BMJ Open

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| Journal: | *BMJ Open* |
| Manuscript ID | bmjopen-2020-047216 |
| Article Type: | Original research |
| Date Submitted by the Author: | 21-Nov-2020 |



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| Keywords: | OCCUPATIONAL & INDUSTRIAL MEDICINE, VIROLOGY, Diagnostic microbiology < INFECTIOUS DISEASES, Public health < INFECTIOUS DISEASES |
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# Seroprevalence of anti-SARS-CoV-2 IgG among health-care workers is not impacted by frontline activity and mirrors the values of general population

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**Abstract electronic words count:** 281

**Main text electronic words count:** 3157

**Abstract**

**Objectives** To assess the seroprevalence of anti-SARS-CoV-2 IgG among HCWs in our University Hospital and verify the risk of acquiring the infection according to work area.

**Design** Cross-sectional observational study

**Setting** Monocentric, Italian third-level university hospital

**Participants** All the employees of the hospital on a voluntary base for a total of 4,055 individuals.

**Primary and secondary outcome measures** Number of anti-SARS-CoV-2 positive serology according to working area. Association of anti-SARS-CoV-2 positive serology according to selected variables (age, gender, country of origin, BMI, smoking, symptoms, contact with confirmed cases).

**Results** From April 27 to June 12, 2020, 4,055 HCWs were tested and 309 (7.6%) had a serologic positive test. No relevant difference was found between men and women (8.3% vs 7.3%), whereas a higher prevalence was observed among foreign-born workers (27/186, 14.5%), employees younger than 30 (64/668, 9.6%) or older than 60 years (38/383, 9.9%) and among healthcare assistants (40/320, 12.5%). Working as frontline HCWs was not associated with an increased frequency of positive serology (p=0.42). A positive association was found with presence and number of symptoms (p<0.001). The symptoms most frequently associated with a positive serology were taste and smell alterations (OR 4.62, 95% CI 2.99-7.15) and fever (OR 4.37, 95%CI 3.11-6.13). No symptoms were reported in 84/309 (27.2%) HCWs with positive IgG levels. Declared exposure to a suspected/confirmed case was more frequently associated with positive serology when the contact was a family member (19/94, 20.2%) than a patient or colleague (78/888, 8.8%).

**Conclusions** SARS-CoV-2 infection occurred undetected in a large fraction of HCWs and it was not associated with working in COVID-19 frontline areas. Beyond the hospital setting, exposure within the community represents an additional source of infection for HCWs.

**Strengths and limitations of this study**

- We assessed the prevalence of SARS-CoV-2 antibodies among healthcare workers, strengthening the fact that working in COVID-19 frontline areas is not associated with an increased risk of being infected which is more related to exposure within the community.
- We performed our study on a large cohort of healthcare workers, from an area with a high incidence of COVID-19.
- Our study was monocentric and performed in Italy, therefore the results may be applicable only to similar scenario (e.g. Western countries with public health system).

**Keywords:** occupational exposure; screening; nosocomial transmission; SARS-COV-2; COVID-19.

**Funding:** none related to the content of this manuscript.

**Conflict of interests:** none related to the content of this manuscript.

## Introduction

As of October 2020, the ongoing pandemic of coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has affected more than 30 million people worldwide resulting in more than 1 million deaths [1]. Since the beginning of the pandemic, healthcare workers (HCWs) has been identified as a group at high risk of infection [2]. The occurrence of nosocomial transmission of SARS-CoV-2 has been well described, emphasizing the adherence to infection control measures among HCWs to protect themselves and avoid nosocomial outbreaks [2–5]. Conversely, other studies did not find differences in SARS-CoV-2 infection rates between frontline and non-frontline HCWs and between HCWs and the general population, suggesting community over nosocomial acquisition as major source of infection [6–8].

In the current pandemic scenario, the optimal method to screen HCWs is still under debate. At present, the most frequently employed testing strategy is the detection of SARS-CoV-2 RNA through reverse transcriptase–polymerase chain reaction (RT-PCR) on upper respiratory specimens in symptomatic individuals or in those exposed to confirmed cases of COVID-19. Unfortunately, the testing strategy based solely on upper respiratory specimens has significant limitations. In a large metanalysis, the rate of positive nasopharyngeal swabs (NPS) ranged from 25% to 80% and decreased with time and in asymptomatic or pauci-symptomatic cases [9]. Of note, no data on test sensitivity in asymptomatic infected individuals exists, and clinical symptoms of COVID-19 among infected HCWs are often relatively mild, with fever and dyspnoea reported in 38-60% and 13-47% of cases, respectively [2,3,7,8,10]. It is also not uncommon for HCWs to work with mild symptoms [8,11], which increases the hazard of nosocomial outbreaks.

More recently, the serologic assessment of SARS-CoV-2 infection has been proposed as screening strategy among both HCWs and the general population. Antibody sensitivity is 30% one week after symptoms onset and rises to 70% and >90% at 2 and 3 weeks, respectively [12]. Hence, the most useful role for serology consists in detecting previous SARS-CoV-2 infection as screening strategy in exposed or high-risk HCWs.

Here we present the results of SARS-CoV-2 serology assessment performed on HCWs from April 27, 2020 to June 12, 2020 at the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico located in Milan, Lombardy, by far the Italian region mostly affected by COVID-19. To cope with the COVID-19 emergency, the organization of our Hospital has been modified, and different wards have been entirely dedicated to the management of COVID-19 patients to accommodate 350 of them [13]. We evaluated the association between positive tests and demographic characteristics, occupation and working environment (frontline *vs* non-frontline HCWs). In addition, we assessed the frequency of positive tests in HCWs with previous symptoms of COVID-19 or who had been quarantined or in contact with suspected or proven COVID-19 cases.

## Methods

We collected occupational and clinical characteristics of all the consecutive HCWs who performed a serologic assay for SARS-CoV-2 at the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico in Milan, Italy from April 27 to June 12, 2020. Policlinico Hospital is one of the leading Italian hospitals in clinical and research activities located in Milan, northern Italy, with more than 4,750 HCWs, 900 beds and 36,000 hospitalization per year. From 21 February 2020, to cope with the COVID-19 emergency, the hospital organization was quickly modified with the installation of four different pavilions entirely dedicated to the management of COVID-19 patients to accommodate 350 patients, of which

50 in intensive care units (13). Specific clinical pathways for COVID-19 patients were created for critical settings (*i.e.,* triage and emergency ward, operating rooms, radiology department) and several internal guidelines were implemented and periodically updated. Trainings on donning and doffing of personal protective equipment (PPE) were provided by the infectious disease specialists and anaesthesiologists to the HCWs working in COVID-19 areas. Trainings were targeted to physicians, nurses and health assistants and consisted in brief reviews on COVID-19 clinical and epidemiological issues, set-up of COVID-19 wards in contaminated, buffer and clean areas, guidance on proper use of PPE in patient daily care and in specific situations (*i.e.,* patient transportation, dialysis, surgical interventions including childbirth).

The serologic assay was offered freely to all hospital HCWs. At blood drawing, HCWs were asked to complete a questionnaire containing demographics, occupational and clinical characteristics. Information on age, gender, nationality, body mass index (BMI), smoking and comorbidities (hypertension, diabetes, immunosuppressive therapies, cardiac, respiratory or renal chronic diseases) was registered. HCWs were stratified by working environment in frontline and non-frontline workers (whether they provided direct assistance to COVID-19 patients or not) and by job title in physicians (including residents), nurses and midwives, healthcare assistants, health technicians, and clerical workers and technicians. The presence of any of the following symptoms since the end of February 2020 was collected: fever, cough, dyspnoea, diarrhoea, nausea or vomit, ageusia/dysgeusia or anosmia/parosmia, rhinorrhoea, ocular symptoms, sore throat, headache, myalgia, asthenia. The presence of any of the following risk factors for previous exposure to SARS-CoV-2 was investigated: performance of NPS (date and results), prophylaxis for SARS-CoV-2 infection (day and type of medication), home quarantine (period), contact with suspected or proven COVID-19 cases (date and type of exposure).

The study was approved by the institutional review board (368_2020bis) of our hospital and was conducted in accordance with the Helsinki Declaration.

*SARS-CoV-2 serology*

SARS-CoV-2 serology was performed with LIAISON® SARS-CoV-2 S1/S2 IgG test on LIAISON® XL (DiaSorin, Saluggia, Italy). The test is a chemiluminescent immunoassay (CLIA) that detects quantitative anti-S1 and anti-S2 specific IgG antibodies against SARS-CoV-2 in human serum. The test has, after >15 days from the infection, a declared sensitivity of 97.4% and a specificity of 98.5%. A test was considered positive when the value observed was equal to or above 15 AU/mL [14].

*Statistical analysis*

We calculated the adjusted seroprevalence using the formula: adjusted prevalence = (observed prevalence + specificity – 1)/(sensitivity + specificity – 1) [15], where sensitivity and specificity were those declared by the manufacturer.

We compared the prevalence of positive tests according to selected variables using chi-squared tests. We then calculated odds ratios (OR) and 95% confidence intervals (CI) by fitting a multivariable logistic regression model containing the following covariates: country of origin, gender, age class, occupation, frontline work, BMI class, and cigarette smoking. For other variables (quarantine, symptoms, contact with COVID-19 case, prophylaxis/therapy, and NPS), we used univariate logistic models. We evaluated the discriminating ability of the number of reported symptoms in a multivariable logistic regression model containing all groups of symptoms. Area under the ROC curve (AUC) was calculated after these models. To verify possible changes in IgG positivity over time, among HCWs with a previous positive nasopharyngeal swab (NPS), we analysed the percentage of subjects with elevated IgG levels according to the days elapsed since the first positive NPs using logistic regression. Statistical analysis was performed with Stata 16 (StataCorp. 2019).

*Patient and Public Involvement*

The serologic assessment was freely offered to all the healthcare workers of our hospital. The majority of them (4,055/4,572, 88.7%) participated and autonomously completed a questionnaire.

**Results**

From April 27 to June 12, 2020, 4,055 HCWs with a mean age of 44.8 years, 2,823 women (69.6%) and 1,232 men (30.4%), provided a blood sample and completed the questionnaire. The majority were physicians/residents (1,292/4,055, 31.9%) and nurses/midwives (1,230/4,055, 30.3%). The overall frequency of workers with a positive test was 309/4,055 (7.6%; 95% CI: 6.8-8.5%) (Table 1). The prevalence adjusted for declared test sensitivity and specificity would be 6.4%. The frequency of positive tests was almost double among workers from abroad (14.5%) compared to those of Italian ancestry (7.3%), whereas women and men had a similar prevalence. The highest frequencies of a positive test were observed in the lowest (<30 years) and highest (≥60 years) age classes. Across HCWs' job titles, a significant higher prevalence was detected among healthcare assistants (40/320, 12.5%), while weak differences were found for the other occupations (6.0% to 8.0%). No difference was observed between frontline and non-frontline HCWs (7.2% vs 7.9%). There was a positive trend of test positivity according to BMI, while current smokers had less than half the prevalence of test positivity than former and never smokers (4.0%, 8.9% and 8.5%, respectively). No association was found between test results and comorbidities (hypertension, diabetes, cardiac, respiratory, or renal chronic diseases) or being on immunosuppressive treatment (data not shown). All findings of the univariate analyses were confirmed in the multivariable analysis.

**Table 1.** Association between selected variables and prevalence of positive tests (anti-SARS-CoV-2 IgG≥15 AU/mL) among healthcare workers in a large University hospital, Milan, Italy, April 27 to June 12, 2020.

| **Variable** | **Workers** | **Positive test** | | | | |
|---|---|---|---|---|---|---|
| | N | N | % | p-value* | OR** | 95% CI** |
| All | 4,055 | 309 | 7.6 | | | |
| Country of origin | | | | | | |
| Italy | 3,869 | 282 | 7.3 | <0.001 | 1.00 | Reference |
| Other | 186 | 27 | 14.5 | | 1.82 | 1.07-3.06 |
| Gender | | | | | | |
| Women | 2,823 | 207 | 7.3 | 0.30 | 1.00 | Reference |
| Men | 1,232 | 102 | 8.3 | | 1.13 | 0.85-1.52 |
| Age (years) | | | | | | |
| <30 | 668 | 64 | 9.6 | 0.02 | 1.00 | Reference |
| 30-39 | 1,018 | 78 | 7.7 | | 0.74 | 0.51-1.07 |
| 40-49 | 858 | 48 | 5.6 | | 0.46 | 0.30-0.72 |
| 50-59 | 1,128 | 81 | 7.2 | | 0.64 | 0.43-0.95 |
| 60+ | 383 | 38 | 9.9 | | 0.83 | 0.50-1.36 |
| Occupation | | | | | | |
| Physicians, including residents | 1,292 | 93 | 7.2 | 0.006 | 0.99 | 0.64-1.53 |
| Nurses, midwives | 1,230 | 99 | 8.0 | | 1.31 | 0.85-2.04 |
| Healthcare assistants | 320 | 40 | 12.5 | | 1.84 | 1.04-3.25 |
| Health technicians*** | 585 | 35 | 6.0 | | 0.84 | 0.50-1.40 |
| Clerical workers, technicians | 628 | 42 | 6.7 | | 1.00 | Reference |
| Frontline HCWs | | | | | | |
| Never | 2,061 | 149 | 7.2 | 0.42 | 1.00 | Reference |
| Ever | 1,730 | 137 | 7.9 | | 0.92 | 0.69-1.24 |
| Missing | 264 | 23 | 8.7 | | | |
| BMI | | | | | | |
| <20 | 684 | 46 | 6.7 | 0.04 | 0.90 | 0.62-1.32 |
| 20-24.99 | 2,035 | 145 | 7.1 | | 1.00 | Reference |
| 25-29.99 | 945 | 79 | 8.4 | | 1.10 | 0.80-1.52 |
| 30+ | 314 | 31 | 9.9 | | 1.52 | 0.98-2.35 |
| Missing | 77 | 8 | 10.4 | | | |
| Cigarette smoking | | | | | | |
| Never | 2,493 | 210 | 8.4 | <0.001 | 1.00 | Reference |
| Former | 552 | 49 | 8.9 | | 1.12 | 0.79-1.58 |
| Current | 842 | 34 | 4.0 | | 0.41 | 0.27-0.61 |
| Missing | 168 | 16 | 9.5 | | | |

Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.
*From chi-squared test. For BMI: from chi-squared test for trend. Missing data not included in analyses.

**From a multivariable logistic regression model including country of origin, gender, age, occupation, frontline area, BMI, and smoking. Missing data not included in analyses.
***Includes biologists, radiology and laboratory technicians, psychologists, other health technicians

Serology results stratified according to risk factors for previous exposure to SARS-CoV-2 are reported in Table 2. A significant higher seropositivity was found among HCWs who had been quarantined (166/426=39.0%, OR=15.6 95% CI: 12.0-20.1), who had taken antiviral drugs as treatment or prophylaxis (44/135=32.3%, OR=6.59, 95%CI: 4.51-9.65) and who had reported any symptom of SARS-Cov-2 infection in the preceding four weeks (225/1,511=14.9%, OR=5.12, 95%CI: 3.95-6.64). We observed a clear monotonic increasing trend in test positivity with number of symptoms, from 56/608 (9.2%) among HCWs with just one symptom to 62/170 (36.5%) in those with five or more. Conversely, no symptom was reported in 84/309 HCWs with positive serological test (27.2%). The prevalence of positive tests was 5.6% (134/2,372) in HCWs who did not report contacts with a person with COVID-19 and 10.1% (154/1,525) in those who reported contacts with suspected or confirmed cases. Of note, prevalence of IgG positivity more than doubled if the reported contact was a family member (19/94=20.2%) compared to a patient or a colleague (78/888=8.8%). HCWs who had undergone SARS-CoV-2 NPS with negative result had a frequency of positive serology of 7.4% (175/2,375), almost the same as the overall hospital seroprevalence. On the contrary, the percentage of IgG positivity was much higher (74.7%, 130/174) in those who had a positive NPS. In 162 subjects NPS had been performed before serology, while in 12 HCWs NPS was performed because of a positive serology. Only four workers among the 1,506 who had never performed NPS (0.3%) had elevated IgG levels.

**Table 2.** Association between quarantine, symptoms contact with COVID-19 patients, and prophylaxis and prevalence of positive tests (anti-SARS-CoV-2 IgG≥15 AU/mL) among healthcare workers in a large University hospital, Milan Italy, April 27 to June 12, 2020.

| Variable | Workers | Positive test | | | | |
|---|---|---|---|---|---|---|
| | N | N | % | p-value* | OR** | 95% CI** |
| Quarantine | | | | | | |
| No | 3,629 | 143 | 3.9 | <0.001 | 1.00 | Reference |
| Yes | 426 | 166 | 39.0 | | 15.6 | 12.0-20.1 |
| Any symptom | | | | | | |
| No | 2,544 | 84 | 3.3 | <0.001 | 1.00 | Reference |
| Yes | 1,511 | 225 | 14.9 | | 5.12 | 3.95-6.64 |
| Number of symptoms | | | | | | |
| 1 | 608 | 56 | 9.2 | <0.001 | 2.97 | 2.09-4.22 |
| 2 | 389 | 45 | 11.6 | | 3.83 | 2.62-5.60 |
| 3 | 226 | 38 | 16.8 | | 5.91 | 3.93-8.93 |
| 4 | 1,118 | 24 | 20.3 | | 7.48 | 4.54-12.3 |
| 5-10 | 170 | 62 | 36.5 | | 16.8 | 11.5-24.6 |
| Contact with COVID-19 case | | | | | | |
| Unknown | 2,372 | 134 | 5.6 | <0.001 | 1.00 | Reference |
| Suspected case | 335 | 34 | 10.1 | | 1.89 | 1.27-2.80 |
| Confirmed case | 1,190 | 120 | 10.1 | | 1.87 | 1.45-2.42 |
| Missing | 158 | 21 | 13.3 | | | |
| Among suspected or confirmed, contact with | | | | | | |
| Patients or colleagues within the hospital | 888 | 78 | 8.8 | <0.001 | 1.00 | Reference |
| Family member | 94 | 19 | 20.2 | | 2.60 | 1.49-4.52 |
| Missing | 543 | 57 | 10.5 | | | |
| Prophylaxis or therapy | | | | | | |
| No | 3,919 | 265 | 6.8 | <0.001 | 1.00 | Reference |
| Yes | 136 | 44 | 32.3 | | 6.59 | 4.51-9.65 |
| Nasopharyngeal swab | | | | | | |
| Negative* | 2,376 | 175 | 7.4 | <0.001 | 1.00 | Reference |
| Positive | 174 | 130 | 74.7 | | 37.1 | 25.5-54.0 |
| Not performed | 1,506 | 4 | 0.3 | | 0.03 | 0.01-0.09 |

Abbreviations: CI, confidence interval; OR, odds ratio.
*From chi-squared test. For number of symptoms: from chi-squared test for trend. Missing data not included in analysis.
**From univariate logistic regression models. Missing data not included in analyses.

There were 162 subjects with a positive NP swab before IgG testing. Among these, IgG testing was performed between 17 and 94 days (Figure 1, left panel), with a peak between 49 and 63 days; the majority (159, 96.1%) were tested at least 21 days since the first positive swab. The percentage of positive IgG tests (N=121) increased linearly (in the logit scale) over time (Figure 1, right panel); it was 50-60% between 17 and 28 days, reaching 80% only after 60 days since the first positive NP swab.

For every specific symptom, there was a positive association with elevated IgG levels (Table 3). Specifically, strong associations emerged with fever (19/374=31.8%) and with taste or smell alterations (64/140=45.7%). In a multivariable model, these two symptoms were confirmed as the strongest predictors of positive test (both ORs>4). Other symptoms associated with positive SARS-CoV-2 serology were asthenia (OR=2.67), coryza (OR=1.90), and cough (OR=1.65), while sore throat was negatively associated with test positivity (OR=0.57). The AUC from the model containing all symptoms was 0.74 (95% CI: 0.74-0.81).



**Table 3.** Association between selected symptoms and prevalence of positive tests (anti-SARS-CoV-2 IgG≥15 AU/mL) among healthcare workers in a large University hospital, Milan, Italy, April 27 to June 12, 2020.

| | **Workers** | **Positive test** | | | | |
|---|---|---|---|---|---|---|
| | N | N | % | p-value* | OR** | 95% CI** |
| **Specific symptom** | | | | | | |
| Cough | | | | | | |
| No | 3,523 | 201 | 5.7 | <0.001 | 1.00 | Reference |
| Yes | 532 | 108 | 20.3 | | 1.65 | 1.18-2.30 |
| Fever | | | | | | |
| No | 3,681 | 190 | 5.2 | <0.001 | 1.00 | Reference |
| Yes | 374 | 119 | 31.8 | | 4.37 | 3.11-6.13 |
| Sore throat | | | | | | |
| No | 3,677 | 261 | 7.1 | <0.001 | 1.00 | Reference |
| Yes | 378 | 48 | 12.7 | | 0.57 | 0.38-0.86 |
| Coryza | | | | | | |
| No | 3,882 | 268 | 6.9 | <0.001 | 1.00 | Reference |
| Yes | 173 | 41 | 23.7 | | 1.90 | 1.21-2.98 |
| Headache | | | | | | |
| No | 3,920 | 277 | 7.1 | <0.001 | 1.00 | Reference |
| Yes | 135 | 32 | 23.7 | | 0.96 | 0.58-1.61 |
| Myalgias | | | | | | |
| No | 3,423 | 216 | 6.3 | <0.001 | 1.00 | Reference |
| Yes | 632 | 93 | 14.7 | | 0.77 | 0.54-1.11 |
| Diarrhoea/nausea/vomit | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| No | 3,633 | 254 | 7.0 | 0.006 | 1.00 | Reference |
| Yes | 422 | 55 | 13.0 | | 0.85 | 0.58-1.24 |
| Asthenia | | | | | | |
| No | 3,619 | 199 | 5.5 | <0.001 | 1.00 | Reference |
| Yes | 436 | 110 | 25.2 | | 2.67 | 1.87-3.80 |
| Ocular symptoms | | | | | | |
| No | 3,847 | 281 | 7.3 | 0.001 | 1.00 | Reference |
| Yes | 208 | 28 | 13.5 | | 0.78 | 0.46-1.32 |
| Dyspnoea | | | | | | |
| No | 3,927 | 275 | 7.0 | <0.001 | 1.00 | Reference |
| Yes | 128 | 34 | 26.6 | | 1.38 | 0.82-2.32 |
| Taste and smell alterations | | | | | | |
| No | 3,915 | 245 | 6.3 | <0.001 | 1.00 | Reference |
| Yes | 140 | 64 | 45.7 | | 4.62 | 2.99-7.15 |

Abbreviations: CI, confidence interval; OR, odds ratio.
*From chi-squared test.
**From a multivariable logistic model including all symptoms.

**Discussion**

In this study of HCWs of a large University hospital located in an area deeply affected by the COVID-19 pandemic, a relevant fraction of the personnel (7.6%) showed anti-SARS-CoV-2 IgG values compatible with a previous infection. The highest rates of seroprevalence were detected among foreign-born workers, those belonging to extreme age groups (below 30 years and above 60 years) and healthcare assistants. SARS-CoV-2 seroprevalence of frontline HCWs did not differ from those who did not report direct contact with COVID-19 patients. Unsurprisingly, a large proportion (84/309, 27.2%) of workers with a positive serology did not report any symptom in the previous four weeks. Yet, HCWs who presented symptoms before the test, were quarantined, or took antiviral drugs as treatment or prophylaxis displayed higher positivity rates compared to those who did not. Interestingly, smokers had a significantly lower prevalence of positive serologies compared to non-smokers and former smokers. Finally, among symptoms, fever and smell and taste alteration were those more frequently associated with IgG positivity.

Our results are in accordance with the data presented by Sandri and colleagues, who described a rate of positive SARS-CoV-2 serologies (in their study defined as IgG>12 AU/mL) ranging from 6.4% to 9% among the HCWs of three different hospitals in Milan [16]. In the same study the authors described a higher seroprevalence, between 35% and 43%, in HCWs from Bergamo district, one of the areas in northern Italy most affected by COVID-19. These results are corroborated by the data provided by the Bergamo Health Authority, which reported a SARS-CoV-2 seroprevalence of 30.6% among HCWs from the Bergamo metropolitan area (15). Noteworthy is thus the fact that seroprevalence among HCWs mirrors the levels encountered in the general population, ranging from 7.1% and 56.9% in the Milan and Bergamo metropolitan area, respectively [17,18]. Wide variations in seroprevalence among HCWs are reported worldwide, reflecting the distinct epidemiologic scenarios occurring in each Country: SARS-CoV-2 seroprevalence of 1.6%, 3.8%, 5.0%, 9.3%, 19.1%, 24.4% and 33% are reported from studies conducted among HCWs in Germany, China, Netherlands, Spain, Sweden, United Kingdom and the USA, respectively [6,19–24].

Contrasting findings exist regarding the role of direct assistance to COVID-19 patients on the risk of SARS-CoV-2 infections in HCWs. Comparing frontline to non-frontline workers, we observed no difference in seroprevalence rates, in line with the findings of Mani and colleagues [7]. At the same time, we observed a significantly higher seroprevalence among healthcare assistants (40/320, 12.5%), with all the other occupations (physician, nurses and midwives, technicians) below 8%. A similar seroprevalence (11.8%) was observed among healthcare assistants during the SARS pandemic in 2004 [25]. These results may suggest that, when nosocomial transmission occurs, it mainly involves those workers who have the closest contact with patients (e.g. healthcare assistants who take care of patients' primary needs) and might therefore be at the highest risk. This condition may also reflect on the higher seroprevalence detected among HCWs from abroad. Indeed, a large fraction of this group is composed by healthcare assistants (46%). When looking at healthcare assistants only, seroprevalence in workers from abroad was twice as high (20%) than in workers of Italian ancestry (9.8%).

What appears from our results is that SARS-CoV-2 transmission largely occurred from close contacts within the hospital in absolute terms (78 HCWs had contact with patients or colleagues, against 19 at home). However, in relative terms the prevalence was higher outside the hospital: in fact, HCWs who reported contacts with suspected or confirmed COVID-19 cases within the family had a prevalence of high IgG more than twice that of workers whose contacts were patients or colleagues (20.2% vs 8.8%, respectively). Similar results of family contacts as likely source of infection were reported by Sandri et al. with even higher percentages (31.2%) [16] and were further corroborated by the molecular analyses performed by Sikkema et al. [6].

Regarding the lower prevalence of positive serologies among smokers, a protective effect of smoking on the risk of infection is unlikely. The lower seroprevalence we observed among smokers might reflect the influence of smoking on major components of both innate and adaptive immune cells [26]. Particularly, a decreased production of IgA, IgG and IgM has been observed in smokers if compared to non-smokers [27].

In our study, the positivity rate of anti-SARS-CoV-2 IgG in HCWs who had a positive NPS (130/174, 74.7%) is sensibly lower than the values reported by the manufacturer, which reports a sensitivity of 90.7% and 97.9% at 5-15 and >15 days after infection, respectively [14]. On the other hand, we found that 7.4% of workers with negative NPS (175/2,375) had IgG≥15 AU/mL. Unfortunately, we are unable to ascertain what proportion is due to lack of NPS sensitivity and what arises from imperfect specificity of IgG test. In fact, our study was not designed to assess the sensitivity of the serologic test. Further reports of real-life data are therefore needed.

Finally, positive serology was associated with a recent history of typical symptoms of SARS-CoV-2 infection, especially taste and smell alterations and fever. These findings corroborate previous observations made by our group who identified taste and smell alterations and fever as the symptoms most frequently reported in HCWs with SARS-Cov-2 positivity on NPS [10]. Other authors confirmed the same observations, suggesting that anosmia is the symptom which better characterizes COVID-19 [16,21,22]. Notably, a large fraction of HCWs with positive serology (84/309, 27.2%) did not report any symptom in the four weeks before the test. This finding is also well-described in COVID-19 epidemiology, where the rate of asymptomatic or pauci-symptomatic infected persons ranges from 1.6% to 56.5% depending on subject characteristics and on the analysed country [28]. Unfortunately, in hospital settings the absence of symptoms makes it difficult to identify infected HCWs and hampers many strategies to control the infection.

The first limitation of our work has been noted above: this study was performed for health surveillance purposes and thus not designed to evaluate serologic test performance (sensitivity and specificity). Secondly, some degree of recall bias, i.e., under-reporting of mild symptoms which occurred many weeks before serologic test, is a possibility. In this case, we may have overestimated the proportion of asymptomatic workers with elevated IgG. Yet, considering that the study started at the end of April 2020, and that the COVID-19 pandemic in Lombardy begun at the end of February, we probably missed only a small percentage of subjects with clinical manifestations. Thirdly, the serologic assessment was not mandatory and was therefore not performed on all HCWs. Nevertheless, considering that the hospital employees are 4,572, our study has involved a large fraction of them (4,055/4,572, 88.7%) and thus provides a fair description of SARS-CoV-2 exposure in HCWs of our

331 Hospital. Finally, we could not evaluate the serologic status of all HCWs in a single day. As
332 the epidemic was still ongoing, even though on a much smaller scale (the zenith of the
333 infection was in March), we may have missed a few new infections.

334 What is suggested by our study, and by those similarly performed in the same area in the
335 context of the ongoing pandemic [16], is that the observed seroprevalence rate reflects the
336 spread of infection in the community served by the hospital. Assuming that PPE is provided
337 and correctly employed by all HCWs, hospitals do not seem to act as an epicentre of the
338 infection. In our study, healthcare assistants showed the highest seroprevalence rate. We do
339 believe that education and training of all HCWs should be strongly supported. Periodic
340 training of correct use of PPE and infection control procedures should be addressed not only
341 to physicians and nurses but also to other healthcare professionals.

342 The fact that more than one quarter of SARS-CoV-2 infections occurred unnoticed supports
343 the implementation of systematic testing strategies among HCWs without an ascertained
344 history of infection. Unfortunately, the best testing strategy as well as the timing and setting
345 in which these tests have the highest performance is still uncertain. Future studies should
346 address these gaps of knowledge. As of now, we deem it is important to monitor periodically
347 SARS-CoV-2 serology in HCWs to correlates the seroprevalence rates with those of general
348 population and detect any discrepancy. This will allow to implement timely and effective
349 infection control measures, thus preventing hospitals to become drivers of future COVID-19
350 outbreaks.

352 **Contributorship statement:** AL, DM, DC, AB and AG conceived the study. LC, PB, APC, BT, MC, GL, ACP, LR and FC collected the data and performed the serologic survey. DC performed the statistical analyses. AL, DM, DC wrote the first draft of the manuscript. All authors revised the final version of the manuscript.

356 **Data sharing statement:** raw data will be provided on reasonable request contacting the corresponding author.

358 **Acknowledgments:** We thank the personnel of SPIO (Servizio Prevenzione e Igiene Ospedaliera), S. Adamoli, F. De Palo, M. Gatti, L. Guerrieri, P. Marazzi, B. Marinelli, M. Polonioli, S. Pratò, E. Radice, and D. Serra for their help in data collection.

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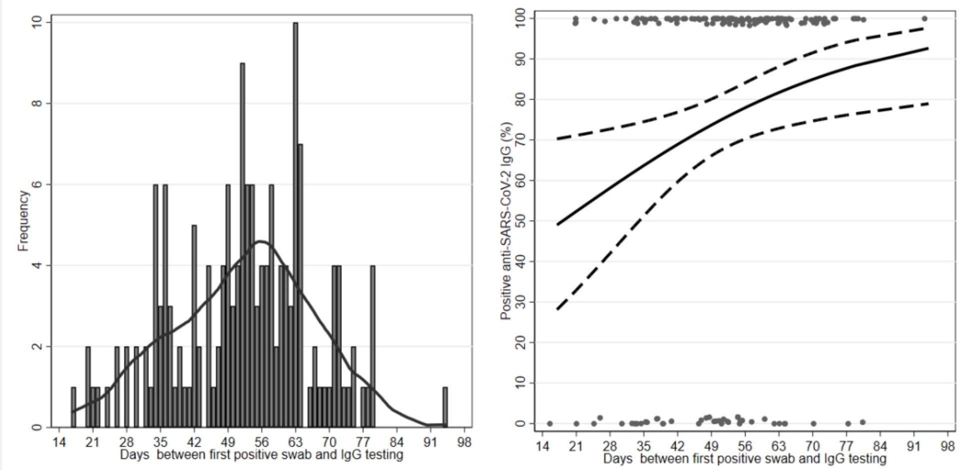
445 **Figures**

446 **Figure 1**

447 Number of IgG tests (left panel) and percentage of positive IgG tests (right panel) in 162
448 subjects with a positive nasopharyngeal swab prior to serological testing, according to days
449 elapsed since day of first positive nasopharyngeal swab.

450 Left panel shows histogram and kernel density smoothing line. In right panel circles indicate
451 subjects with negative (lower circles, N=41) or positive (upper circles, N=121) anti-SARS-
452 CoV-2 IgG, solid and dashed lines are the predicted percentages calculated with a logistic
453 regression model, and dashed lines are 95% bands around the predicted.




434x316mm (72 x 72 DPI)

# ✓STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

| | Item No | Recommendation |
|---|---|---|
| **Title and abstract** | 1✓ | (*a*) Indicate the study's design with a commonly used term in the title or the abstract |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found |
| **Introduction** | | |
| Background/rationale | 2✓ | Explain the scientific background and rationale for the investigation being reported |
| Objectives | 3✓ | State specific objectives, including any prespecified hypotheses |
| **Methods** | | |
| Study design | 4✓ | Present key elements of study design early in the paper |
| Setting | 5✓ | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection |
| Participants | 6✓ | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up |
| | | (*b*) For matched studies, give matching criteria and number of exposed and unexposed |
| Variables | 7✓ | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable |
| Data sources/ measurement | 8*✓ | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group |
| Bias | 9✓ | Describe any efforts to address potential sources of bias |
| Study size | 10✓ | Explain how the study size was arrived at |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why |
| Statistical methods | 12✓ | (*a*) Describe all statistical methods, including those used to control for confounding |
| | | (*b*) Describe any methods used to examine subgroups and interactions |
| | | (*c*) Explain how missing data were addressed |
| | | (*d*) If applicable, explain how loss to follow-up was addressed |
| | | (*e*) Describe any sensitivity analyses |
| **Results** | | |
| Participants | 13*✓ | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed |
| | | (b) Give reasons for non-participation at each stage |
| | | (c) Consider use of a flow diagram |
| Descriptive data | 14*✓ | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders |
| | | (b) Indicate number of participants with missing data for each variable of interest |
| | | (c) Summarise follow-up time (eg, average and total amount) |
| Outcome data | 15*✓ | Report numbers of outcome events or summary measures over time |
| Main results | 16✓ | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included |
| | | (*b*) Report category boundaries when continuous variables were categorized |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period |

| | | |
|---|---|---|
| Other analyses | 17✓ | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses |
| **Discussion** | | |
| Key results | 18✓ | Summarise key results with reference to study objectives |
| Limitations | 19✓ | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias |
| Interpretation | 20✓ | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence |
| Generalisability | 21✓ | Discuss the generalisability (external validity) of the study results |
| **Other information** | | |
| Funding | 22✓ | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based |

*Give information separately for exposed and unexposed groups.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

BMJ Open

# Seroprevalence of anti-SARS-CoV-2 IgG among health-care workers of a large university Hospital in Milan, Lombardy, Italy

| | |
|---|---|
| Journal: | *BMJ Open* |
| Manuscript ID | bmjopen-2020-047216.R1 |
| Article Type: | Original research |
| Date Submitted by the Author: | 30-Jan-2021 |
| Complete List of Authors: | Lombardi, Andrea; Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Infectious Diseases<br>Mangioni, Davide; Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Infectious Diseases<br>Consonni, Dario; Fondazione IRCCS Ca' Granda – Ospedale Maggiore Policlinico, Epidemiology Unit<br>Cariani, Lisa; Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Clinical Laboratory<br>Bono, Patrizia; Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Clinical Laboratory<br>Cantù, Anna; Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Medical Direction<br>Tiso, Basilio; Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Medical Direction<br>Carugno, Michele; Università degli Studi di Milano, Department of Clinical Sciences and Community Health<br>Muscatello, Antonio; Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Infectious Diseases<br>Lunghi, Giovanna; Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Infectious Diseases<br>Pesatori, Angela; University of Milan, Italy, Occupational and Environmental Health<br>Riboldi, Luciano; Fondazione IRCCS Ca' Granda – Ospedale Maggiore Policlinico, Department of Preventive Medicine, Unit of Epidemiology<br>Ceriotti, Ferruccio; Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Clinical Laboratory<br>Bandera, Alessandra; Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Infectious Diseases<br>Gori, Andrea; Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Infectious Diseases |
| <b>Primary Subject Heading</b>: | Epidemiology |
| Secondary Subject Heading: | Infectious diseases |
| Keywords: | OCCUPATIONAL & INDUSTRIAL MEDICINE, VIROLOGY, Diagnostic microbiology < INFECTIOUS DISEASES, Public health < INFECTIOUS DISEASES |

# Seroprevalence of anti-SARS-CoV-2 IgG among health-care workers of a large university Hospital in Milan, Lombardy, Italy

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**Abstract electronic words count:** 293

**Main text electronic words count:** 3,353

**Abstract**

**Objectives** To assess the seroprevalence of anti-SARS-CoV-2 IgG among HCWs in our university hospital and verify the risk of acquiring the infection according to work area.

**Design** Cross-sectional observational study

**Setting** Monocentric, Italian third-level university hospital

**Participants** All the employees of the hospital on a voluntary base for a total of 4,055 participants among 4,572 HCWs (88.7%).

**Primary and secondary outcome measures** Number of anti-SARS-CoV-2 positive serology according to working area. Association of anti-SARS-CoV-2 positive serology according to selected variables (age, gender, country of origin, BMI, smoking, symptoms, contact with confirmed cases).

**Results** From April 27 to June 12, 2020, 4,055 HCWs were tested and 309 (7.6%) had a serologic positive test. No relevant difference was found between men and women (8.3% vs 7.3%, p=0.3), whereas a higher prevalence was observed among foreign-born workers (27/186, 14.5%, p<0.001), employees younger than 30 (64/668, 9.6%, p=0.02) or older than 60 years (38/383, 9.9%, p=0.02) and among healthcare assistants (40/320, 12.5%, p=0.06). Working as frontline HCWs was not associated with an increased frequency of positive serology (p=0.42). A positive association was found with presence and number of symptoms (p<0.001). The symptoms most frequently associated with a positive serology were taste and smell alterations (OR 4.62, 95% CI 2.99-7.15) and fever (OR 4.37, 95% CI 3.11-6.13). No symptoms were reported in 84/309 (27.2%) HCWs with positive IgG levels. Declared exposure to a suspected/confirmed case was more frequently associated (p<0.001) with positive serology when the contact was a family member (19/94, 20.2%) than a patient or colleague (78/888, 8.8%).

**Conclusions** SARS-CoV-2 infection occurred undetected in a large fraction of HCWs and it was not associated with working in COVID-19 frontline areas. Beyond the hospital setting, exposure within the community represents an additional source of infection for HCWs.

**Strengths and limitations of this study**

- The serologic test employed in our study has, after >15 days from the infection, a declared sensitivity of 97.4% and a specificity of 98.5%.
- We performed our study on a large cohort of healthcare workers, from an area with a high incidence of COVID-19.
- Our study was monocentric and performed in Italy, therefore the results may be applicable only to similar scenarios (e.g. Western countries with public health system).

**Keywords:** occupational exposure; screening; nosocomial transmission; SARS-COV-2; COVID-19.

**Funding:** none related to the content of this manuscript.

**Conflict of interests:** none related to the content of this manuscript.

## Introduction

As of January 2021, the ongoing pandemic of coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has affected more than 100 million people worldwide resulting in more than 2 million deaths [1]. Since the beginning of the pandemic, healthcare workers (HCWs) has been identified as a group at high risk of infection [2]. The occurrence of nosocomial transmission of SARS-CoV-2 has been well described, emphasizing the adherence to infection control measures among HCWs to protect themselves and avoid nosocomial outbreaks [2–5]. Conversely, other studies did not find differences in SARS-CoV-2 infection rates between frontline and non-frontline HCWs and between HCWs and the general population, suggesting community over nosocomial acquisition as major source of infection [6–8].

In the current pandemic scenario, the optimal method to screen HCWs is still under debate. At present, the most frequently employed testing strategy is the detection of SARS-CoV-2 RNA through reverse transcriptase–polymerase chain reaction (RT-PCR) on upper respiratory specimens in symptomatic individuals or in those exposed to confirmed cases of COVID-19. Unfortunately, the testing strategy based solely on upper respiratory specimens has significant limitations. In a large meta-analysis, the rate of positive nasopharyngeal swabs (NPS) ranged from 25% to 80% and decreased with time and in asymptomatic or pauci-symptomatic cases [9]. Of note, no data on test sensitivity in asymptomatic infected individuals exists, and clinical symptoms of COVID-19 among infected HCWs are often relatively mild, with fever and dyspnoea reported in 38-60% and 13-47% of cases, respectively [2,3,7,8,10]. It is also not uncommon for HCWs to work with mild symptoms [8,11], which increases the hazard of nosocomial outbreaks.

More recently, the serologic assessment of SARS-CoV-2 infection has been proposed as screening strategy among both HCWs and the general population. Antibody sensitivity is 30% one week after symptoms onset and rises to 70% and >90% at 2 and 3 weeks, respectively [12]. Hence, the most useful role for serology consists in detecting previous SARS-CoV-2 infection as screening strategy in exposed or high-risk HCWs. Little is known about the duration of humoral immune response to SARS-CoV-2 infection. In some studies antibody titers did not decline within 6 months after diagnosis [13–15]. Conversely, others have reported a rapid waning over 3–4 months [16,17].

Here we present the results of SARS-CoV-2 serology assessment performed on HCWs from April 27, 2020 to June 12, 2020 at the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico located in Milan, Lombardy, by far the Italian region mostly affected by COVID-19. To cope with the COVID-19 emergency, the organization of our hospital has been modified, and different wards have been entirely dedicated to the management of COVID-19 patients to accommodate 350 of them [18]. We evaluated the association between positive tests and demographic characteristics, occupation and working environment (frontline *vs* non-frontline HCWs). In addition, we assessed the frequency of positive tests in HCWs with previous symptoms of COVID-19 or who had been quarantined or in contact with suspected or proven COVID-19 cases.

## Methods

We collected occupational and clinical characteristics of all the consecutive HCWs who performed a serologic assay for SARS-CoV-2 at the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico in Milan, Italy from April 27 to June 12, 2020. Of note, the first documented case of COVID-19 in our hospital occurred on February 23, 2020. Policlinico hospital is one of the leading Italian hospitals in clinical and research activities located in Milan, northern Italy, with more than 4,750 HCWs, 900 beds and 36,000 hospitalization per year. From 21 February 2020, to cope with the COVID-19 emergency, the hospital organization was quickly modified with the installation of four different pavilions entirely dedicated to the management of COVID-19 patients to accommodate 350 patients, of which 50 in intensive care units (13). Specific clinical pathways for COVID-19 patients were created for critical settings (*i.e.,* triage and emergency ward, operating rooms, radiology department) and several internal guidelines were implemented and periodically updated. Trainings on donning and doffing of personal protective equipment (PPE) were provided by the infectious disease specialists and anaesthesiologists to the HCWs working in COVID-19 areas. Trainings were targeted to physicians, nurses and health assistants and consisted in brief reviews on COVID-19 clinical and epidemiological issues, set-up of COVID-19 wards in contaminated, buffer and clean areas, guidance on proper use of PPE in patient daily care and in specific situations (*i.e.,* patient transportation, dialysis, surgical interventions including childbirth).

The serologic assay was offered freely to all hospital HCWs. At blood drawing, HCWs were asked to complete a questionnaire containing demographics, occupational and clinical characteristics. Information on age, gender, nationality, body mass index (BMI), smoking and comorbidities (hypertension, diabetes, immunosuppressive therapies, cardiac, respiratory or renal chronic diseases) was registered. HCWs were stratified by working environment in frontline and non-frontline workers (whether they provided direct assistance to COVID-19 patients or not) and by job title in physicians (including residents), nurses and midwives, healthcare assistants, health technicians, and clerical workers and technicians. The presence of any of the following symptoms since the end of February 2020 was collected: fever, cough, dyspnoea, diarrhoea, nausea or vomit, ageusia/dysgeusia or anosmia/parosmia, rhinorrhoea, ocular symptoms, sore throat, headache, myalgia, and asthenia. The presence of any of the following indicators of previous exposure to SARS-CoV-2 was investigated: previous NPS (date and results), prophylaxis for SARS-CoV-2 infection (day and type of medication), home quarantine (period), and contact with suspected or proven COVID-19 cases (date and type of exposure).

The study was approved by the institutional review board (368_2020bis) of our hospital and was conducted in accordance with the Helsinki Declaration.

*SARS-CoV-2 serology*

SARS-CoV-2 serology was performed with LIAISON® SARS-CoV-2 S1/S2 IgG test on LIAISON® XL (DiaSorin, Saluggia, Italy). The test is a chemiluminescent immunoassay (CLIA) that detects quantitative anti-S1 and anti-S2 specific IgG antibodies against SARS-CoV-2 in human serum. The test has, after >15 days from the infection, a declared sensitivity of 97.4%, and a specificity of 98.5%. A test was considered positive when the value observed was equal to or above 15 AU/mL [19].

*Statistical analysis*

We calculated the adjusted seroprevalence using the formula: adjusted prevalence = (observed prevalence + specificity – 1)/(sensitivity + specificity – 1) [20], where sensitivity and specificity were those declared by the manufacturer.

We compared the prevalence of positive tests according to selected variables using chi-squared tests. We then calculated odds ratios (OR) and 95% confidence intervals (CI) by fitting a multivariable logistic regression model containing the following covariates: country of origin, gender, age class, occupation, frontline work, BMI class, and cigarette smoking. For other variables (quarantine, symptoms, contact with COVID-19 case, prophylaxis/therapy, and NPS), we used univariate logistic models. We evaluated the discriminating ability of the number of reported symptoms in a multivariable logistic regression model containing all groups of symptoms. Area under the ROC curve (AUC) was calculated after these models. To verify possible changes in IgG positivity over time, among HCWs with a previous positive NPS, we analysed the percentage of subjects with elevated IgG levels according to the days elapsed since the first positive NPS using logistic regression. Statistical analysis was performed with Stata 16 (StataCorp. 2019).

*Patient and Public Involvement*

The serologic assessment was freely offered to all the healthcare workers of our hospital. The majority of them (4,055/4,572, 88.7%) participated and autonomously completed a questionnaire.

## Results

From April 27 to June 12, 2020, 4,055 HCWs with a mean age of 44.8 years, 2,823 women (69.6%) and 1,232 men (30.4%), provided a blood sample and completed the questionnaire. The majority were physicians/residents (1,292/4,055, 31.9%) and nurses/midwives (1,230/4,055, 30.3%). The overall frequency of workers with a positive test was 309/4,055 (7.6%; 95% CI: 6.8-8.5%) (Table 1). The prevalence adjusted for declared test sensitivity and specificity would be 6.4%. The frequency of positive tests was almost double among workers from abroad (14.5%) compared to those of Italian ancestry (7.3%), whereas women and men had a similar prevalence. The highest frequencies of a positive test were observed in the lowest (<30 years) and highest (≥60 years) age classes. Across HCWs' job titles, a significant higher prevalence was detected among healthcare assistants (40/320, 12.5%), while weak differences were found for the other occupations (6.0% to 8.0%). No difference was observed between frontline and non-frontline HCWs (7.2% vs 7.9%). There was a positive trend of test positivity according to BMI, while current smokers had less than half the prevalence of test positivity than former and never smokers (4.0%, 8.9% and 8.5%, respectively). No association was found between test results and comorbidities (hypertension, diabetes, cardiac, respiratory, or renal chronic diseases) or being on immunosuppressive treatment (data not shown). All findings of the univariate analyses were confirmed in the multivariable analysis.

**Table 1.** Association between selected variables and prevalence of positive tests (anti-SARS-CoV-2 IgG≥15 AU/mL) among healthcare workers in a large university hospital, Milan, Italy, April 27 to June 12, 2020.

| **Variable** | **Workers** | **Positive test** | | | | |
|---|---|---|---|---|---|---|
| | N | N | % | p-value* | OR** | 95% CI** |
| All | 4,055 | 309 | 7.6 | | | |
| Country of origin | | | | | | |
| Italy | 3,869 | 282 | 7.3 | <0.001 | 1.00 | Reference |
| Other | 186 | 27 | 14.5 | | 1.82 | 1.07-3.06 |
| Gender | | | | | | |
| Women | 2,823 | 207 | 7.3 | 0.30 | 1.00 | Reference |
| Men | 1,232 | 102 | 8.3 | | 1.13 | 0.85-1.52 |
| Age (years) | | | | | | |
| <30 | 668 | 64 | 9.6 | 0.02 | 1.00 | Reference |
| 30-39 | 1,018 | 78 | 7.7 | | 0.74 | 0.51-1.07 |
| 40-49 | 858 | 48 | 5.6 | | 0.46 | 0.30-0.72 |
| 50-59 | 1,128 | 81 | 7.2 | | 0.64 | 0.43-0.95 |
| 60+ | 383 | 38 | 9.9 | | 0.83 | 0.50-1.36 |
| Occupation | | | | | | |
| Physicians, including residents | 1,292 | 93 | 7.2 | 0.006 | 0.99 | 0.64-1.53 |
| Nurses, midwives | 1,230 | 99 | 8.0 | | 1.31 | 0.85-2.04 |
| Healthcare assistants | 320 | 40 | 12.5 | | 1.84 | 1.04-3.25 |
| Health technicians*** | 585 | 35 | 6.0 | | 0.84 | 0.50-1.40 |
| Clerical workers, technicians | 628 | 42 | 6.7 | | 1.00 | Reference |
| Frontline HCWs | | | | | | |
| Never | 2,061 | 149 | 7.2 | 0.42 | 1.00 | Reference |
| Ever | 1,730 | 137 | 7.9 | | 0.92 | 0.69-1.24 |
| Missing | 264 | 23 | 8.7 | | | |
| BMI | | | | | | |
| <20 | 684 | 46 | 6.7 | 0.04 | 0.90 | 0.62-1.32 |
| 20-24.99 | 2,035 | 145 | 7.1 | | 1.00 | Reference |
| 25-29.99 | 945 | 79 | 8.4 | | 1.10 | 0.80-1.52 |
| 30+ | 314 | 31 | 9.9 | | 1.52 | 0.98-2.35 |
| Missing | 77 | 8 | 10.4 | | | |
| Cigarette smoking | | | | | | |
| Never | 2,493 | 210 | 8.4 | <0.001 | 1.00 | Reference |

| | | | | | | |
|---|---|---|---|---|---|---|
| Former | 552 | 49 | 8.9 | | 1.12 | 0.79-1.58 |
| Current | 842 | 34 | 4.0 | | 0.41 | 0.27-0.61 |
| Missing | 168 | 16 | 9.5 | | | |

Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.
*From chi-squared test. For BMI: from chi-squared test for trend. Missing data not included in analyses.
**From a multivariable logistic regression model including country of origin, gender, age, occupation, frontline area, BMI, and smoking. Missing data not included in analyses.
***Includes biologists, radiology and laboratory technicians, psychologists, other health technicians

Serology results stratified according to risk factors for previous exposure to SARS-CoV-2 are reported in Table 2. A significant higher seropositivity was found among HCWs who had been quarantined (166/426=39.0%, OR=15.6 95% CI: 12.0-20.1), who had taken antiviral drugs as treatment or prophylaxis (44/135=32.3%, OR=6.59, 95%CI: 4.51-9.65) and who had reported any symptom of SARS-Cov-2 infection in the preceding four weeks (225/1,511=14.9%, OR=5.12, 95%CI: 3.95-6.64). We observed a clear monotonic increasing trend in test positivity with number of symptoms, from 56/608 (9.2%) among HCWs with just one symptom to 62/170 (36.5%) in those with five or more. Conversely, no symptom was reported in 84/309 HCWs with positive serologic test (27.2%). The prevalence of positive tests was 5.6% (134/2,372) in HCWs who did not report contacts with a person with COVID-19 and 10.1% (154/1,525) in those who reported contacts with suspected or confirmed cases. Of note, prevalence of IgG positivity more than doubled if the reported contact was a family member (19/94=20.2%) compared to a patient or a colleague (78/888=8.8%). HCWs who had undergone SARS-CoV-2 NPS with negative result had a frequency of positive serology of 7.4% (175/2,375), almost the same as the overall hospital seroprevalence. On the contrary, the percentage of IgG positivity was much higher (74.7%, 130/174) in those who had a positive NPS. In 162 subjects NPS had been performed before serology, while in 12 HCWs NPS was performed after the detection of a positive serology. Only four workers among the 1,506 who had never performed NPS (0.3%) had elevated IgG levels.

**Table 2.** Association between quarantine, symptoms contact with COVID-19 patients, and prophylaxis and prevalence of positive tests (anti-SARS-CoV-2 IgG≥15 AU/mL) among healthcare workers in a large university hospital, Milan Italy, April 27 to June 12, 2020.

| **Variable** | **Workers** | **Positive test** | | | | |
|---|---|---|---|---|---|---|
| | N | N | % | p-value* | OR** | 95% CI** |
| Quarantine | | | | | | |
| No | 3,629 | 143 | 3.9 | <0.001 | 1.00 | Reference |
| Yes | 426 | 166 | 39.0 | | 15.6 | 12.0-20.1 |
| Any symptom | | | | | | |
| No | 2,544 | 84 | 3.3 | <0.001 | 1.00 | Reference |
| Yes | 1,511 | 225 | 14.9 | | 5.12 | 3.95-6.64 |
| Number of symptoms | | | | | | |
| 1 | 608 | 56 | 9.2 | <0.001 | 2.97 | 2.09-4.22 |
| 2 | 389 | 45 | 11.6 | | 3.83 | 2.62-5.60 |
| 3 | 226 | 38 | 16.8 | | 5.91 | 3.93-8.93 |
| 4 | 1,118 | 24 | 20.3 | | 7.48 | 4.54-12.3 |
| 5-10 | 170 | 62 | 36.5 | | 16.8 | 11.5-24.6 |
| Contact with COVID-19 case | | | | | | |
| Unknown | 2,372 | 134 | 5.6 | <0.001 | 1.00 | Reference |
| Suspected case | 335 | 34 | 10.1 | | 1.89 | 1.27-2.80 |
| Confirmed case | 1,190 | 120 | 10.1 | | 1.87 | 1.45-2.42 |
| Missing | 158 | 21 | 13.3 | | | |
| Among suspected or confirmed, contact with | | | | | | |
| Patients or colleagues within the hospital | 888 | 78 | 8.8 | <0.001 | 1.00 | Reference |
| Family member | 94 | 19 | 20.2 | | 2.60 | 1.49-4.52 |
| Missing | 543 | 57 | 10.5 | | | |
| Prophylaxis or therapy | | | | | | |
| No | 3,919 | 265 | 6.8 | <0.001 | 1.00 | Reference |
| Yes | 136 | 44 | 32.3 | | 6.59 | 4.51-9.65 |
| Nasopharyngeal swab | | | | | | |
| Negative* | 2,376 | 175 | 7.4 | <0.001 | 1.00 | Reference |
| Positive | 174 | 130 | 74.7 | | 37.1 | 25.5-54.0 |
| Not performed | 1,506 | 4 | 0.3 | | 0.03 | 0.01-0.09 |

Abbreviations: CI, confidence interval; OR, odds ratio.
*From chi-squared test. For number of symptoms: from chi-squared test for trend. Missing data not included in analysis.
**From univariate logistic regression models. Missing data not included in analyses.

There were 162 subjects with a positive NPS before IgG testing. Among these, IgG testing was performed between 17 and 94 days (Figure 1, left panel), with a peak between 49 and 63 days; the majority (159, 96.1%) were tested at least 21 days since the first positive swab. The percentage of positive IgG tests (N=121) increased linearly (in the logit scale) over time (Figure 1, right panel); it was 50-60% between 17 and 28 days, reaching 80% only after 60 days since the first positive NPS.

For every specific symptom, there was a positive association with elevated IgG levels (Table 3). Specifically, strong associations emerged with fever (19/374=31.8%) and with taste or smell alterations (64/140=45.7%). In a multivariable model, these two symptoms were confirmed as the strongest predictors of positive test (both ORs>4). Other symptoms associated with positive SARS-CoV-2 serology were asthenia (OR=2.67), coryza (OR=1.90), and cough (OR=1.65), while sore throat was negatively associated with test positivity (OR=0.57). The AUC from the model containing all symptoms was 0.74 (95% CI: 0.74-0.81).

**Table 3.** Association between selected symptoms and prevalence of positive tests (anti-SARS-CoV-2 IgG≥15 AU/mL) among healthcare workers in a large university hospital, Milan, Italy, April 27 to June 12, 2020.

| | **Workers** | **Positive test** | | | | |
|---|---|---|---|---|---|---|
| | N | N | % | p-value* | OR** | 95% CI** |
| **Specific symptom** | | | | | | |
| Cough | | | | | | |
| No | 3,523 | 201 | 5.7 | <0.001 | 1.00 | Reference |
| Yes | 532 | 108 | 20.3 | | 1.65 | 1.18-2.30 |
| Fever | | | | | | |
| No | 3,681 | 190 | 5.2 | <0.001 | 1.00 | Reference |
| Yes | 374 | 119 | 31.8 | | 4.37 | 3.11-6.13 |
| Sore throat | | | | | | |
| No | 3,677 | 261 | 7.1 | <0.001 | 1.00 | Reference |
| Yes | 378 | 48 | 12.7 | | 0.57 | 0.38-0.86 |
| Coryza | | | | | | |
| No | 3,882 | 268 | 6.9 | <0.001 | 1.00 | Reference |
| Yes | 173 | 41 | 23.7 | | 1.90 | 1.21-2.98 |
| Headache | | | | | | |
| No | 3,920 | 277 | 7.1 | <0.001 | 1.00 | Reference |
| Yes | 135 | 32 | 23.7 | | 0.96 | 0.58-1.61 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Myalgias | | | | | | |
| No | 3,423 | 216 | 6.3 | <0.001 | 1.00 | Reference |
| Yes | 632 | 93 | 14.7 | | 0.77 | 0.54-1.11 |
| Diarrhoea/nausea/vomit | | | | | | |
| No | 3,633 | 254 | 7.0 | 0.006 | 1.00 | Reference |
| Yes | 422 | 55 | 13.0 | | 0.85 | 0.58-1.24 |
| Asthenia | | | | | | |
| No | 3,619 | 199 | 5.5 | <0.001 | 1.00 | Reference |
| Yes | 436 | 110 | 25.2 | | 2.67 | 1.87-3.80 |
| Ocular symptoms | | | | | | |
| No | 3,847 | 281 | 7.3 | 0.001 | 1.00 | Reference |
| Yes | 208 | 28 | 13.5 | | 0.78 | 0.46-1.32 |
| Dyspnoea | | | | | | |
| No | 3,927 | 275 | 7.0 | <0.001 | 1.00 | Reference |
| Yes | 128 | 34 | 26.6 | | 1.38 | 0.82-2.32 |
| Taste and smell alterations | | | | | | |
| No | 3,915 | 245 | 6.3 | <0.001 | 1.00 | Reference |
| Yes | 140 | 64 | 45.7 | | 4.62 | 2.99-7.15 |

Abbreviations: CI, confidence interval; OR, odds ratio.
*From chi-squared test.
**From a multivariable logistic model including all symptoms.

**Discussion**

In this study of HCWs of a large university hospital located in an area deeply affected by the COVID-19 pandemic, in a period ranging from 2 to 4 months after the first reported case in the hospital, a relevant fraction of the personnel (7.6%) showed anti-SARS-CoV-2 IgG values compatible with a previous infection. The highest rates of seroprevalence were detected among foreign-born workers, those belonging to extreme age groups (below 30 years and above 60 years) and healthcare assistants. SARS-CoV-2 seroprevalence of frontline HCWs did not differ from those who did not report direct contact with COVID-19 patients. Unsurprisingly, a large proportion (84/309, 27.2%) of workers with a positive serology did not report any symptom in the previous four weeks. Yet, HCWs who presented symptoms before the test, were quarantined, or took antiviral drugs as treatment or prophylaxis displayed higher positivity rates compared to those who did not. Interestingly, smokers had a significantly lower prevalence of positive serologies compared to non-smokers and former smokers. Finally, among symptoms, fever and smell and taste alteration were those more frequently associated with IgG positivity.

Our results are in accordance with the data presented by Sandri and colleagues, who described a rate of positive SARS-CoV-2 serologies (in their study defined as IgG>12 AU/mL) ranging from 6.4% to 9% among the HCWs of three different hospitals in Milan [21]. In the same study the authors described a higher seroprevalence, between 35% and 43%, in HCWs from Bergamo district, one of the areas in northern Italy most affected by COVID-19. These results are corroborated by the data provided by the Bergamo Health Authority, which reported a SARS-CoV-2 seroprevalence of 30.6% among HCWs from the Bergamo metropolitan area (15). Noteworthy is thus the fact that seroprevalence among HCWs mirrors the levels encountered in the general population, ranging from 7.1% and 56.9% in the Milan and Bergamo metropolitan area, respectively [22,23]. Wide variations in seroprevalence among HCWs are reported worldwide, reflecting the distinct epidemiologic scenarios occurring in each Country: SARS-CoV-2 seroprevalence of 1.6%, 3.8%, 5.0%, 9.3%, 19.1%, 24.4% and 33% are reported from studies conducted among HCWs in Germany, China, Netherlands, Spain, Sweden, United Kingdom and the USA, respectively [6,24–29].

Contrasting findings exist regarding the role of direct assistance to COVID-19 patients on the risk of SARS-CoV-2 infections in HCWs. Comparing frontline to non-frontline workers, we observed no difference in seroprevalence rates, in line with the findings of Mani and colleagues [7]. At the same time, we observed a significantly higher seroprevalence among healthcare assistants (40/320, 12.5%), with all the other occupations (physician, nurses and midwives, technicians) below 8%. A similar seroprevalence (11.8%) was observed among healthcare assistants during the SARS pandemic in 2004 [30]. These results may suggest that, when nosocomial transmission occurs, it mainly involves those workers who have the closest contact with patients (e.g. healthcare assistants who take care of patients' primary needs) and might therefore be at the highest risk. This condition may also reflect on the higher seroprevalence detected among HCWs from abroad. Indeed, a large fraction of this group is composed by healthcare assistants (46%). When looking at healthcare assistants only, seroprevalence in workers from abroad was twice as high (20%) than in workers of Italian ancestry (9.8%).

What appears from our results is that SARS-CoV-2 transmission largely occurred from close contacts within the hospital in absolute terms (78 HCWs had contact with patients or colleagues, against 19 at home). However, in relative terms the prevalence was higher outside the hospital: in fact, HCWs who reported contacts with suspected or confirmed COVID-19 cases within the family had a prevalence of high IgG more than twice that of workers whose contacts were patients or colleagues (20.2% vs 8.8%, respectively). Similar results of family contacts as likely source of infection were reported by Sandri et al. with even higher percentages (31.2%) [21] and were further corroborated by the molecular analyses performed by Sikkema et al. [6].

Regarding the lower prevalence of positive serologies among smokers, a protective effect of smoking on the risk of infection is unlikely. The lower seroprevalence we observed among smokers might reflect the influence of smoking on major components of both innate and adaptive immune cells [31]. Particularly, a decreased production of IgA, IgG and IgM has been observed in smokers if compared to non-smokers [32].

In our study, the positivity rate of anti-SARS-CoV-2 S1/S2 IgG in HCWs who had a positive NPS (130/174, 74.7%) is sensibly lower than the values reported by the manufacturer, which reports a sensitivity of 90.7% and 97.9% at 5-15 and >15 days after infection, respectively [19]. Of note, 53/162 (32.7%) of the tested workers performed serology 2 or more months after first NPS positivity (Figure 1, left panel), and it is currently unknown for how long antibodies persist following SARS-CoV-2 infection. While in some studies antibody titres did not decline within 6 months after diagnosis [13–15], others reported a rapid waning over 3–4 months [16,17]. In our cohort the percentage of positive IgG tests increased monotonically over time (Figure 1, right panel), supporting the persistence of anti-SARS-CoV-2 S1/S2 IgG up to 3 months from NPS positivity. On the other hand, we found that 7.4% of workers with negative NPS (175/2,375) had IgG≥15 AU/mL. Unfortunately, we are unable to ascertain what proportion is due to lack of NPS sensitivity and what arises from imperfect specificity of IgG test. In fact, our study was not designed to assess the accuracy of the serologic test. Further reports of real-life data are therefore needed.

Finally, positive serology was associated with a recent history of typical symptoms of SARS-CoV-2 infection, especially taste and smell alterations and fever. These findings corroborate previous observations made by our group who identified taste and smell alterations and fever as the symptoms most frequently reported in HCWs with SARS-Cov-2 positivity on NPS [10]. Other authors confirmed the same observations, suggesting that anosmia is the symptom which better characterizes COVID-19 [21,26,27]. Notably, a large fraction of HCWs with positive serology (84/309, 27.2%) did not report any symptom in the four weeks before the test. This finding is also well-described in COVID-19 epidemiology, where the rate of asymptomatic or pauci-symptomatic infected persons ranges from 1.6% to 56.5% depending on subject characteristics and on the analysed country [33]. Unfortunately, in hospital settings the absence of symptoms makes it difficult to identify infected HCWs and hampers many strategies to control the infection.

The first limitation of our work has been noted above: this study was performed for health surveillance purposes and thus not designed to evaluate serologic test performance (sensitivity and specificity). Secondly, some degree of recall bias, i.e., under-reporting of mild symptoms which occurred many weeks before serologic test, is a possibility. In this case, we may have overestimated the proportion of asymptomatic workers with elevated IgG. Yet, considering that the study started at the end of April 2020, and that the COVID-19 pandemic in Lombardy begun at the end of February, we probably missed only a small percentage of subjects with clinical manifestations. Thirdly, the serologic assessment was not mandatory and was therefore not performed on all HCWs. Nevertheless, considering that the hospital employees are 4,572, our study has involved a large fraction of them (4,055/4,572, 88.7%) and thus provides a fair description of SARS-CoV-2 exposure in HCWs of our hospital. Finally, we could not evaluate the serologic status of all HCWs in a single day. As the epidemic was still ongoing, even though on a much smaller scale (the zenith of the infection was in March), we may have missed a few new infections.

What is suggested by our study, and by those similarly performed in the same area in the context of the ongoing pandemic [21], is that the observed seroprevalence rate reflects the spread of infection in the community served by the hospital. Assuming that PPE is provided and correctly employed by all HCWs, hospitals do not seem to act as an epicentre of the infection. In our study, healthcare assistants showed the highest seroprevalence rate. We do believe that education and training of all HCWs should be strongly supported. Periodic training of correct use of PPE and infection control procedures should be addressed not only to physicians and nurses but also to other healthcare professionals.

The fact that more than one quarter of SARS-CoV-2 infections occurred unnoticed supports the implementation of systematic testing strategies among HCWs without an ascertained history of infection. Unfortunately, the best testing strategy as well as the timing and setting in which these tests have the highest performance is still uncertain. Future studies should address these gaps of knowledge. As of now, we deem it is important to monitor periodically SARS-CoV-2 serology in HCWs to correlates the seroprevalence rates with those of general population and detect any discrepancy. This will allow to implement timely and effective infection control measures, thus preventing hospitals to become drivers of future COVID-19 outbreaks.

**Contributorship statement:** AL, DM, DC, AB and AG conceived the study. LC, PB, APC, BT, MC, GL, ACP, LR, AM and FC collected the data and performed the serologic survey. DC performed the statistical analyses. AL, DM, DC wrote the first draft of the manuscript. All authors revised the final version of the manuscript.

**Data sharing statement:** raw data will be provided on reasonable request contacting the corresponding author.

**Acknowledgments:** This work is dedicated to the memory of GL, which sadly passed away during the revision of this paper. We thank the personnel of SPIO (Servizio Prevenzione e Igiene Ospedaliera), S. Adamoli, F. De Palo, M. Gatti, L. Guerrieri, P. Marazzi, B. Marinelli, M. Polonioli, S. Pratò, E. Radice, and D. Serra for their help in data collection.

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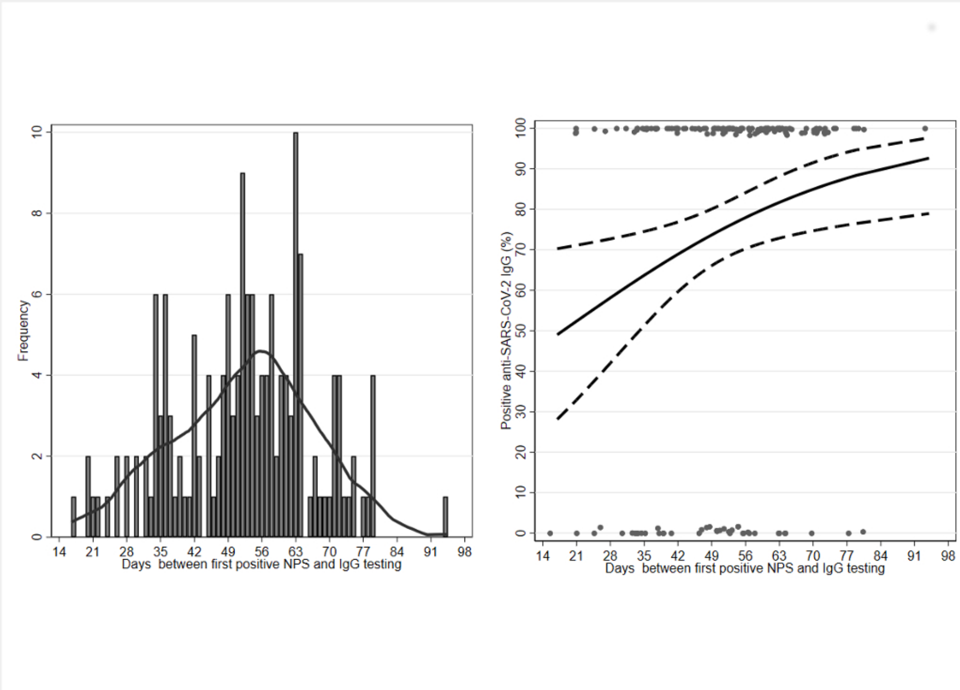
## Figures

### Figure 1

Number of IgG tests (left panel) and percentage of positive IgG tests (right panel) in 162 subjects with a positive nasopharyngeal swab prior to serologic testing, according to days elapsed since day of first positive nasopharyngeal swab.

Left panel shows histogram and kernel density smoothing line. In right panel circles indicate subjects with negative (lower circles, N=41) or positive (upper circles, N=121) anti-SARS-CoV-2 IgG, solid and dashed lines are the predicted percentages calculated with a logistic regression model, and dashed lines are 95% bands around the predicted.




Number of IgG tests (left panel) and percentage of positive IgG tests (right panel) in 162 subjects with a positive nasopharyngeal swab prior to serologic testing, according to days elapsed since day of first positive nasopharyngeal swab.

90x65mm (300 x 300 DPI)

✓STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

| | Item No | Recommendation |
|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract **p. 2** |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **p. 2-3** |
| **Introduction** | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported **p. 4-5** |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses **p. 5** |
| **Methods** | | |
| Study design | 4✓ | Present key elements of study design early in the paper **p. 5-6** |
| Setting | 5✓ | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **p. 5-6** |
| Participants | 6✓ | (*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up **p. 5-6** |
| | | (*b*) For matched studies, give matching criteria and number of exposed and unexposed **n/a** |
| Variables | 7✓ | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable **p. 6-8** |
| Data sources/ measurement | 8*✓ | For each variable of interest, give sources of data and details of methods of assessment (measurement). **p. 6-8** Describe comparability of assessment methods if there is more than one group **n/a** |
| Bias | 9✓ | Describe any efforts to address potential sources of bias **p. 16** |
| Study size | 10✓ | Explain how the study size was arrived at **p. 5-6** |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **p. 7-8** |
| Statistical methods | 12✓ | (*a*) Describe all statistical methods, including those used to control for confounding **p. 7-8** |
| | | (*b*) Describe any methods used to examine subgroups and interactions **p. 7-8** |
| | | (*c*) Explain how missing data were addressed **n/a** |
| | | (*d*) If applicable, explain how loss to follow-up was addressed **n/a** |
| | | (*e*) Describe any sensitivity analyses **p. 7-8** |
| **Results** | | |
| Participants | 13*✓ | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed **p. 8** |
| | | (b) Give reasons for non-participation at each stage **n/a** |
| | | (c) Consider use of a flow diagram **n/a** |
| Descriptive data | 14*✓ | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **p. 8-13** |
| | | (b) Indicate number of participants with missing data for each variable of interest **n/a** |
| | | (c) Summarise follow-up time (eg, average and total amount) **p. 8-13** |
| Outcome data | 15*✓ | Report numbers of outcome events or summary measures over time **p. 8-13** |
| Main results | 16✓ | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were |

| | | |
|---|---|---|
| | | adjusted for and why they were included **p. 8-13** |
| | | (*b*) Report category boundaries when continuous variables were categorized **p. 8-13** |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period **p. 8-13** |
| Other analyses | 17✓ | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **p. 8-13** |
| **Discussion** | | |
| Key results | 18✓ | Summarise key results with reference to study objectives **p. 13** |
| Limitations | 19✓ | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias **p. 16-17** |
| Interpretation | 20✓ | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence **p. 14-16** |
| Generalisability | 21✓ | Discuss the generalisability (external validity) of the study results **p. 17** |
| **Other information** | | |
| Funding | 22✓ | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based **p. 3** |

*Give information separately for exposed and unexposed groups.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.