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SARS-CoV-2 serosurvey in health care workers of the Veneto Region

<https://doi.org/10.1515/cclm-2020-1236>

Received August 13, 2020; accepted August 17, 2020;

published online August 26, 2020

Abstract

Objectives: The ongoing outbreak of coronavirus disease (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) poses formidable challenges to all health care systems. Serological assays may be used for improving disease management when appropriately applied, for investigating the antibody responses mounted against SARS-CoV-2 infection and for assessing its real prevalence. Although testing the whole population is impractical, well-designed serosurveys in selected subpopulations in specific risk groups may provide valuable information. We evaluated the prevalence of SARS-CoV-2 infection in health care workers (HCW) who underwent molecular testing with reverse transcription real-time polymerase chain reaction (rRT-PCR) in the main hospitals of the Veneto Region of Italy by measuring specific antibodies (Abs).

Methods: Both immunoglobulin (IgM and IgG antibodies against SARS-CoV-2 S-antigen and N-protein were measured using a validated chemiluminescent analytical system (CLIA) called Maglumi™ 2000 Plus (New Industries Biomedical Engineering Co., Ltd [Snibe], Shenzhen, China).

Results: A total of 8,285 HCW were tested. SARS-CoV-2 specific antibodies (IgM, IgG or both) were detectable in 378 cases (4.6%, 95% CI 4.1–5.0%). Seroconversion was observed in 4.4% of women vs. 5.0% of men, but this difference was not significant. Although detectable antibodies were found in all HCW who developed severe COVID-19 infection (100%), lower seropositivity was found in mild disease (83%) and the lowest prevalence (58%) was observed in asymptomatic subjects.

Conclusions: Seroprevalence surveys are of utmost importance for understanding the rate of population that has already developed antibodies against SARS-CoV-2. The present study defined precisely the circulation of SARS-CoV-2 in a cohort of HCW in the Veneto Region, with its prevalence (4.6%) reflecting a relatively low circulation. Symptomatic individuals or those hospitalized for medical care were 100% antibody positive, whilst Abs were only detectable in 58% of asymptomatic carriers.

Keywords: COVID-19; health care workers; SARS-CoV-2 antibody; seroprevalence.

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Introduction

The spread of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a pandemic, with sustained human-to-human transmission. Since the initial identification of COVID-19 in December 2019, there has been an exponential rise in the number of cases worldwide, especially in Italy [1]. The reasons for the rapid spread include the high transmissibility of the virus, especially among asymptomatic or minimally symptomatic carriers, the apparent absence of cross-protective immunity from related coronavirus infections, as well as the tardy public health response measures [2, 3]. Nucleic acid tests for detecting SARS-CoV-2 RNA genome are widely employed for diagnosing COVID-19 [4], but cannot be deployed for

prevalence investigations or for sensing prior exposure to an infectious agent. Therefore, there remains a great need for assays that measure antibody responses and determine seroconversion [5]. In fact, inadequate knowledge about the extent of COVID-19 pandemic challenges public health response and planning. Although it is impractical to test the entire population, well-designed serosurveys play a relevant role to determine how prevalent COVID-19 may be in the general population, or in selected high risk groups (e.g., health care workers [HCW]) [6, 7]. The several serological assays developed and currently available on the market differ by two major variables: (1) the format used, in particular, whether the test is a quantitative laboratory-based immunoassays such as enzyme linked immunosorbent assay (ELISA), chemiluminescent analytical system (CLIA) or a qualitative point-of-care test (POCT) and (2) the SARS-CoV-2 antigen targeted by the assay, in particular if the antibodies are against the spike surface protein (s) (namely subunit 1 and 2), and/or the spike receptor binding domain (RBD), and/or the nucleocapsid protein (N) [6]. In addition, they may detect total antibodies (tAb), and/or one or more specific immunoglobulins such as immunoglobulin (Ig)A, IgM, and IgG according to the main purposes of the measurements. Therefore, this study was aimed to determine the prevalence of SARS-CoV-2 antibodies in the HCW of the Veneto Region of Italy using a validated chemiluminescent assay (CLIA) [8, 9].

Materials and methods

Subjects included in the study

This multicenter study involved several Structures of the National Healthcare Service of the Veneto Region, in which an early cluster of SARS-CoV-2 infection in Italy was reported on February 21st, 2020. In particular, serological determinations were performed at the University Hospital of Padova, the University Hospital of Verona and the S. Bortolo Hospital of Vicenza laboratories. A total of 8,285 healthcare workers were included in the study which was performed between February 22nd and May 29th. For each patient, a serum sample was collected and subsequently heat inactivated at 56 °C for 30 min prior to analyses. To evaluate sensitivity, molecular rRT-PCR tests were performed according to Corman et al. [4]. Briefly, RNA was extracted using an automated platform (Magna Pure 96 Instrument, Roche Diagnostics, USA) and then used for rRT-PCR, which was performed in separate reactions with SARS-CoV-2 gene *E* and RNaseP analyses by ABI prism® AB 7900 or 7900HT or QuantStudio™ 5 Real-Time PCR Systems (Applied Biosystems, USA). The threshold cycle (Ct) of SARS-CoV-2 gene *E* and of RNaseP was obtained after standardization of the rRT-PCR instruments software settings as follows: baseline calculated in the cycle range 3–15; fixed threshold at 0.2 [10]. All

subjects underwent at least one nasopharyngeal swab test; serological testing was performed within a 13-days timeframe from the first molecular test on the basis of the current knowledge of seroconversion time and antibody kinetics [8, 11–13]. For estimating specificity, a subject was considered SARS-CoV-2 negative if at least three sequential negative results rRT-PCR were found (one of that within 12 days before serological testing). The association between sensitivity of serological test and severity of SARS-Cov-2 infection was assessed through linkage with a regional centralized surveillance system including all infection cases and related hospitalizations. This study was approved by the Regional Committee for Bioethics (prot. n. 178357) and the Ethics Committee of the University Hospital of Verona (prot. n. 22851) and all subjects recruited to the study expressed an informed consent for being enrolled.

Measurement of SARS-CoV-2 antibodies

Maglumi™ 2000 Plus (New Industries Biomedical Engineering Co., Ltd [Snibe], Shenzhen, China) is a chemiluminescent analytical system (CLIA) for the detection of both IgM and IgG antibodies against SARS-Cov-2 S-antigen and N-protein. According to the manufacturer's inserts (271 2019-nCoV IgM, V2.0, 2020-03 and 272 2019-nCoV IgG, V1.2, 2020-02), the clinical sensitivities of IgM and IgG were 78.7% and 91.2%, respectively, while the specificities of IgM and IgG were 97.5% and 97.3%, respectively. Manufacturers' reported thresholds were 1.0 kAU/L for IgM and 1.1 kAU/L for IgG, respectively. The analytical and clinical performances of the assay have been evaluated and described elsewhere [8].

Statistical analyses

Statistical analyses were carried out using SAS software, version 9.4 on the SAS System for Windows (SAS Institute Inc., Cary, NC). Mean and standard deviation and Student's t-test were used to describe subjects age and to evaluate group's differences. The χ^2 -test was used to evaluate proportions differences across groups. The Cuzick test was used as proportion test for trend across ordered groups.

Results

A total of 8,285 HCW from the University-Hospital of Padova and Verona, from the Istituto Oncologico Veneto, from the Hospital of Vicenza and from other healthcare structures were investigated; 5,933 (71.6%) were women and 2,352 were men (28.4%). The mean age was 43.2 ± 11.6 years, 43.6 ± 11.4 years for women and 42.2 ± 12.5 years for men, ($t=5.06$, $p<0.001$).

SARS-CoV-2 specific antibodies (IgM, IgG or both) were detectable in 378 healthcare workers (4.6%, 95% CI: 4.1–5.0%). In particular, IgM antibodies were detected in 82 subjects, while IgG antibodies were found in 343 HCW. Seroconversion was observed in 4.4% of women versus 5.0% of men, but the difference did not achieve statistical significance ($\chi^2=1.41$, $p=0.493$). Table 1 shows the distribution

Table 1: Total number and percentages of positive tests with 95% confidence intervals (CI), subdivided by age classes.

Age classes, years	Total number of tests	Percentage (%) of positive tests	Percentage 95% CI
<30 years	1512	4.1%	3.2–5.2%
30–39 years	1826	3.5%	2.7–4.4%
40–49 years	1962	4.4%	3.6–5.4%
50–59 years	2389	6.0%	5.1–7.1%
>60 years	596	3.7%	2.3–5.5%

Table 2: Total number and percentages of positive tests with 95% confidence intervals (CI), subdivided by the different health care figures.

Healthcare figures	Total number of tests	Percentage (%) of positive tests	Percentage 95% CI
Physicians	2337	3.6%	2.8–4.4%
Nurses	3230	4.7%	4.0–5.5%
Healthcare assistants	1040	6.0%	4.6–7.6%
Others	1678	4.8%	3.8–5.9%

according to different age groups ($\chi^2=18.82$, $p=0.001$). Table 2 shows the seroprevalence distribution according to the different health care role (physicians, nurses and health care assistants, $\chi^2=10.55$, $p=0.014$). A significant higher seroprevalence could be observed in health care assistants compared to other groups ($\chi^2=5.34$, $p=0.021$).

To evaluate the diagnostic specificity, 3,110 health care operators with at least three nasopharyngeal swabs negative results, were evaluated: 3,049 resulted negative thus leading to an estimated specificity of 98% (CI 97.5–98.5%). Figure 1 shows the prevalence of seropositivity in three different groups of subjects with positive nasopharyngeal swabs according to disease severity (group A hospitalized with severe disease; group B mild disease; group C asymptomatic subjects). While detectable antibodies were found in all severe COVID-19 patients (100%), a lower seropositivity was found in mild disease (83%) and the lowest prevalence (58%) was observed in asymptomatic subjects. The differences were found to be significant with the Cuzick test ($z=4.31$, $p<0.001$). An intermediate seropositivity (66%) was found in quarantined at home subjects with “undefined mild or asymptomatic” disease.

Discussion

Serological testing is an essential tool in the management of infectious diseases, including for the diagnosis of

infection, measurement of protective antibody titers upon vaccination, and seroprevalence assessments of immunity in a population. In the case of COVID-19, many issues are still unclear. It is unknown, for example, whether the presence of binding antibody correlates with virus neutralization, whether antibody titers correlate with protection against reinfection and if there is a difference in antibody responses in individuals presenting with severe, mild and asymptomatic COVID-19 [6]. While we have to better understand and clarify all these issues, the role of serological testing in assuring information on prevalence of SARS-CoV-2 infection in different populations is unquestionable [7]. The present study is a large seroprevalence investigation dealing with exposure to SARS-CoV-2 of personnel attending hospitalized and critically ill patients suffering from COVID-19. The survey was carried out with a CLIA assay endowed with high analytical performances [8, 9, 14] and capable to measure (semi)-quantitatively IgM and IgG that recognize both the N and S proteins.

Although the study population cannot be considered representative of the entire healthcare workforce in the Veneto Region, results indicate that, on average, 4.6% of this multicenter cohort seroconverted by production of SARS-CoV-2 IgM and IgG. IgGs were present more often than IgMs, a result that is in keeping with a number of other reports [8–17], thus indicating that this isotype is the predominant in term of anti-SARS-CoV-2 immune response. Other papers have reported very different prevalence of SARS-CoV-2 antibodies in HCW, ranging from 6% [18, 19] to 9.8% [20] and 13.7% [21], respectively.

The prevalence observed in such at risk population reflects the relatively low circulation of SARS-CoV-2 in Veneto Region, much lower compared to other Italian areas where the virus was more widely spread [22, 23]. This reflects the overall incidence data of SARS-CoV-2 in the Veneto Region, with 20,000 cases out of 5 million inhabitants. On these grounds one could reasonably argue that a minority of general population has been exposed to the virus (2–3%), as demonstrated in a recently performed seroprevalence survey in all Italian regions [23]. As a consequence, more than 90% of residents are still likely susceptible to viral infection, an estimate that instructs future preventive strategies in the case of a new wave of the pandemic.

The specificity of the assay was as high as 98%, as assessed in more than 3,000 healthcare workers who repeatedly tested negative by rRT-PCR. A subset of 286 rRT-PCR positive health care professionals, whose blood could be sampled at least 13 days after the nasopharyngeal swab, offered the possibility to test sensitivity of the antibody assay. Only 210 out of 286 blood samples contained

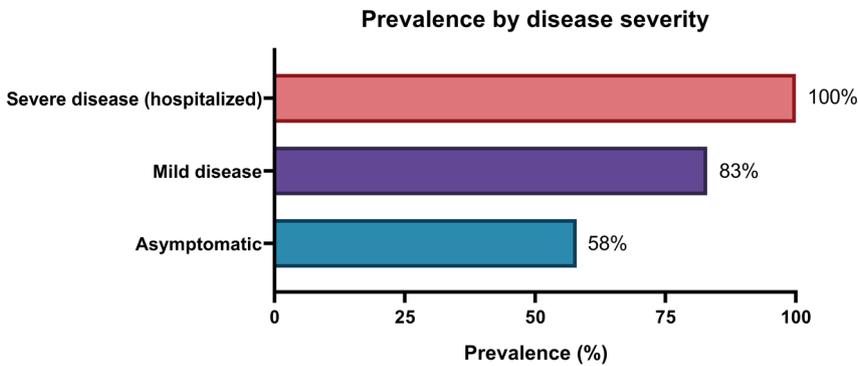


Figure 1: Prevalence of seropositivity in three different groups of subjects, according to disease severity.

detectable anti SARS-CoV-2 IgG and IgM, a result yielding an overall sensitivity of 73%.

Noteworthy, however, a fraction of these HCW failing to produce antibodies were asymptomatic or with mild symptoms of COVID-19, i.e., 42 and 17%, respectively. Symptomatic individuals or those admitted to hospital for medical care has scored 100% antibody positive. This finding confirms previous literature data [24, 25] that indicate a clear correlation between burden of symptoms or disease phenotype and capacity to mount an adaptive immune response. This feature is not unique of COVID-19, but is common to many viral syndromes. In fact, there are always subjects that do not only respond to a viral infection with antibody production, but also fail to show signs of disease. If this is due to a low sensitivity of the available serological assays or to an individual genetic background is a matter of further investigation that also deals with the nature and strength of innate and adaptive immune responses.

Recent data seem to indicate a relevant role of CD4 and CD8 T cells in helping production of antibodies against coronaviruses and clearing viral infection from involved tissues [26, 27]. The study of cell mediated immunity combined with serology could hence be helpful in predicting COVID-19 evolution.

The present study has some limitations. First, the study population cannot be considered representative of the whole healthcare workforce in the Veneto Region, and a selection bias cannot be excluded. Second, the relationships of currently measured antibodies with neutralizing activity against SARS-CoV-2 were not evaluated. A body of evidence, however, demonstrates that immunoassays which recognize antibodies targeting different domains of S protein, including S1, RBD and S2, correlate well with virus neutralization activity [28, 29]. Finally, follow-up data on subjects with detectable SARS-CoV-2 antibodies are not yet available as this follow-up requires several more months to better understand the duration of immune response and protection from reinfection.

Conclusions

The present study defined precisely the circulation of SARS-CoV-2 in a cohort of health workers. These data, and in particular seroprevalence in our setting, may allow to calculate the predictive positive and negative values, thus overcoming the limitations of diagnostic sensitivity and specificity in clinical practice [9, 30]. In addition, our results carry relevant implications for public health policy and control of virus spread in a region that was badly hit by COVID-19. Moreover, the availability of an assay such as that described in this study can help define the immune status of an infected host once its values have been normalized to titers obtained by a classical neutralization assay. Quantitative serological assays will be also extremely helpful in better understanding the immune response to SARS-CoV-2 and in assisting development of new vaccines and further developments of new diagnostic and therapeutic tools.

Research funding: None declared.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: This study was approved by the Regional Committee for Bioethics (prot. n. 178357) and the Ethics Committee of the University Hospital of Verona (prot. n. 22851).

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