread of variants such as Delta and Omicron. We do not know in what epidemiological scenario we will find ourselves during the fieldwork of our study. Thus, estimations of associations between exposure to vaccines or infection and clinical outcomes will depend on the incidence rate of cases during data collection. Notwithstanding, neutralising antibody level data should be available for a large proportion of the cohort who will have been vaccinated or boosted relatively recently.

No funding is currently available to create a serum bank to store the aliquoted serum samples gathered during the conduct of the study. However, we will maximise efforts to raise the necessary funds to purchase a deep freezer for long-term storage of the biological samples.

Twitter Vivienne C Bachelet @V_Bachelet and Ignacio Silva-Ayarza @Ignacio_Silva_A

Contributors VCB—conceptualisation, methodology, original draft and preparation, review and editing, visualisation, project administration and funding acquisition. IS-A—original draft preparation, methodology, review and editing. FJL and DS-V—methodology, review and editing. PG—methodology and project administration. MSN— conceptualisation, methodology, original draft preparation, review and editing.

Funding This study is financially supported by the Ministry of Science of Chile (grant number: ANID FONIS SA21I0014). The grant includes the provision of the assays by Siemens Healthineers and the laboratory processing by BioNet.

Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; peer reviewed for ethical and funding approval prior to submission.

ORCID iDs
Vivienne C Bachelet http://orcid.org/0000-0002-5715-9755
Ignacio Silva-Ayarza http://orcid.org/0000-0002-6996-3695
Diego Silva-Villalobos http://orcid.org/0000-0002-8986-669X
María S Navarrete http://orcid.org/0000-0002-5411-4498

REFERENCES

1 Huang AT, Garcia-Carreras B, Hitchings MDT, *et al*. A systematic review of antibody mediated immunity to coronaviruses: kinetics, correlates of protection, and association with severity. *Nat Commun* 2020;11:4704.
2 Townsend JP, Hassler HB, Wang Z, *et al*. The durability of immunity against reinfection by SARS-CoV-2: a comparative evolutionary study. *Lancet Microbe* 2021;2:e666–75.
3 Milne G, Hames T, Scotton C, *et al*. Does infection with or vaccination against SARS-CoV-2 lead to lasting immunity? *Lancet Respir Med* 2021;9:1450–66.

4 Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese center for disease control and prevention. *JAMA* 2020;323:1239–42.
5 Cui J, Li F, Shi Z-L. Origin and evolution of pathogenic coronaviruses. *Nat Rev Microbiol* 2019;17:181–92.
6 Hanson KE, Caliendo AM, Arias CA, *et al*. Infectious diseases Society of America guidelines on the diagnosis of coronavirus disease 2019 (COVID-19): serologic testing. *Clinical Infectious Diseases* 2020;5.
7 Dan JM, Mateus J, Kato Y, *et al*. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science* 2021;371:eabf4063.
8 World Health Organization. COVID-19 natural immunity: scientific brief. Report No.: WHO/2019-nCoV/Sci_Brief/Natural_immunity/2021.1, 2021. Available: https://apps.who.int/iris/handle/10665/341241
9 Pang NY-L, Pang AS-R, Chow VT, *et al*. Understanding neutralising antibodies against SARS-CoV-2 and their implications in clinical practice. *Mil Med Res* 2021;8:47.
10 Arkhipova-Jenkins I, Helfand M, Armstrong C, *et al*. Antibody response after SARS-CoV-2 infection and implications for immunity: a rapid living review. *Ann Intern Med* 2021;174:811–21.
11 Weisblum Y, Schmidt F, Zhang F, *et al*. Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants. *eLife* 2020;9:61312. doi:10.7554/eLife.61312
12 Plotkin SA. Updates on immunologic correlates of vaccine-induced protection. *Vaccine* 2020;38:2250–7.
13 Pulendran B, Ahmed R. Immunological mechanisms of vaccination. *Nat Immunol* 2011;12:509–17.
14 Stephens DS, McElrath MJ. COVID-19 and the path to immunity. *JAMA* 2020;324:1279–81.
15 Wheatley AK, Juno JA, Wang JJ, *et al*. Evolution of immune responses to SARS-CoV-2 in mild-moderate COVID-19. *Nat Commun* 2021;12:1162.
16 Robbiani DF, Gaebler C, Muecksch F, *et al*. Convergent antibody responses to SARS-CoV-2 in convalescent individuals. *Nature* 2020;584:437–42.
17 Lynch KL, Whitman JD, Lacanienta NP, *et al*. Magnitude and kinetics of anti-severe acute respiratory syndrome coronavirus 2 antibody responses and their relationship to disease severity. *Clin Infect Dis* 2021;72:301–8.
18 Rijkers G, Murk J-L, Wintermans B, *et al*. Differences in antibody kinetics and functionality between severe and mild severe acute respiratory syndrome coronavirus 2 infections. *J Infect Dis* 2020;222:1265–9.
19 Gallais F, Gantner P, Bruel T, *et al*. Evolution of antibody responses up to 13 months after SARS-CoV-2 infection and risk of reinfection. *EBioMedicine* 2021;71:103561.
20 Lumley SF, O'Donnell D, Stoesser NE, *et al*. Antibody status and incidence of SARS-CoV-2 infection in health care workers. *N Engl J Med* 2021;384:533–40.
21 Harvey RA, Rassen JA, Kabelac CA, *et al*. Association of SARS-CoV-2 seropositive antibody test with risk of future infection. *JAMA Intern Med* 2021;181:672–9.
22 Letizia AG, Ge Y, Vangeti S, *et al*. SARS-CoV-2 seropositivity and subsequent infection risk in healthy young adults: a prospective cohort study. *Lancet Respir Med* 2021;9:712–20.
23 Wang Z, Muecksch F, Schaefer-Babajew D, *et al*. Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. *Nature* 2021;595:426–31.
24 Planas D, Veyer D, Baidaliuk A, *et al*. Reduced sensitivity of SARS-CoV-2 variant delta to antibody neutralization. *Nature* 2021;596:276–80.
25 Wall EC, Wu M, Harvey R, *et al*. Neutralising antibody activity against SARS-CoV-2 VOCs B.1.617.2 and B.1.351 by BNT162b2 vaccination. *Lancet* 2021;397:2331–3.
26 Wang P, Nair MS, Liu L, *et al*. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. *Nature* 2021;593:130–5.
27 Schmidt F, Muecksch F, Weisblum Y. Plasma neutralization properties of the SARS-CoV-2 omicron variant 2021.
28 Addetia A, Crawford KHD, Dingens A, *et al*. Neutralizing antibodies correlate with protection from SARS-CoV-2 in humans during a fishery vessel outbreak with a high attack rate. *J Clin Microbiol* 2020;58. doi:10.1128/JCM.02107-20. [Epub ahead of print: 21 10 2020].
29 Ju B, Zhang Q, Ge J, *et al*. Human neutralizing antibodies elicited by SARS-CoV-2 infection. *Nature* 2020;584:115–9.
30 Banga Ndzouboukou J-L, Zhang Y-di, Fan X-L. Recent developments in SARS-CoV-2 neutralizing antibody detection methods. *Curr Med Sci* 2021;41:1052–64.
31 Schmidt F, Weisblum Y, Muecksch F, *et al*. Measuring SARS-CoV-2 neutralizing antibody activity using pseudotyped and chimeric viruses. *J Exp Med* 2020;217.
32 National SARS-CoV-2 Serology Assay Evaluation Group. Performance characteristics of five immunoassays for SARS-CoV-2: a head-to-head benchmark comparison. *Lancet Infect Dis* 2020;20:1390–400.
33 Liu L, Wang P, Nair MS, *et al*. Potent neutralizing antibodies against multiple epitopes on SARS-CoV-2 spike. *Nature* 2020;584:450–6.
34 Valdivia A, Torres I, Latorre V, *et al*. Inference of SARS-CoV-2 spike-binding neutralizing antibody titers in sera from hospitalized COVID-19 patients by using commercial enzyme and chemiluminescent immunoassays. *Eur J Clin Microbiol Infect Dis* 2021;40:485–94.
35 Suhandynata RT, Hoffman MA, Huang D, *et al*. Commercial serology assays predict neutralization activity against SARS-CoV-2. *Clin Chem* 2021;67:404–14.
36 Khoury DS, Cromer D, Reynaldi A, *et al*. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med* 2021;27:1205–11.
37 Gilbert PB, Montefiori DC, McDermott AB, *et al*. Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial. *Science* 2022;375:43–50.
38 Munro APS, Janani L, Cornelius V, *et al*. Safety and immunogenicity of seven COVID-19 vaccines as a third dose (booster) following two doses of ChAdOx1 nCov-19 or BNT162b2 in the UK (COV-BOOST): a blinded, multicentre, randomised, controlled, phase 2 trial. *Lancet* 2021;398:2258–76.
39 Wajnberg A, Amanat F, Firpo A, *et al*. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. *Science* 2020;370:1227–30.
40 Gudbjartsson DF, Norddahl GL, Melsted P, *et al*. Humoral immune response to SARS-CoV-2 in Iceland. *N Engl J Med* 2020;383:1724–34.
41 Levin EG, Lustig Y, Cohen C, *et al*. Waning immune humoral response to BNT162b2 Covid-19 vaccine over 6 months. *N Engl J Med* 2021;385:e84.
42 Nadesalingam A, Cantoni D, Wells DA, *et al*. Paucity and discordance of neutralising antibody responses to SARS-CoV-2 VOCs in vaccinated immunodeficient patients and health-care workers in the UK. *Lancet Microbe* 2021;2:e416–8.
43 Planas D, Bruel T, Grzelak L, *et al*. Sensitivity of infectious SARS-CoV-2 B.1.1.7 and B.1.351 variants to neutralizing antibodies. *Nat Med* 2021;27:917–24.
44 Wang P, Wang M, Yu J. Increased resistance of SARS-CoV-2 variant P.1 to antibody neutralization. *bioRxiv* 2021.
45 Zhou D, Dejnirattisai W, Supasa P, *et al*. Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera. *Cell* 2021;184:2348–61.
46 Pegu A, O'Connell SE, Schmidt SD, *et al*. Durability of mRNA-1273 vaccine-induced antibodies against SARS-CoV-2 variants. *Science* 2021;373:1372–7.
47 Cromer D, Steain M, Reynaldi A, *et al*. Neutralising antibody titres as predictors of protection against SARS-CoV-2 variants and the impact of boosting: a meta-analysis. *Lancet Microbe* 2022;3:e52–61.
48 Goldberg Y, Mandel M, Bar-On YM, *et al*. Waning immunity after the BNT162b2 vaccine in Israel. *N Engl J Med Overseas Ed* 2021;385:e85.
49 Mahase E. Covid-19: omicron and the need for boosters. *BMJ* 2021;375:n3079.
50 Leung K, Wu JT. Managing waning vaccine protection against SARS-CoV-2 variants. *Lancet* 2022;399:2–3.
51 Chemaitelly H, Tang P, Hasan MR, *et al*. Waning of BNT162b2 vaccine protection against SARS-CoV-2 infection in Qatar. *N Engl J Med Overseas Ed* 2021;385:e83.
52 Fiolet T, Kherabi Y, MacDonald C-J, *et al*. Comparing COVID-19 vaccines for their characteristics, efficacy and effectiveness against SARS-CoV-2 and variants of concern: a narrative review. *Clin Microbiol Infect* 2022;28:202–21.
53 Lopez Bernal J, Andrews N, Gower C, *et al*. Effectiveness of Covid-19 vaccines against the B.1.617.2 (delta) variant. *N Engl J Med Overseas Ed* 2021;385:585–94.
54 Nasreen S, Chung H, He S. Effectiveness of mRNA and ChAdOx1 COVID-19 vaccines against symptomatic SARS-CoV-2 infection and severe outcomes with variants of concern in Ontario 2021.
55 Wu K, Choi A, Koch M. Preliminary analysis of safety and immunogenicity of a SARS-CoV-2 variant vaccine booster 2021:2021.05.05.21256716.
56 Chen T, Wu D, Chen H, *et al*. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. *BMJ* 2020;368:m1091.

57 Zhou F, Yu T, Du R, *et al*. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* 2020;395:1054–62.
58 Chidambaram V, Tun NL, Haque WZ, *et al*. Factors associated with disease severity and mortality among patients with COVID-19: a systematic review and meta-analysis. *PLoS One* 2020;15:e0241541.
59 Grasselli G, Greco M, Zanella A, *et al*. Risk factors associated with mortality among patients with COVID-19 in intensive care units in Lombardy, Italy. *JAMA Intern Med* 2020;180:1345.
60 Killerby ME, Link-Gelles R, Haight SC, *et al*. Characteristics associated with hospitalization among patients with COVID-19 - metropolitan Atlanta, Georgia, March-April 2020. *MMWR Morb Mortal Wkly Rep* 2020;69:790–4.
61 Williamson EJ, Walker AJ, Bhaskaran K, *et al*. Factors associated with COVID-19-related death using OpenSAFELY. *Nature* 2020;584:430–6.
62 Harrison SL, Fazio-Eynullayeva E, Lane DA, *et al*. Comorbidities associated with mortality in 31,461 adults with COVID-19 in the United States: a federated electronic medical record analysis. *PLoS Med* 2020;17:e1003321.
63 Petrilli CM, Jones SA, Yang J, *et al*. Factors associated with hospital admission and critical illness among 5279 people with coronavirus disease 2019 in New York City: prospective cohort study. *BMJ* 2020;369:m1966.
64 Long J, Luo Y, Wei Y, *et al*. The effect of cardiovascular disease and acute cardiac injury on fatal COVID-19: a meta-analysis. *Am J Emerg Med* 2021;48:128–39.
65 Gupta S, Hayek SS, Wang W, *et al*. Factors associated with death in critically ill patients with coronavirus disease 2019 in the US. *JAMA Intern Med* 2020;180:1436.
66 Zhao Q, Meng M, Kumar R, *et al*. The impact of COPD and smoking history on the severity of COVID-19: a systemic review and meta-analysis. *J Med Virol* 2020;92:1915–21.
67 Patanavanich R, Glantz SA. Smoking is associated with COVID-19 progression: a meta-analysis. *Nicotine Tob Res* 2020;22:1653–6.
68 Millett GA, Jones AT, Benkeser D, *et al*. Assessing differential impacts of COVID-19 on black communities. *Ann Epidemiol* 2020;47:37–44.
69 World Health Organization. WHO COVID-19 dashboard, 2020. Available: https://covid19.who.int [Accessed 21 Jan 2022].
70 Our World in Data. Coronavirus (COVID-19) vaccinations - statistics and research, 2021. Available: https://ourworldindata.org/covid-vaccinations [Accessed 21 Jan 2022].
71 World Health Organization. The unity studies: WHO sero-epidemiological investigations protocols. Available: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/early-investigations [Accessed 12 Mar 2021].
72 STROBE [online]. Available: https://www.strobe-statement.org/ [Accessed 1 Dec 2021].
73 Subsecretaría de Redes Asistenciales, División de Atención Primaria, Ministerio de Salud de Chile. *Orientaciones para La implementación del modelo de atención integral de salud familiar Y comunitaria*, 2012.
74 Ministerio de Desarrollo Social y Familia. Observatorio social. Available: http://observatorio.ministeriodesarrollosocial.gob.cl/pobreza-comunal-2020 [Accessed 28 Dec 2021].
75 Polanco SG. Región metropolitana de Santiago índice de prioridad social de comunas 2020 Seremi de Desarrollo social Y familia Metropolitana: 22.
76 Ministerio de Salud de Chile. Orientación Técnica Programa de Salud cardiovascular, 2017. Available: http://www.repositoriodigital.minsal.cl/bitstream/handle/2015/862/OT-PROGRAMA-DE-SALUD-CARDIOVASCULAR_05.pdf
77 Duggan J, Andrews N, Otter A. Evaluation of Siemens Atellica-IM IgG (sCOVG) SARS-CoV-2 serology assay for the detection of anti-SARS-CoV-2 IgG antibodies. *Public Health England* 2021.
78 Conklin J, Freeman J, Patel S, *et al*. *Evaluation of the enhanced SARS-CoV-2 IgG (sCOVG) assay on the Atellica Im analyzer*. Undated: Siemens Healthineers.
79 Siemens Healthineers. SARS-CoV-2 IgG (sCOVG) product information, 2021. Available: https://www.fda.gov/media/146931/download [Accessed 22 Dec 2021].