Epidemiology

Community seroprevalence of COVID-19 in probable and possible cases at primary health care centres in Spain

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Abstract

Background: There is a scarcity of information about patients with mild or moderate symptoms during the coronavirus disease 2019 (COVID-19). This is especially true for those who attended and were followed up at primary care settings.

Objectives: We aim to measure the seroprevalence of antibodies against SARS-CoV-2 infection in a community sample of possible cases and among probable cases followed in primary care.

Methods: We selected a random sample of 600 individuals stratified by age groups from a total population of 19,899 individuals from a community area in Barcelona. We also invited all the patients that had been followed by General Practitioners (GPs). For both populations, we used COVID-19 rapid lateral flow immunoassays, which qualitatively assess the presence of patient-generated Immunoglobulins G (IgG) and Immunoglobulin M (IgM).

Results: Three hundred and eleven asymptomatic individuals from the randomly selected sample participated in the study. The mean age was 43.7 years (standard deviation (SD) = 21.79) and 55% were women. Seventeen individuals were seropositive for IgM and/or IgG, resulting in an overall prevalence of 5.47% (95% confidence interval = 3.44–8.58). Six hundred and thirty-four symptomatic patients were followed up by GPs. The mean age was 46.97 years (SD = 20.05) and 57.73% were women. Of these, 244 patients (38.49%) were seropositive. Results of the multivariate logistic regression analysis showed that the odds ratio for a positive test was significantly increased in patients who had fever, ageusia and contact with a patient diagnosed with COVID-19.

Conclusions: The seroprevalence of antibodies against SARS-CoV-2 among possible cases was lower than expected. Approximately, 40% of the symptomatic patients followed up by GPs during the peak months of the pandemic were positive.

Key words: General practice, infectious diseases, practice management, primary care, public health, screening.
Background
The COVID-19 pandemic is a major challenge for health systems, citizens and policymakers worldwide (1). An escalation in the contagiousness, severity and pervasiveness of the disease prompted the World Health Organization (WHO) to declare COVID-19 as a pandemic on 11 March 2020 (2). A report of the London School of Hygiene and Tropical medicine on 30 April estimated that since Spain has recently seen a large increase in the number of deaths, 15% of the population could be infected (3).

Spain had not devised, until very recently, an intensive testing strategy for suspected cases of COVID-19 infections. This renewed effort includes the utilization of reverse transcriptase polymerase chain reaction (RT-PCR) swab testing or rapid antibody testing in the primary care setting. The major contributing factors to the rapid spread and the high number of coronavirus deaths in the population could be traced back to the lack of: (i) intensive testing, (ii) identification of close contacts and (iii) insufficient screening of these close contacts.

In order to mitigate the effect of these obstacles, we created a comprehensive primary health care program that comprises the following: (i) a seroprevalence study in possible cases, (ii) a follow-up study in probable cases, (iii) a survey in institutionalized patients in nursing homes and (iv) a survey in health care workers. Monitorization of patients reporting mild or moderate symptoms compatible with COVID-19 consisted of a close follow-up of patients by General Practitioners (GPs) every 24 or 48 hours through telephone contacts to ensure that patients maintained self-isolation from Day 1 to 14 until symptoms disappeared. Patients developing severe symptoms were promptly referred to the emergency department (ED) at one of the hospitals in the health care area. We present here results of the seroprevalence and follow-up studies.

Study objectives
This study aims to estimate the seroprevalence of antibodies against SARS-CoV-2 in possible cases (without symptoms) in a community setting and to outline the antibody profiles in probable cases (with mild or moderate symptoms) that were followed up by GPs since the beginning of the pandemic.

Methods
Study design
The seroprevalence study in asymptomatic patients used a cross-sectional survey approach to recruit individuals for participation in the study (Study population A). The follow-up study used an observational prospective follow-up approach for monitoring suspected cases of COVID-19 followed by primary care physicians (Study population B).

Study population
For the seroprevalence study, we selected a simple random sampling of 600 individuals 1 year or older from a total population of 19 899 individuals registered at a primary health care centre from a community area of Barcelona, Spain. Institutionalized patients, terminally ill patients and suspected cases of COVID-19 were excluded because they were confined either at home or at the community residences. Also, patients who tested positive for COVID-19 prior to recruitment were excluded.

For the follow-up study, we included all patients aged 1 year or older consulting the primary care physician either face-to-face or by phone with mild or moderate symptoms (without a confirmed diagnosis) during the COVID-19 pandemic from 2 March to 24 April 2020. These patients were followed up mostly by phone every 24 or 48 hours until the resolution of symptoms or referral to a hospital as appropriate.

Setting
Individuals from both study populations were invited to attend a municipality centre for the elderly located nearby the primary health care centre. A team of trained GPs, nurses and medical students carried out the survey from 21 April to 24 April 2020 (Study population A) and from 29 April to 5 May 2020 (Study population B).
We used three different rapid IgM–IgG tests for COVID-19: Livoslon, Lysine and Sure Screen tests. All of them are visually read lateral flow immunoassay used to qualitatively detect Immunoglobulins G (IgG) and Immunoglobulins M (IgM) antibodies directed against the novel SARS-CoV-2 in human finger-stick (capillary).

Data were collected through a standardized questionnaire using a web-based platform designed for the study (studies4Covid) by UniversalDoctor and by the Sardenya Primary Health Care Center. The questionnaire was installed in tablet computers to facilitate the collection of the data.

All adult participants provided consent to participate in the survey. For children and adolescents, written consent from a legal representative was required. The study design was reviewed and approved by the ethics committee of the Institut Universitari d’Investigació en Atenció Primària (IDIAP Jordi Gol; number 20/104-P).

Main study endpoints
The prevalence of infection (past and current) in possible cases was defined by antibody seropositivity. For the symptomatic patients with COVID-19-compatible symptoms followed by GPs, the endpoints of interest were the proportion of patients with antibody seropositivity, the proportion of patients seeing at the ED, the proportion of hospitalized patients and proportion of deaths.

Statistical analysis
For the seroprevalence study, a sample size of 322 subjects randomly selected were sufficient to estimate with a 95% confidence and an accuracy of ±5 per cent units, a population percentage that is expected to be around 15%. A replacement rate of 40% was anticipated. The sample was drawn by fixing a random sample seed and then extracting a random sample. It was done by stratifying by age groups (1–14, 15–29, 30–39, 40–49, 50–59, 60–69 and 70–80 years).

Key Messages
- Seroprevalence of SARS-CoV-2’s antibodies in possible cases is 5.5%.
- Seroprevalence of SARS-CoV-2’s antibodies in symptomatic patients is 40%.
- Thirteen per cent of symptomatic patients were hospitalized and 2% died.
Descriptive statistics were calculated using means and standard deviations (SDs) for continuous variables and frequencies and percentages with 95% confidence interval (CI) for categorical variables. We used chi-square test and independent student’s t-test for categorical and quantitative comparisons, respectively, between positive cases versus negative cases.

Significant variables were included in a multivariate logistic regression analysis (using a stepwise procedure selection) to identify factors influencing positive antibodies test results in symptomatic population. Regression analysis was done globally and by gender. Independent variables included in the model were: age, gender, contact with a positive case and all symptoms. Results were considered statistically significant at a $P$-value of <0.05. All the analyses, including the extract of the random sample, were conducted using Statistics and Data Analysis program (STATA version 14/MP; STATA Corp.).

Results

Seroprevalence study (Study population A)

Three hundred and eleven asymptomatic individuals agreed to participate (response rate of 52%). Main reasons for non-responses included, among others, difficulties in reaching people through telephone calls, concerns related to violating lockdown restrictions and important chronic conditions that limit the participation. The overall mean age of participants was 43.7 years (SD = 21.79, range = 1–94) and 55% were women. None of the participants had been tested previously for SARS-CoV-2 by RT-PCR.

Of the 311 participants, 17 were seropositive for IgM and/or IgG, resulting in a prevalence of 5.47% (95% CI = 3.44–8.58). Six patients were both IgG and IgM positive, 6 patients were only IgM positive and 5 patients were only IgG positive. Women had a higher seroprevalence than men (6.43% versus 4.28%). Prevalence data by age groups is shown in Figure 1. The highest prevalence was found in the age group of 80 years of age or older.

Observational study and patient follow-up (Study population B)

Seven hundred and forty-three symptomatic patients were followed up by GPs for 2 months, and 634 underwent the serological test (participation rate of 85.33%). The overall mean age was 46.97 years (SD = 20.0, range = 0–92) and 57.73% were women. Of these, 244 patients (38.49%, 95% CI = 34.78%–42.33%) were seropositive for IgM and/or IgG.

During the follow-up period, 27.13% of symptomatic patients attended the ED, 11.83% were hospitalized and 1.61% died. Forty-one patients had a previous SARS-CoV-2 RT-PCR performed, 25 were positive and 16 negative. Sixteen out of the 25 PCR positives were seropositive for IgG.

Characteristics of patients in Study population 2 by test result are shown in Table 1. Positive cases were more likely to have had contact with other positive cases and to have been admitted to the ED or hospitalized.

Individuals with a positive antibody positive test were more likely to have had symptoms in the last 2 months than individuals with a negative antibody test (88.11 versus 81.03%, $P = 0.019$). Mean number of symptoms was higher in individuals with positive antibody test than in individuals with negative antibody test (4.50 versus 3.32, $P < 0.001$). The most frequent symptoms in both groups were cough (53%), tiredness (48.58%), headache (42.27%) and fever (32.49%).

Individuals with antibody positive tests were more likely to have tiredness, cough, fever, ageusia, anosmia and headache (Fig. 2), with some differences between males and females. Direct logistic regression was performed to assess the impact of a number of factors on the likelihood of getting a positive antibody test result. The model contained 16 independent variables (sex, age, contact with positive cases and symptoms). Results of the multivariate logistic regression analysis, including the independent variables age, contact with positive cases and symptoms, showed (Table 2) that the odds ratio for a positive test was significantly increased in patients who had fever ($>38^\circ$C), ageusia, contact with a positive patient, tiredness (only men), anosmia (only in women) and decreased in patients suffering from headache (in men), sore throat (in women) and shaking chills (in women).

The global model containing all predictors and using backward stepwise method for selecting predictors (fixing the significance level for addition to the model of 0.05 and the significance level for removal from the model of 0.1) was statistically significant, $X^2 (8, n = 623) = 108.64, P < 0.001$, indicating that the model was able to distinguish between positive and negative results. The model as a whole explained between 15.9% (McFadden’s adjusted $R$ square) and 29.1% (Cragg and Uhler’s $R$ square) of the variance in test result and correctly classified 73.03% of the cases.

Discussion

Main study findings

We found an overall population prevalence of 5.47%. Of those probable cases followed up in primary care, 38.49% were seropositive, and the risk of being positive was increased in patients who had fever, ageusia and contact with a patient diagnosed with COVID-19.

Comparison with other studies

Several studies analysing SARS-CoV-2 seroprevalence in the community have shown largely inconsistent results. Studies conducted in Santa Clara (CA), Heinsberg (Germany), Geneva (Switzerland), Split-Dalmatia and Šibenik-Knin County (Croatia) and in the Island of Jersey (UK) showed a range of results of seroprevalence between 1.27% and 15.5% (4–9).

Each study differs in the methods employed and in the strategy for selecting the sample. The results are, therefore, not entirely comparable.
While the results of our study are not representative of other parts of the city, they have important implications for the implementation of preventive measures to contain the COVID-19 pandemic.

Recent results from a country-wide seroprevalence study of nearly 70,000 participants in Spain, using antibody blood tests, have shown that only 5% of the population have been infected with the coronavirus (10). This result is in line with the prevalence observed in our study performed at a local level, which is far below the rate that would provide the population with the so-called herd immunity, which experts place at 60% at the very least.

With regard to the frequency of symptoms, we observed in our study that anosmia and ageusia are the fourth and the fifth more frequent symptoms in infected patients. Results from the logistic regression analysis showed that fever (>38°C), anosmia, ageusia and contact with a positive patient were risk factors associated with a positive test result. A multicentre cross-sectional cohort study in primary care patients in Germany also showed that patients who reported anosmia had a 4-fold increase for a positive test result (11).

A recent study done in 2153 consecutive ambulatory and hospitalized patients with positive results on PCR testing at 18 European hospitals found that a total of 1754 patients (87%) reported loss of smell, whereas 1136 (56%) reported taste dysfunction (12). The results of this study highlight the importance of considering loss of smell and taste in the diagnosis of mild to moderate COVID-19.

| Table 1. Characteristics of suspected cases in a Spanish population by test result (2020) |
|-----------------------------------------------|---------------|---------------|---------------|---------------|
|                                          | **Test positive** | **Test negative** | **Total** | **P-value** |
|                                          | **n = 244 (38.49%)** | **n = 390 (61.51%)** | **n = 634** |               |
| Gender male, n (%)                        | 109 (44.67%)     | 159 (40.77%)     | 268 (42.27%) | 0.333        |
| Contact with positive cases, n (%)        | 124 (50.82%)     | 152 (38.97%)     | 276 (43.53%) | 0.003        |
| Visit to ED, n (%)                        | 88 (36.07%)      | 84 (21.54%)      | 172 (27.13%) | <0.001       |
| Hospital admission, n (%)                 | 59 (24.18%)      | 16 (4.10%)       | 75 (11.83%)  | <0.001       |
| Symptoms (last 2 months), n (%)           | 215 (88.11%)     | 316 (81.03%)     | 531 (83.75%) | 0.019        |
| N° of symptoms, mean (SD)                | 4.50 (2.99)      | 3.32 (2.69)      | 3.77 (2.87)  | <0.001       |
| Cough, n (%)                              | 128 (52.46%)     | 208 (53.33%)     | 336 (53.00%) | 0.830        |
| Tiredness, n (%)                          | 144 (59.02%)     | 164 (42.05%)     | 308 (48.58%) | <0.001       |
| Headache, n (%)                           | 98 (40.16%)      | 170 (43.59%)     | 268 (42.27%) | 0.396        |
| Fever (>38°C), n (%)                      | 120 (49.18%)     | 86 (22.05%)      | 206 (32.49%) | <0.001       |
| Diarrhoea, n (%)                          | 87 (35.66%)      | 108 (27.69%)     | 195 (30.76%) | 0.035        |
| Dyspepsia, n (%)                          | 72 (29.51%)      | 98 (25.13%)      | 170 (26.81%) | 0.226        |
| Ageusia, n (%)                            | 107 (43.85%)     | 60 (15.38%)      | 167 (26.34%) | <0.001       |
| Anosmia, n (%)                            | 104 (42.62%)     | 62 (15.90%)      | 166 (26.18%) | <0.001       |
| Sore throat, n (%)                        | 51 (20.90%)      | 108 (27.69%)     | 159 (25.08%) | 0.055        |
| Low grade fever (37.5–38°C), n (%)        | 63 (25.82%)      | 82 (21.03%)      | 145 (22.87%) | 0.162        |
| Shaking chills, n (%)                     | 52 (21.31%)      | 72 (18.46%)      | 124 (19.56%) | 0.379        |
| Nausea, vomiting, n (%)                   | 50 (20.49%)      | 45 (11.54%)      | 95 (14.98%)  | 0.002        |
| Skin lesions, n (%)                       | 23 (9.43%)       | 31 (7.95%)       | 54 (8.52%)   | 0.317        |

| Table 2. Factors associated with positive antibodies test result in a Spanish symptomatic population (2020) |
|---------------------------------------------------------------|----------------|----------------|---------------|
|                                                                | **Total** | **Men** | **Women** |
|                                                                | **OR**  | **95% CI** | **OR**  | **95% CI** | **OR**  | **95% CI** |
| Age                                                            | 1.02    | 1.01–1.03 | 1.02    | 1.00–1.03 | 1.03    | 1.02–1.05 |
| Contact with positive cases                                    | 1.81    | 1.24–2.64 | 1.82    | 1.01–3.27 | 1.88    | 1.11–3.18 |
| Low grade fever (37.5–38°C)                                   | 1.61    | 1.04–2.49 | 2.18    | 1.05–4.55 |         |            |
| Fever (>38°C)                                                  | 4.15    | 2.76–6.24 | 2.87    | 1.55–5.32 | 4.55    | 2.58–8.03 |
| Nausea, vomiting                                               |         |           | 4.01    | 1.84–8.75 | 2.61    | 1.27–5.65 |
| Anosmia                                                        | 2.08    | 1.10–3.93 | 2.52    | 1.23–5.27 |         |            |
| Ageusia                                                        | 2.51    | 1.34–4.69 | 4.01    | 1.84–8.75 | 2.61    | 1.27–5.65 |
| Headache                                                       | 0.54    | 0.36–0.83 | 0.28    | 0.13–0.59 |         |            |
| Tiredness                                                      | 2.32    | 1.22–4.41 |         |           |         |            |
| Shaking chills                                                 | 0.60    | 0.38–0.96 | 0.42    | 0.20–0.85 | 0.57    | 0.31–1.05 |

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**Strengths and limitations**

One of the limitations of our study is that characteristics of serological immunoassay tests may not be sufficiently explored and validated (13). We did not perform PCR to the IgM positive cases; thus, we could not confirm if these individuals were infected. The WHO stated that serological tests could be susceptible to cross-reaction with other frequent infections, such as human coronaviruses causing common cold (14). Nonetheless, despite their limitations, serology testing for COVID-19 is useful to quantify the number of cases of COVID-19, including those that may be asymptomatic or have recovered (13). According to published data (15–17), sensitivities of tests used in our study (Livzon, Lysune and SureScreen tests) are 91.2%, 98.6% and 91%, respectively.

Another limitation of this study is the response rate of 52% by Thus, the study sample might be different from the target population, potentially resulting in biased estimates. We compare the results of responders and non-responders by age (43.7 versus 43.1) and sex (54.98% of women versus 51.05%) and the results were not statistically significant.
Despite such limitations, we are reasonably confident of the generalizability of our results as the sample of participants was randomly selected and represented an urban community in primary care in Spain. Another important strength of this study is that it was a survey that followed one protocol, personnel were specifically trained, it was conducted in a primary care setting and probable cases were followed up entirely by GPs working in primary health centres. Most of the cases of COVID-19 infection having mild or moderate symptoms are likely to be seen and managed at a primary care setting (18). Therefore, effective management of COVID-19 pandemic from a primary care perspective requires an accurate estimation of the seroprevalence in a specific community area, as well as registration and follow-up of suspected or confirmed cases.

Conclusions
The seroprevalence of antibodies against SARS-CoV-2 among asymptomatic individuals in the general population was 5.47%, which was lower than expected. Approximately, 40% of the symptomatic patients followed by GPs during the peak months of the pandemic in Barcelona were positive for antibodies against SARS-CoV-2. Thirty per cent of symptomatic patients attended the ED, 13% were hospitalized and about 2% died. Fever (>38°C), anosmia, ageusia and contact with a patient diagnosed with COVID-19 were associated with a positive test result.

Data availability
The data underlying this article will be shared on reasonable request to the corresponding author.

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