ABSTRACT

Introduction SARS-CoV-2 seroprevalence studies are currently being recommended and implemented in many countries. Forming part of the COVID-19 monitoring and evaluation plan of the Catalan Government Health Department, our network aims to initiate a primary healthcare sentinel monitoring system as a surrogate of SARS-CoV-2 exposure in the Barcelona Metropolitan Area. Methods and analysis The seroCAP is a serial cross-sectional study, which will be performed in the Barcelona Metropolitan Area to estimate antibodies against SARS-CoV-2. From February 2021 to March 2022, the detection of serum IgG antibodies against SARS-CoV-2 trimeric spike protein will be performed on a monthly basis in blood samples collected for diverse clinical purposes in three reference hospitals from the three Barcelona healthcare areas (BCN areas). The samples (n=2588/month) will be from patients attended by 30 primary healthcare teams at 30 basic healthcare areas (BHA). A lab software algorithm will systematically select the samples by age and sex. Seroprevalence will be estimated and monitored by age, sex, BCN area and BHA. Descriptive and cluster analysis of the characteristics and distribution of SARS-CoV-2 infections will be performed. Sociodemographic, socioeconomic and morbidity-associated factors will be determined using logistic regression. We will explore the association between seroprevalence, SARS-CoV-2 confirmed cases and the implemented measures using interrupted time series analysis. Ethics and dissemination Ethical approval was obtained from the University Institute Foundation for Primary Health Care Research Jordi Gol i Gurina ethics committee. An informed consent is not required regarding the approval of the secondary use of biological samples within the framework of the COVID-19 pandemic. A report will be generated quarterly. The final analysis, conclusions and recommendations will be shared with the stakeholders and communicated to the general public. Manuscripts resulting from the network will be submitted for publication in peer-reviewed journals.

INTRODUCTION

Background

In November 2019, the first outbreak of COVID-19 was reported in China (Wuhan Province). Subsequently, WHO declared a SARS-CoV-2 pandemic and 191 countries confirmed new cases. A state of alarm was proclaimed in Spain on 13 March 2020, and lockdown commenced. By 27 April
2020, Spain globally ranked ninth with the quantity of confirmed cases (n=3 488 469) and had recorded 77 799 deaths since the pandemic had been announced.1 On 5 April 2020, Spain registered the highest percentage of COVID-19 excess mortality in Europe (156%).2

With respect to Catalonia, the second Spanish autonomous community with the highest number of confirmed cases,3 when the first COVID-19 wave peaked on 26 March 2020, there were 23,56 confirmed cases (3667 suspected cases). At the time of the second wave peak on 21 October 2020, there were 6143 confirmed cases (28 697 suspected ones).

Since the end of 2020, the healthcare systems of many countries including Spain have once again been under extreme pressure due to the new rise in incidence rates. Such a situation has led to the adoption of new measures to control the spread of the virus until vaccination campaigns have become universal. Most countries have population-based data regarding SARS-CoV-2 infections by means of serological and molecular markers integrated into their COVID-19 surveillance systems. Although, from an epidemiological perspective, interpretation of SARS-CoV-2 seroprevalence data is hindered by the unknown duration of both natural and vaccine protection, seroprevalence studies, as part of COVID-19 surveillance strategies, are recommended.1 Currently, many countries are trying to ascertain the number of individuals exposed/infected, the way the disease is spread, and how to identify SARS-CoV-2 infection changes over time.2 A systematic review and meta-analysis performed by Rostami et al3 analysed 47 studies involving 399 265 individuals from 23 countries. The SARS-CoV-2 seroprevalence in the general population varied from 0.37% to 22.1%. Few representative population-based prospective studies at a national or regional level have been published so far. In Switzerland, Stringhini et al,4 between 6 April 2020 and 9 May 2020, observed an increasing seroprevalence from 4.8% to 10.8% in 2766 participants from 1339 households.4 A retrospective, repeated cross-sectional analysis of anti-SARS-CoV-2 spike antibodies in weekly intervals from the beginning of February to July 2020 in New York city showed a stabilised seroprevalence (EN-19.12 In Japan, research on the characteristics and distribution of cases has permitted the design of more effective strategies to specifically target high-risk population groups for the prevention and control of SARS-CoV-2 infection.13 Thus, as the number of cases decreases, the monitoring of population subgroups at higher risks becomes crucial in controlling the spread of COVID-19.12

The propagation pattern of SARS-CoV-2 infection is made up of many biological, behavioural and structural factors, including vaccine coverage. Nevertheless, social inequalities have a profound impact on COVID-19 morbidity and mortality. Indeed, social determinants, such as poverty, physical environment and race/ethnicity, can considerably affect COVID-19 outcomes.9 The mechanisms are totally different, yet, in a similar manner to the HIV epidemic in the 1980s when transmission shifted from specific core groups to marginalised, discriminated individuals with overlapping vulnerabilities,10 such subgroups are currently very sensitive to SARS-CoV-2.

A number of institutions, including public health agencies, have highlighted the necessity to analyse and address health inequalities in the COVID-19 pandemic. In the UK, the risk of dying has been reported to be higher among older adults, ethnic minorities and those living in areas with worse socioeconomic deprivation indices.11 In Catalunya, higher mortality rates occurred in individuals aged 80 or older, and were greater in men than in women at all socioeconomic levels. Nevertheless, the lower the socioeconomic level the greater the mortality for both sexes.12 In Barcelona, the elderly and those living in areas with higher deprivation indices presented higher incidence and mortality rates for COVID-19.13 In Japan, research on the characteristics and distribution of cases has permitted the design of more effective strategies to specifically target high-risk population groups for the prevention and control of SARS-CoV-2 infection. Infection13 Thus, as the number of cases decreases, the monitoring of population subgroups at higher risks becomes crucial in controlling the spread of COVID-19.12

Aim

Our aim is to establish a network of primary healthcare teams in the Barcelona Metropolitan Area to systematically monitor SARS-CoV-2 seroprevalence over time. The information obtained will be linked to sociodemographical, epidemiological and clinical variables to identify potential individual and associated factors, and thus better design and implement preventive and control measures. This initial pilot network will be later expanded with systematised methodology to other Catalan health regions and institutions.

Objectives

Main objectives

1. To describe SARS-CoV-2 seroprevalence over time by age group, sex and geographical area among primary healthcare attendees in the Barcelona Metropolitan Area, from February 2021 to March 2022.
2. To identify multilevel associated factors with SARS-CoV-2 seroprevalence among primary healthcare attendees in the Barcelona Metropolitan Area.
3. To differentiate between previously infected participants and those that have received the SARS-CoV-2 vaccine with individuals who presented a positive result for SARS-CoV-2 IgG antibodies in our study.
4. To correlate SARS-CoV-2 seroprevalence over time with population-based preventive and control interventions, including lockdowns, mobility restrictions and vaccine coverage.

5. To identify and characterise clusters of SARS-CoV-2 infection using both seroprevalence and epidemiological surveillance from the Barcelona Metropolitan Area.

METHODS AND ANALYSIS

Setting

In 2019, the Barcelona Metropolitan Area had a population of 5,047,597 and was composed of three healthcare areas (BCN areas): Barcelona city, North Metropolitan Area and South Metropolitan Area. Each of the BCN areas has a different number of BHAs. The BHAs are located in specified geographical areas where a PCT is in charge of attending the population. The BHA is the smallest administrative territorial health unit in Catalonia, and covers territories with a population of between 5,000 and 25,000 individuals. For the purpose of this study, the BHA was used as the minimum geographical study unit.

Study design

A serial monthly cross-sectional study to detect IgG antibodies to SARS-CoV-2 in biological samples from primary healthcare attendees in the Barcelona Metropolitan Area between February 2021 and March 2022.

Sample size calculation

As there are no SARS-CoV-2 seroprevalence estimations available for each area of the Barcelona Metropolitan Area, we used those seroprevalences reported for the different Catalan provinces by the Spanish Ministry of Health in December 2020. Taking into account population and estimated seroprevalence, we have calculated the necessary sample size for each BCN area with a 95% confidence and an accuracy of ±1.27%. This represents 2,588 blood samples/month per BCN area (7,764 in total) (online supplemental table 1).

The sample size was distributed by age group (25–49, 50–64, 65–79, ≥80) and sex, in order to achieve a quarterly precision of ±2.07% (monthly precision of ±3.59%) for each stratum of sex/age in each BCN area (online supplemental table 2).

Study population and participants

The total sample size calculated for each BCN area was distributed among nine specific BHAs in the North and South Metropolitan area and 12 in BCN city (30 PCTs located in 30 different BHAs in total) (online supplemental table 3). The BHAs were previously chosen taking into account tertiles of BHA socioeconomic deprivation index and tertiles of SARS-CoV-2 incidence (ensuring at least nine BHAs for each BCN area, the result of combining both tertile variables in a cross-table). Such strategy permits a heterogeneous population, in terms of both variables, in the different BCN areas in all the sampling campaigns. Online supplemental table 3 depicts the chosen BHA in each BCN area.

According to each of the BCN areas, the biological samples are processed at the respective reference laboratory: Vall d’Hebron University Hospital for Barcelona city, Germans Trias i Pujol University Hospital for North Barcelona, and Bellvitge University Hospital for South Barcelona.

All patients with a blood extraction performed for any health reason in the participant BHA could potentially be chosen to be included in the analysis. Through the design and implementation of an automatic algorithm, the information technology (IT) department of each hospital will systematically select a specific number of samples distributed among the different BCN areas and BHA as mentioned above. Starting day 1 of each month (when each sampling campaign will begin) and until the sample size has been reached in each BCN area for each sex-age-BHA stratum, all potential eligible blood samples will be selected and tested.

Patient and public involvement

No patient involved

Circuits of samples and results

The main output from the network will be the serology results for IgG against SARS-CoV-2. They will be added to the original blood test request indicating that the test is serology monitoring and the research project number. No additional samples need to be taken. In this way, the laboratory personnel can identify the samples when the test needs to be performed. The results are uploaded as usual to the hospital and primary care patient’s electronic health record by the IT teams.

This procedure avoids the study interfering with the usual daily laboratory routine.

All individuals who have had blood samples extracted for any clinical purpose, and who are potentially eligible to participate in the study, will be informed about the possibility of inclusion. In each BHA, a healthcare professional has been chosen as a reference and contact person for the research team members in charge of controlling data collection and management.

The study’s purpose and objectives will be communicated to the patients at the BHA by: (1) general information displayed in posters and screens located at the BHA waiting rooms and (2) through the BHA professionals. In this way, we can ensure that patients are informed and may decline to participate at any time. General practitioners, nurses and other staff will inform individuals about the study when the blood test is ordered, scheduled and at sample extraction. In the case that patients express to any BHA professional their refusal to participate, this person, via the BHA contact, will inform the research team. The BHA contact will note in the patients’ electronic health record their refusal to participate.

Phone calls and emails will be done on a daily basis to the research team (including the contact individual in the laboratory corresponding to its BCN area) with the list of patients...
declining to participate. The research team will inform the IT software managers to not admit these patients in the selection algorithm. Patients whose algorithm has been selected will be told by the PCT that their SARS-CoV-2 serological results (IgG) can be consulted in the online health patient portal (La Meva Salut app). Those patients with a positive result will be informed through the online health patient portal and by a phone call of their general practitioner (GP) or nurse, that a positive result indicates natural exposure to the SARS-CoV-2 virus or as a result of the vaccine, not active infection. They should contact their GP only if they have recently been in close contact with a confirmed case and have not have already been studied or vaccinated or if they currently have COVID-19 compatible symptoms such as cough, shortness of breath or fever. They are reminded that protective measures must be continued.

Data collection and study variables

Individual data
Sociodemographic data (sex, date of birth and BHA where the sample was taken), IgG results (qualitatively as positive/negative) and date of sample extraction will be collected by the microbiology research team at the reference hospitals, and provided anonymously to the network data managers. The IT teams of each hospital will create a unique, anonymous hospital identifier. The laboratory team in each hospital will provide the BHA where the samples are taken every week, a list of the analysed samples’ serological results, including the newly created identifiers and the variables specified above.

As participants could be identified in the laboratory databases (only by the microbiology research team at the reference hospitals), we will perform a data linkage with the Data Analytics Programme for Health Research and Innovation (PADRIS), explained in detail elsewhere. We will thus obtain information related to SARS-CoV-2 vaccination (manufacturer, number of doses, date of doses, refusal to vaccination (date and reason)), SARS-CoV-2 diagnosis made by a clinician, and/or SARS-CoV-2 positive result by a nucleic acid amplification test from respiratory samples, and/or antigen or antibodies detection according to the Spanish Ministry of Health guidelines, 1 year after project commencement.

Aggregated data
These data, collected and provided by the Primary Care Services Information System, will include information from different sources in order to obtain the following information:

1. COVID-19 surveillance system data: (1) Number of new COVID-19 cases confirmed or tested positive for SARS-CoV-2/total residents (BHA—BCN area—health region); (2) Median age (and IQR) and sex of the COVID-19 cases confirmed or tested positive for SARS-CoV-2/total residents (BHA—BCN area—health region); (3) Number of new COVID-19 cases confirmed or tested positive for SARS-CoV-2/total people tested (BHA—BCN area—health region); (4) Number of new COVID-19 cases confirmed or tested positive for SARS-CoV-2/Number of suspected cases (BHA—health region); (5) Number of patients hospitalised for COVID-19/total residents (BHA—BCN area—health region) and (6) Number of deaths by COVID-19 as a main cause of death/total residents (BHA—BCN area—health region).

2. Sociodemographic, socioeconomic and comorbidity variables by BHA: (1) Median age, nationality and sex; (2) Deprivation index, values from 0 to 100 (calculated by the Agency for Health Quality and Assessment of Catalonia); (3) urbanicity, rural or urban (from MEDEA index); (4) Comorbidities: Charlson Index and adjusted Morbidity Groups (GMA) classification (number of comorbidities, five severity levels and seven different categories).

No missing data are expected.

Microbiological analysis
Determination of IgG serum antibodies against SARS-CoV-2 trimeric Spike protein
Serum samples were centrifuged 10 min at 3000 rpm and stored at 4°C until processing.

Screening will be performed by a new generation of chemiluminescence immunoassay (CLIA) intended for detection of IgG antibodies to SARS-CoV-2 trimeric S in human serum with 99.4% sensitivity and 99.8% specificity, and 93.6% sensitivity and 100% specificity according an independent evaluator.

Serum testing will be conducted in the microbiology laboratories of each BCN area using the LIAISON SARS-CoV-2 TrimericS IgG (DiaSorin, Vercelli, Italy) on the LIAISON XL platform. This test discriminates between negative (<13 binding antibody units (BAU/mL) and positive (≥13 BAU/mL) samples. If the test is positive, the subject is considered seropositive. The LIAISON SARS-CoV-2 TrimericS IgG Assay is an indirect CLIA for the detection of IgG antibodies against SARS-CoV-2 in human serum or plasma samples. The main components of the test are magnetic particles (solid phase) coated with recombinant trimeric spicular protein of SARS-CoV-2 and a reagent conjugated with mouse monoclonal antibodies against human IgG, linked to an isoluminol derivative. Antibody concentrations are expressed as BAU/mL are referenced in relation to the First International Standard of the WHO for immunoglobulin against SARS-CoV-2 (20/136). A positive result indicates the patient has been infected by SARS-CoV-2 in the past and/or he or she has received at least one dose of a COVID-19 vaccine.

Analysis plan and statistical analysis
Estimation of SARS-CoV-2 seroprevalence, description of the sociodemographic characteristics of seroprevalent cases and factors associated with seroprevalence
To answer objectives 1–3, we will calculate seroprevalence and its 95% CI by age, sex, BCN area and BHA of residence.

In addition to the crude seroprevalence of SARS-CoV-2 in the primary care attendees, prevalence will be adjusted
for 99.4% test sensitivity % and 99.8% specificity by LIAISON SARS-CoV-2 TrimericS IgG (DiaSorin, Vercelli, Italy). The 95% CI will be set for the crude and adjusted estimates.

Every 3 months, and at the end of the study, we will compare the seroprevalence results in the different subgroups (by age and sex) and areas (BCN areas and BHA) using the \( \chi^2 \) test for qualitative variables and one-way analysis of variance test for quantitative ones. Moreover, for the same groups, subgroups and areas we will perform a descriptive analysis of the characteristics and distribution of SARS-CoV-2 seroprevalence. Finally, we will analyse the associated factors with SARS-CoV-2 seroprevalence. First, we will fit a selection method based on logistic regression to assess the association between SARS-CoV-2 seroprevalence as a dependent variable and each of the factors mentioned above. Second, multivariable logistic regression models will be performed.

We will describe the proportion of seroprevalent cases for SARS-CoV-2 individuals who have received the SARS-CoV-2 vaccine, and the proportion of participants with a previous SARS-CoV-2 diagnosis and/or a SARS-CoV-2 positive result.

### Time series analysis to explore associations between SARS-CoV-2 seroprevalence and SARS-CoV-2 confirmed infected cases.

To answer objective 4, we will perform a time series analysis of SARS-CoV-2 confirmed infected cases and SARS-CoV-2 seroprevalence. We will explore the association between the time series of the incidence rate of SARS-CoV-2 infection confirmed by laboratory (PCR) and rapid antigen test (assumed as confirmed cases), both extracted from the PADRIS database; and the seroprevalence repeated measurements resulting from our study (for the Barcelona health region and for all three BCN areas). We will perform the analysis using lag times of 2 and 3 weeks between both time series, the incidence rate of SARS-CoV-2 infection confirmed cases and seroprevalence, according to the time average for developing antibodies against SARS-CoV-2 after being exposed to the virus. Analysis of interrupted time series of SARS-CoV-2 seroprevalence and SARS-CoV-2 confirmed infected cases will be carried out to test the public health implemented measures including vaccination programmes. The confirmed cases will be modelled as Autoregressive Integrated Moving Average (ARIMA) processes to estimate the expected numbers to be compared with those observed, and assess the impact of the different analysed measures. To do so, absolute and relative changes between expected-observed confirmed cases at each time point of the implemented measures will be calculated.

### K-means and spatial-temporal cluster analysis

In order to address objective 5, we will perform a cluster analysis using two different approaches: K-means and spatial-temporal cluster analysis. By employing both we can better identify and describe clusters of SARS-CoV-2 incidence and SARS-CoV-2 seroprevalence by BHA. K-means methodology is a machine-learning technique that identifies and groups analysis units (in our case BHA) based on their similarities of characteristics. K-means methodology will be used to identify clusters of SARS-CoV-2 incidence by BHA, taking into account the rest of the variables described above. In addition, we will identify the spatial-temporal cluster of SARS-CoV-2 infection incidence and seroprevalence by BHA using SaTScan. Finally, in order to characterise the identified BHA clusters, we will perform a descriptive analysis using the variables by BHA: individual variables, related to the samples used to calculate seroprevalence, and aggregated ones, related to the population’s characteristics.

### Study limitations

This network will provide neither a population-representative sample nor a population seroprevalence estimate. Its objective is to establish a sentinel SARS-CoV-2 infection surveillance of individuals attending primary care in the Barcelona health region. Nevertheless, a high percentage of the population at some point request a test, thus the seroCAP network will provide relevant information about seroprevalence by age group and sex in the targeted population. The relative lack of representativeness will be counterbalanced by combining the seroprevalence results with population data from SARS-CoV-2 infection confirmed cases. With these findings, time series and cluster analyses will be carried out to monitor the epidemic, and evaluate interventions such as vaccination campaigns. The current campaigns, and the as yet unknown duration of antibodies after infection or vaccination, may also alter our seroprevalence results. Nevertheless, the joint analysis of seroprevalence with these and other factors will provide a useful picture for the monitoring and evaluation of the pandemic. An issue that may have an impact on our results is the differences in the patients’ characteristics between those who take part and those who decline, which could lead to a selection bias. However, as participation does not require any additional procedure, it is expected that most patients will take part. While we will not monitor samples from children and adolescents, SARS-CoV-2 seroprevalence data will be available from ongoing projects focusing on paediatric populations promoted by the Catalan Department of Health.

We will not have information for each individual regarding comorbidities and socioeconomic status. Nevertheless, aggregated information from the BHA will allow us to characterise SARS-CoV-2 infections by health area. A dynamic picture of the BHA heterogeneous sample, with different sociodemographic, socioeconomic and medical characteristics, will provide vital knowledge to prevent and control COVID-19. A strength of our study is using the BHA—the smallest administrative territorial health unit in Catalonia—as a geographical study unit. This approach could potentially facilitate future management of the COVID-19 epidemic.
Data confidentiality and other ethical considerations will be managed under the recommendations of Law 14/2007 on Biomedical Research, and the Royal Decree (RD) 1716/2011. These stipulate the basic requirements for the authorisation and operation of biobanks for biomedical research purposes and the treatment of human biological samples. Our project complies with Article 58 of Law 14/2007 and article 24 of RD 1716/2011 which deal with the exceptional treatment of human biological samples for biomedical research in the absence of consent from the source subject, as it is of general interest within the framework of the COVID-19 pandemic. The Health Department of the Catalan Generalitat data protection office is preparing an agreement to be signed by all the research team organisations to align with the ethical considerations mentioned above, and recommended by the same office. We strongly believe that the project’s data and results will be valuable within the current context of the international public health emergency as declared by the WHO for the COVID-19 pandemic. In addition to taking into account the urgent need for information coming from seroprevalence studies. Our approach is, moreover, reinforced by Spanish Bioethics Committee recommendations issued in a resolution concerning the secondary use of health data and biological samples without informed consent within the framework of the COVID-19 pandemic of 28 April 2020. For all the reasons mentioned above, the Ethics Committee consider informed consent will not be required. However, all potential participants will be informed about the study and refusal to take part is considered.

As previously mentioned, the IT hospital teams will create unique, anonymous patient and hospital identifiers that will allow unlinked anonymous testing in the sentinel population. The laboratory data (serology results and the other individual variables) will be sent every month to the research team of the Catalan Centre of Epidemiological Studies on Sexually Transmitted Infections and AIDS (CEEISCAT) using these anonymous identifiers. The study data will be kept on the Microsoft SQL Server database server of CEEISCAT. Patients’ reidentification will not be possible by the members of CEEISCAT, as only the microbiologist investigators have the link between the anonymous identifiers and the personal identifier. Data will be stored until the project is finished. This includes all postdata analysis and the development of reports, presentations at conferences or scientific articles. The microbiologist investigators and the research team of IDIAP Jordi Gol will send the weekly serology results to the referent individual at the respective BHA. This individual will inform the general practitioners of those patients with positive results, they will then decide how to proceed according to each BHA clinical protocols. The results will also be available for the participating patients in their electronic medical registers. We believe that such procedures, with prior ethical committee evaluation, will permit further studies employing individual identification to gather more information. The Health Department of the Catalan Government and the Ministry of Health of Spain will be duly informed about the preliminary and final results of the study. The study could be easily replicated and promoted to the whole territory of Catalonia and other autonomous regions of Spain, for the latter it would be necessary to have access to the vaccination data as we have in Catalonia.

**Study registration**

Ethical approval was obtained from the University Institute Foundation for Primary Health Care Research Jordi Gol I Gurina (IDIAPJGol) ethics committee with code 20/167-PCV on 22 October 2020.

**Publication plan**

This network will provide information on how the virus is spreading, help to characterise the exposed population, and identify possible clusters of transmission. All of which is crucial information to better design effective strategies for the prevention and control of the COVID-19 epidemic in Catalonia.

**CEEISCAT** will quarterly generate a report with preliminary results to give feedback to the stakeholders. The final analysis, conclusions and recommendations will be shared with all the stakeholders and communicated to the general public. Any manuscript that arises from the network will be submitted for publication in peer-reviewed journals.

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**Correction notice** This article has been corrected since it was first published. The name of the authors Miguel Ángel Munoz and Gema Fernández has been corrected.

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