Acta Biomed 2021; Vol. 92, N. 1: e2021070 DOI: 10.23750/abm.v92i1.10847

Seroprevalence of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection in an Italian cohort in Marche Region, Italy

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Abstract

Background and aim: The COVID-19 is an infectious disease caused by the novel coronavirus SARS-CoV-2, declared a public health emergency by the World Health Organization. In this study, we evaluated the seroconversion of SARS-CoV-2 antibodies to find predictors of infection in terms of symptoms, health status, and professions. *Methods:* Serological samples of 341 volunteers in a cohort in Marche Region, Italy, were analyzed for the presence of IgM and/or IgG immunoglobulins specific for the SARS-CoV-2. Contextually, an anamnestic questionnaire was administered. The binary logistic regression analysis was used to find the predictors of seroconversion. *Results:* Forty-nine subjects (14.4 %) were found positive, without significant differences between gender and age groups. The predictors identified inside the variable categories "symptoms," "risk factors" (smoking habit and established pathologies), and "professions" were the loss of taste and smell (OR, 8.563), cardiovascular diseases (OR, 2.912), and police officers profession (OR, 3.875), respectively. *Conclusions*: Although the limited number of subjects recruited in this study, our results could give important findings to be considered for planning preventive strategies in the view of the next COVID-19 waves. (www.actabiomedica.it)

Key words: COVID-19, SARS-CoV-2 antibodies, seroprevalence, Italy, risk factors

Introduction

The COVID-19 is an infectious disease caused by the novel coronavirus SARS-CoV-2, declared a public health emergency by the World Health Organization (1). The rapid global spread of SARS-CoV-2 may have been facilitated by unrecognized asymptomatic infection along with high viral load early in the course of infection before people become ill. How the virus is transmitted in the workplace or which workers are principally involved in the virus spread is not clear (2). This information is critical to devise optimal infection prevention strategies to protect both frontline healthcare workers (HCWs)(3) or other work categories during the probable second wave of the COVID-19 pandemic (4,5).

The early identification of SARS-CoV-2 infection is crucial to prevent severe-critical conditions and improve the prognosis. In this context, the identification of symptoms specific to the infection could represent a useful strategy.

The purpose of this study was to a) analyze serological samples in a cohort in the Marche Region, Italy, for the presence of IgM and/or IgG immunoglobulins specific for the SARS-CoV-2, and b) find predictors of infection in terms of symptoms, health status, and professions. Even though the serological tests are not as effective as PCR during the acute

infection (6,7)known as COVID-19. SARS-CoV-2 was discovered in late December 2019 and, since then, has become a global pandemic. Timely and accurate COVID-19 laboratory testing is an essential step in the management of the COVID-19 outbreak. To date, assays based on the reverse-transcription polymerase chain reaction (RT-PCR, they can detect antibodies for a long period after disease recovery. From an epidemiologic point of view, the knowledge of the previous infection and its predictors is crucial and is currently a good tool to plan future preventive strategies.

Methods

The serological survey was conducted in Terre Roveresche, a municipality (70.37 km^2) situated in the province of Pesaro e Urbino (PU) in Le Marche region, Italy, from March to June 2020. Terre Roveresche has a population of 5,189 inhabitants, with a population density of 73.7 inhabitants/km^2. The recruitment was conducted by family and occupational doctors firstly involving workers at greater risk of contagion (i.e. HCWs, shop assistants, and police officers) selected by the Local Health Authority and the municipality, and secondly involving all volunteers who wanted to participate in the survey. A total of 341 subjects were recruited, 176 female, 165 males, aged between 20 and 82 years. A written informed consent was obtained from all of them. The subjects recruited were artisans (N, 29; 8.5 %), bartenders and waiters (N, 13; 3.8 %), homemakers and pensioners (N, 27; 7.9 %), shopkeepers (N, 52; 15.2 %), shop assistants (N, 36; 10.6 %), police officers (N, 22; 6.5 %), HCWs (N, 32; 9.4 %), factory workers (N, 14; 4.1 %), office workers (N, 64; 18.8 %), others (N, 52; 15.2 %).

Serological analysis

Serum samples were collected and analyzed by the social clinic of the no-profit association "Impresa Sociale Fondazione Art32 ONLUS", Montefelcino, PU, Italy, during a monitoring campaign in the working population for the seroprevalence of SARS-CoV-2 specific immunoglobulin. The social clinic of Art32 ONLUS was responsible for organizing the appointments and carrying out blood sampling and analysis. Samples were analyzed for the detection of IgG and IgM anti-SARS-CoV-2 antibodies using two commercially available CE-approved enzyme-linked immunosorbent assays (ELISAs) (Diagnostic Bioprobes Srl, Milano, Italy). The tests contain microplates coated with antigens specific for COVID-19 IgG and IgM. The assays give a ratio between sample OD450nm/620-630nm and the Cut-Off value (calculated by the negative control); the results are interpreted as negative, equivocal, or positive according to values < 0.9, 0.9 – 1.1, or > 1.1, respectively. In this study, only values > 1.1 have been considered indicative of IgM/IgG positivity. The specificity reported is > 98% and > 90% for IgM and IgG assays, respectively; the sensitivity reported is about 98% for both IgM and IgG assays. The assays were conducted following the manufacturer's instructions. Positive subjects were managed by the Local Health Authorities.

Questionnaire

An anamnestic questionnaire specifically designed for this study, was administered to the participants, asking for eventually contact at risk of SARS-CoV-2 contagion, symptoms (fever, cough, cold/sore throat, conjunctivitis, loss of taste/smell, respiratory difficulties), risk factors such as smoking habit, and established pathologies (cardiovascular diseases, diabetes, respiratory diseases, digestive tract diseases), anthropometric values (weight, eight), and profession.

Statistical analysis

The results from the serological survey and questionnaires were blinded analyzed for the statistical evaluation. The chi-square test was used to find differences in the distribution of SARS-CoV-2 positivity in the population divided by gender and age. Spearman's rho coefficients were calculated to find correlations between IgM/IgG positivity and the variables showed in table 2. The binary logistic regression analysis with backward stepwise elimination was performed using IgM/IgG positivity as the dependent variable. Predictors were divided into three groups and included in three different regression models: symptoms (fever,

cough, loss of taste/smell, and respiratory difficulties), risk factors (smoking habit, cardiovascular diseases, diabetes, and respiratory diseases), and professions (artisans, homemakers and pensioners, shopkeepers, shop assistants, police officers, HCWs, and office workers). Odds ratios (OR), corresponding 95% confidence intervals (CI) and p values were estimated. All statistical analyses were performed using the statistical package SPSS (version 17; SPSS Inch., Chicago, IL, USA).

Results

A total of 341 volunteers were recruited in the present study, with a median age of 47 (20-82).

The positivity for IgG and IgM was found in 19 (5.6 %) and 35 (10.3 %) subjects, respectively. Five subjects were found positive for both IgM and IgG. As shown in Table 1, the IgM was mainly revealed in March (22.7 %), reflecting the major circulation of SARS-CoV-2 in this month. On the other hand, the IgG prevalence did not reveal a temporal trend, probably due to the different work categories enrolled during the survey. Hereafter, "positive" subjects were considered if positive to IgM and/or IgG.

In table 2 have been reported the positive subjects stratified for gender and age, revealing any significative differences in both groups ($p<0.05$). Six subjects did the nasopharyngeal swab before the serological test (1-2 months), 4 of which were found positive. All subjects positive for the nasopharyngeal swab were found positive for the SARS-CoV-2 antibodies (IgG: 4 out of 4; IgM: 3 out of 4).

The most represented working classes were artisans (N, 29), bartenders and waiters (N, 13), homemakers and pensioners (N, 27), shopkeepers (N, 52), shop assistants (N, 36), police officers (N, 22), HCWs (N, 32), factory workers (N, 14), office workers (N, 64). The SARS-CoV-2 antibody positivity were found in artisans (N, 4; 13.8%), homemakers and pensioners (N, 6; 22.2 %), shopkeepers (N, 4; 7.7 %), shop assistants (N, 7; 19.4 %), police officers (N, 8; 36.4 %), HCWs (N, 4; 12.5 %), and office workers (N, 9; 14.1 %).

The Spearman's rho coefficients were calculated to find correlations between SARS-CoV-2 antibody positivity and contacts at risk, symptoms, and risk factors. As shown in Table 2, the IgM/IgG positivity was

Table 1. IgM and IgG seroprevalence per month.

	N	IgM	IgG
March	66	15 (22.7)	6 (9.1)
April	52	1 (1.9)	9 (17.3)
May	201	1 (0.5)	16 (8.0)
June	22	2 (9.1)	4 (18.2)
Total	341	19 (5.6)	35 (10.3)

Table 2. Seroprevalence in recruited subjects stratified by gender, age-group, symptoms, and risk factors.

	Tot	Positive (%) [a]	*p–value*
Total subjects	341	49 (14.4)	
Male	165	25 (15.2)	
Female	176	24 (13.6)	*0.690* [b]
Age groups			
20-29	38	4 (10.5)	
30-39	59	8 (13.6)	
40-49	92	10 (10.9)	
50-59	87	14 (16.1)	
60-69	43	9 (20.9)	
>70	10	3 (30.0)	*0.403* [b]
Risk contacts	11	8 (72.7)	*0.192 (0.000)* [c]
Symptoms			
Yes/no	25	9 (36.0)	*0.173 (0.001)* [c]
Fever	16	6 (37.5)	*0.146 (0.007)* [c]
Cough	15	6 (40.0)	*0.157 (0.004)* [c]
Cold/Sore throat	13	1 (7.7)	*ns* [c]
Conjunctivitis	3	0 (0.0)	*ns* [c]
Loss taste/smell	7	4 (57.1)	*0.177 (0.001)* [c]
Respiratory difficulties	5	2 (40.0)	*ns* [c]
Risk factors			
Smoking habit	88	7 (8.0)	*- 0.108 (0.047)* [c]
Cardiovascular diseases	32	9 (28.1)	*0.126 (0.020)* [c]
Diabetes	7	1 (14.3)	*ns* [c]
Respiratory diseases	11	2 (18.2)	*ns* [c]
Digestive tract diseases	7	0 (0.0)	*ns* [c]

[a] IgM and/or IgG positivity
[b] Chi-squared test
[c] Spearman's test

significantly correlated with subjects who had a contact at risk ($p<0.001$), as expected, in a time window of two weeks to 4 months from the blood sampling.

The presence of symptoms significantly correlates with SARS-CoV-2 antibody positivity, particularly with fever, cough, and loss of taste and smell ($p<0.01$). Any significant correlation was found considering cold and sore throat, conjunctivitis, and respiratory difficulties. Forty positive subjects (81.6 %) were found asymptomatic.

Considering risk factors, there was a significant correlation between SARS-CoV-2 antibody positivity and cardiovascular diseases, and a slight but significant inverse correlation was found between positive subjects and smoking habits.

Lastly, the binary logistic regression analysis was conducted to find predictors of SARS-CoV-2 antibody positivity in the variable groups. The IgM/IgG positivity was used as the dependent variable. Variables were divided into three groups and included in three backward stepwise elimination regression models. Variables included were fever, cough, loss of taste/smell, and respiratory difficulties (symptoms group); smoking habit, cardiovascular diseases, diabetes, respiratory diseases (risk factors group); artisans, homemakers and pensioners, shopkeepers, shop assistants, police officers, HCWs, and office workers (professions group). For each group, the identified predictors, 95% CI, and p values are shown in Table 3. The predictors identified for SARS-CoV-2 antibody positivity were loss of taste and smell (OR, 8.563) for symptoms group, cardiovascular diseases (OR, 2.912) for risk factors group, and police officers (OR, 3.875) for profession group. Only significantly associated variables are reported.

Table 3. Binary logistic regression analysis. The backward stepwise elimination regression models were applied to the variable groups *symptoms*, *risk factors*, and *professions*.

Predictors	**Odds Ratio (95% CI)**	***p–value***
Symptoms [a]		
Fever	–[b]	–
Cough	–[b]	–
Loss of taste/smell	8.563 (1.855–39.527)	*0.006*
Respiratory difficulties	–[b]	–
Risk factors [a]		
Smoking habit	0.440 (0.189–1.024)	*ns*
Cardiovascular diseases	2.587 (1.110–6.030)	*0.028*
Diabetes	–[b]	–
Respiratory diseases	–[b]	–
Professions [a]		
Artisans	–[b]	–
Homemakers and pensioners	–[b]	–
Shopkeepers	–[b]	–
Shop assistants	–[b]	–
Police officers	3.875 (1.531–9.805)	*0.004*
HCWs	–[b]	–
Office workers	–[b]	–

[a] Variables entered in the regression models.
[b] Variables removed from the regression models.

Discussion

In this study, the seroprevalence of SARS-CoV-2 antibodies in a cohort of 341 volunteers has been evaluated. As the antibody kinetics is complex – the seroconversion of IgM and IgG could occur simultaneously or sequentially (8) – we considered as positive the subjects tested positive for IgM only, IgG only, or both. We found a seroprevalence of 14.4 %, without significant differences between gender and age groups.

For the period from 1st March to 30th June 2020, the number of positive nasopharyngeal swabs tested for SARS-Cov-2 in the PU province was 2,746 in a total population of 357,137 (0.77 %). In our study group there were 4 subjects positive to the nasopharyngeal swab (1.17 %); considering that the PU population aged between 20-100+ is 294,853 the percentage of the positive nasopharyngeal swab was of 0.93 %, that is in line with our results.

As expected, all subjects positive for the nasopharyngeal swab were found positive for the SARS-CoV-2 antibodies. As reported by others (9), we found that the antibody anti-Sars-Cov-2 could be revealed up to four months after a positive nasopharyngeal swab.

Until now, there are many studies reporting the spread of the new coronavirus in the healthcare system

(10–12), but little is known about other worker categories at risk of infection such as supermarket shop assistants and police officers, who continued to work and have contacts during the lockdown of April and March 2020. Moreover, there is no information on people not working during this period, who may have risky contacts due to people's personal needs to move out of their house. The heterogeneous jobs conducted by the subjects involved in this study allows us to find the riskiest one. The logistic regression analysis revealed the police officers as the work with the great predictive role for the SARS-CoV-2 infection. Moreover, homemakers, pensioners, and supermarket shop assistants were found highly exposed to the infection. These results suggest carrying out checks by the police officers with the utmost caution to reduce the risk of infection both for the agents themselves and for the citizens. It remains equally important to continue with the attention to hygiene in supermarkets, which are confirmed as places where it is easy for both staff and customers to come into contact with the virus. These results also highlight the importance to improve the knowledge about the contagion modalities, planning training courses about the use of the Personal Protective Equipment and preventive behaviors, making attention to the workers outside the healthcare system.

Our data is consistent with recent Italian studies that also show a relatively low seroconversion in HCWs (13,14); other studies also show that the risk of seroconversion is higher outside than within the healthcare system (2,15). This is probably due to the strict infection control measures that are in place in the healthcare system, as suggested in a recent large study in Boston that showed a decrease in the rate of seroconversion followed by the institution of infection control measures (16).

We found that the presence of symptoms was correlated with seroconversion, however, we also found more than 80 % of the seropositive subjects were asymptomatic, confirming that many people do not have symptoms when infected with SARS-CoV-2 and there is a high number of undocumented cases (17). As reported by others, the loss of sense of taste or smell was a strong predictor of infection (2). Even though correlated with the seroconversion, cough, and fever were not identified as predictors. Cold, sore throat, conjunctivitis, and respiratory difficulties were not associated with seroconversion. These results may reflect the wide variability in symptoms experienced during COVID-19 infection and sampling.

Even though these results could be of some importance, several limitations need to be pointed out. First, the limited number of the recruited subjects due to the local setting of this survey is not representative at a regional and national level. Although statistically significative, these findings should be interpreted with caution. Second, the percentage of SARS-CoV-2 positive subjects tested in this study should be compared with caution with the COVID-19 prevalence in the entire population, as the possible over-representation of positivity in the earlier stages of the sampling due to more urgent need to check their status of at-risk workers. Third, excluding the HCWs, police officers, and supermarket shop assistants we have no information on the non-occupational interactions of the participants; for this reason, we could only speculate that personal habits such as supermarket shopping could represent a risky behavior. Fourth, the technical limitation of the kits used in this study should be considered, taking into account a possible presence of a 2 % of false-negative for both IgM and IgG, and a possible presence of a 2 % and 10 % of false-positive for IgM and IgG, respectively, and avoiding the use of these evidence for clinical decision-making(18).

In conclusion, taking into account the limitations of the present study, our results could give important findings to be considered for planning preventive strategies in the view of the imminent COVID-19 second wave.

Acknowledgments: Authors gratefully acknowledge the no-profit association "Impresa Sociale Fondazione Art32 ONLUS" Montefelcino, PU, Italy, and its President Fabio Gant, Dr. Massimo Agostini as Consultant, the municipality of "Terre Roveresche", PU, Italy, and its Major Antonio Sebastianelli.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

Informed consent: A written informed consent was obtained from all subjects.

Funding: This project was partially supported by the no-profit association "Impresa Sociale Fondazione Art32 ONLUS" Montefelcino, PU, Italy, and the municipality of "Terre Roveresche", PU, Italy.

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Received: 23/10/2020
Accepted: 06/11/2020

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