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Seroprevalence of anti-SARS-CoV-2 IgG among healthcare workers of a large university hospital in Milan, Lombardy, Italy: a cross-sectional study

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ABSTRACT

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

Design Cross-sectional study.

Setting Monocentric, Italian, third-level university hospital.

Participants All the employees of the hospital on a voluntary base, for a total of 4055 participants among 4572 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 to selected variables (age, gender, country of origin, body mass index, smoking, symptoms and contact with confirmed cases).

Results From 27 April 2020 to 12 June 2020, 4055 HCWs were tested and 309 (7.6%) had a serological 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 to 7.15) and fever (OR 4.37, 95% CI: 3.11 to 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 serological 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 (eg, Western countries with public health system).

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) have been identified as a group at high risk of infection.[2] The occurrence of nosocomial transmission of SARS-CoV-2 has been well described, emphasising 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 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% to 60% and 13% to 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 serological assessment of SARS-CoV-2 infection has been proposed as screening strategy among both HCWs and the general population. Antibody sensitivity is 30% 1 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 titres did not decline within 6 months after diagnosis.[13–15] Conversely, others have reported a rapid waning over 3 to 4 months.[16 17]

Here we present the results of SARS-CoV-2 serology assessment performed on HCWs from 27 April 2020 to 12 June 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 organisation of our hospital has been modified, and different wards have been entirely dedicated to the management of patients with COVID-19 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 serological assay for SARS-CoV-2 at the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico in Milan, Italy, from 27 April 2020 to 12 June 2020. Of note, the first documented case of COVID-19 in our hospital occurred on 23 February 2020. Policlinico Hospital is one of the leading Italian hospitals in clinical and research activities located in Milan, northern Italy, with more than 4750 HCWs, 900 beds and 36 000 hospitalisations per year. From 21 February 2020, to cope with the COVID-19 emergency, the hospital organisation was quickly modified with the installation of four different pavilions entirely dedicated to the management of patients with COVID-19 to accommodate 350 patients, of which 50 in intensive care units. Specific clinical pathways for patients with COVID-19 were created for critical settings (ie, 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 (ie, patient transportation, dialysis, surgical interventions including childbirth).

The serological 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 patients with COVID-19 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 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 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 χ^2 tests. We then calculated ORs and 95% CIs 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 (4055/4572, 88.7%) participated and autonomously completed a questionnaire.

RESULTS

From 27 April 2020 to 12 June 2020, 4055 HCWs with a mean age of 44.8 years, 2823 women (69.6%) and 1232 men (30.4%), provided a blood sample and completed the questionnaire. The majority were physicians/residents (1292/4055, 31.9%) and nurses/midwives (1230/4055, 30.3%). The overall frequency of workers with a positive test was 309/4055 (7.6%; 95% CI: 6.8% to 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 with 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.

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 to 20.1), who had taken antiviral drugs as treatment or prophylaxis (44/135=32.3%, OR=6.59, 95% CI: 4.51 to 9.65) and who had reported any symptom of SARS-Cov-2 infection in the preceding 4 weeks (225/1511=14.9%, OR=5.12, 95% CI: 3.95 to 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% (134/2372) in HCWs who did not report contacts with a person with COVID-19 and 10.1% (154/1525) 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 with 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% (175/2375), 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 1506 who had never performed NPS (0.3%) had elevated IgG levels.

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% to 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 to 0.81).

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

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, 27 April 2020 to 12 June 2020

Variable	Workers	Positive test				
	N	N	%	P value*	OR†	95% CI†
All	4055	309	7.6			
Country of origin						
Italy	3869	282	7.3	<0.001	1.00	Reference
Other	186	27	14.5		1.82	1.07 to 3.06
Gender						
Women	2823	207	7.3	0.30	1.00	Reference
Men	1232	102	8.3		1.13	0.85 to 1.52
Age (years)						
<30	668	64	9.6	0.02	1.00	Reference
30–39	1018	78	7.7		0.74	0.51 to 1.07
40–49	858	48	5.6		0.46	0.30 to 0.72
50–59	1128	81	7.2		0.64	0.43 to 0.95
60+	383	38	9.9		0.83	0.50 to 1.36
Occupation						
Physicians, including residents	1292	93	7.2	0.006	0.99	0.64 to 1.53
Nurses, midwives	1230	99	8.0		1.31	0.85 to 2.04
Healthcare assistants	320	40	12.5		1.84	1.04 to 3.25
Health technicians‡	585	35	6.0		0.84	0.50 to 1.40
Clerical workers, technicians	628	42	6.7		1.00	Reference
Frontline healthcareworkers						
Never	2061	149	7.2	0.42	1.00	Reference
Ever	1730	137	7.9		0.92	0.69 to 1.24
Missing	264	23	8.7			
BMI						
<20	684	46	6.7	0.04	0.90	0.62 to 1.32
20–24.99	2035	145	7.1		1.00	Reference
25–29.99	945	79	8.4		1.10	0.80 to 1.52
30+	314	31	9.9		1.52	0.98 to 2.35
Missing	77	8	10.4			
Cigarette smoking						
Never	2493	210	8.4	<0.001	1.00	Reference
Former	552	49	8.9		1.12	0.79 to 1.58
Current	842	34	4.0		0.41	0.27 to 0.61
Missing	168	16	9.5			

*From χ^2 test. For BMI: from χ^2 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 and other health technicians.
BMI, body mass index.;

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 patients with COVID-19. Unsurprisingly, a large proportion (84/309, 27.2%) of workers with a positive serology did not report

Table 2 Association between quarantine, symptoms contact with patients with COVID-19, 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, 27 April 2020 to 12 June 2020

Variable	Workers	Positive test				
	N	N	%	P value*	OR†	95% CI†
Quarantine						
No	3629	143	3.9	<0.001	1.00	Reference
Yes	426	166	39.0		15.6	12.0 to 20.1
Any symptom						
No	2544	84	3.3	<0.001	1.00	Reference
Yes	1511	225	14.9		5.12	3.95 to 6.64
Number of symptoms						
1	608	56	9.2	<0.001	2.97	2.09 to 4.22
2	389	45	11.6		3.83	2.62 to 5.60
3	226	38	16.8		5.91	3.93 to 8.93
4	1118	24	20.3		7.48	4.54 to 12.3
5–10	170	62	36.5		16.8	11.5 to 24.6
Contact with COVID-19 case						
Unknown	2372	134	5.6	<0.001	1.00	Reference
Suspected case	335	34	10.1		1.89	1.27 to 2.80
Confirmed case	1190	120	10.1		1.87	1.45 to 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 to 4.52
Missing	543	57	10.5			
Prophylaxis or therapy						
No	3919	265	6.8	<0.001	1.00	Reference
Yes	136	44	32.3		6.59	4.51 to 9.65
Nasopharyngeal swab						
Negative*	2376	175	7.4	<0.001	1.00	Reference
Positive	174	130	74.7		37.1	25.5 to 54.0
Not performed	1506	4	0.3		0.03	0.01 to 0.09

*From χ^2 test. For number of symptoms: from χ^2 test for trend. Missing data not included in analysis.
†From univariate logistic regression models. Missing data not included in analyses.

any symptom in the previous 4 weeks. Yet, HCWs who presented symptoms before the test, were quarantined, or took antiviral drugs as treatment or prophylaxis displayed higher positivity rates compared with those who did not. Interestingly, smokers had a significantly lower prevalence of positive serologies compared with 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. 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 epidemiological scenarios occurring in each Country: SARS-CoV-2 seroprevalence of 1.6%, 3.8%, 5.0%, 9.3%, 19.1%, 24.4%

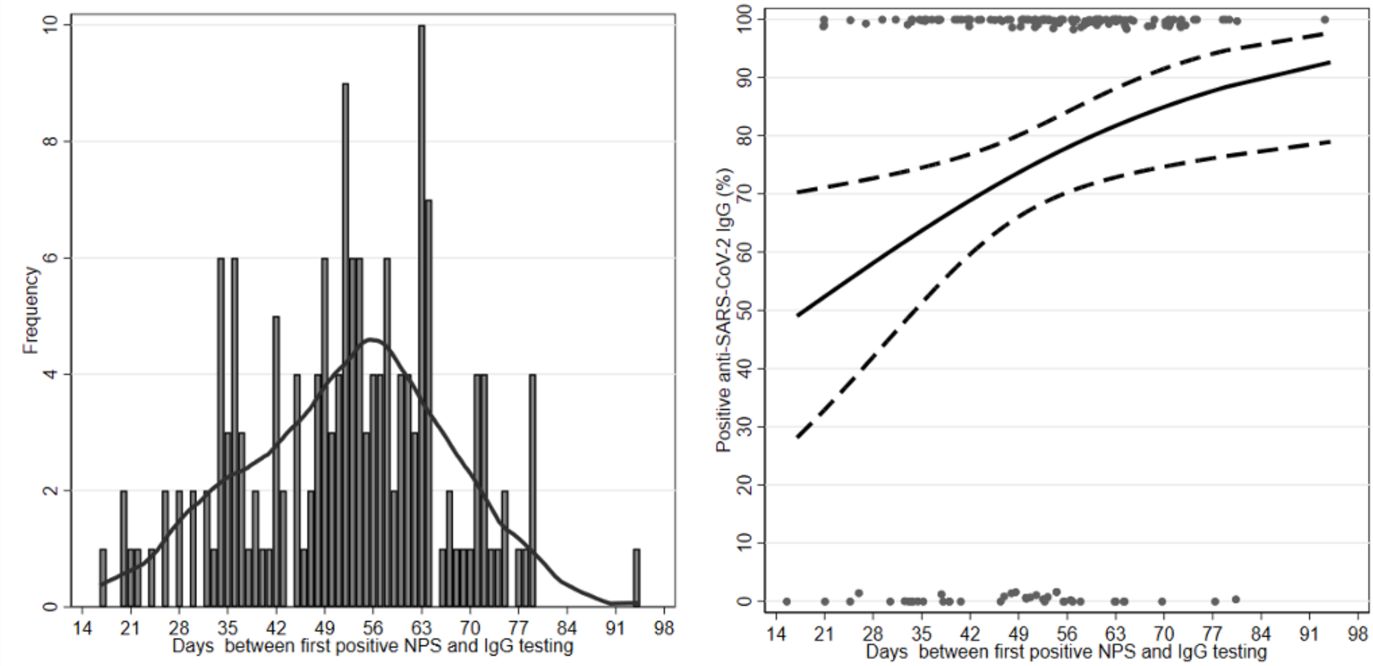

Figure 1 Number of IgG tests (left panel) and percentage of positive IgG tests (right panel) in 162 subjects with a positive nasopharyngeal swab (NPS) prior to serological testing, according to days elapsed since day of first positive NPS. Left panel shows histogram and kernel density smoothing line. 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.

and 33% are reported from studies conducted among HCWs in Germany, China, Netherlands, Spain, Sweden, UK and the USA, respectively.[6 24–29]

Contrasting findings exist regarding the role of direct assistance to patients with COVID-19 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 (eg, 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 with 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 to 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

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, 27 April 2020 to 12 June 2020

	Workers	Positive test				
	N	N	%	P value*	OR†	95% CI†
Specific symptom						
Cough						
No	3523	201	5.7	<0.001	1.00	Reference
Yes	532	108	20.3		1.65	1.18 to 2.30
Fever						
No	3681	190	5.2	<0.001	1.00	Reference
Yes	374	119	31.8		4.37	3.11 to 6.13
Sore throat						
No	3677	261	7.1	<0.001	1.00	Reference
Yes	378	48	12.7		0.57	0.38 to 0.86
Coryza						
No	3882	268	6.9	<0.001	1.00	Reference
Yes	173	41	23.7		1.90	1.21 to 2.98
Headache						
No	3920	277	7.1	<0.001	1.00	Reference
Yes	135	32	23.7		0.96	0.58 to 1.61
Myalgias						
No	3423	216	6.3	<0.001	1.00	Reference
Yes	632	93	14.7		0.77	0.54 to 1.11
Diarrhoea/nausea/vomit						
No	3633	254	7.0	0.006	1.00	Reference
Yes	422	55	13.0		0.85	0.58 to 1.24
Asthenia						
No	3619	199	5.5	<0.001	1.00	Reference
Yes	436	110	25.2		2.67	1.87 to 3.80
Ocular symptoms						
No	3847	281	7.3	0.001	1.00	Reference
Yes	208	28	13.5		0.78	0.46 to 1.32
Dyspnoea						
No	3927	275	7.0	<0.001	1.00	Reference
Yes	128	34	26.6		1.38	0.82 to 2.32
Taste and smell alterations						
No	3915	245	6.3	<0.001	1.00	Reference
Yes	140	64	45.7		4.62	2.99 to 7.15

*From χ^2 test.
†From a multivariable logistic model including all symptoms.

rapid waning over 3 to 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 (175/2375) 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 serological 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 characterises 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 4weeks 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.[32] 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 serological test performance (sensitivity and specificity). Second, some degree of recall bias, that is, under-reporting of mild symptoms which occurred many weeks before serological 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. Third, the serological assessment was not mandatory and was therefore not performed on all HCWs. Nevertheless, considering that the hospital employees are 4572, our study has involved a large fraction of them (4055/4572, 88.7%) and thus provides a fair description of SARS-CoV-2 exposure in HCWs of our hospital. Finally, we could not evaluate the serological 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 correlate 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.

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