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# BMJ Open

## 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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3 1 **Seroprevalence of anti-SARS-CoV-2 IgG among health-care workers is not impacted by**  
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5 2 **frontline activity and mirrors the values of general population**  
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## 23 Abstract

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

26 **Design** Cross-sectional observational study

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

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

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

34 **Results** From April 27 to June 12, 2020, 4,055 HCWs were tested and 309 (7.6%) had a  
35 serologic positive test. No relevant difference was found between men and women (8.3% vs  
36 7.3%), whereas a higher prevalence was observed among foreign-born workers (27/186,  
37 14.5%), employees younger than 30 (64/668, 9.6%) or older than 60 years (38/383, 9.9%)  
38 and among healthcare assistants (40/320, 12.5%). Working as frontline HCWs was not  
39 associated with an increased frequency of positive serology ( $p=0.42$ ). A positive association  
40 was found with presence and number of symptoms ( $p<0.001$ ). The symptoms most frequently  
41 associated with a positive serology were taste and smell alterations (OR 4.62, 95% CI 2.99-  
42 7.15) and fever (OR 4.37, 95%CI 3.11-6.13). No symptoms were reported in 84/309 (27.2%)  
43 HCWs with positive IgG levels. Declared exposure to a suspected/confirmed case was more  
44 frequently associated with positive serology when the contact was a family member (19/94,  
45 20.2%) than a patient or colleague (78/888, 8.8%).

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3 46 **Conclusions** SARS-CoV-2 infection occurred undetected in a large fraction of HCWs and it  
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5 47 was not associated with working in COVID-19 frontline areas. Beyond the hospital setting,  
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7 48 exposure within the community represents an additional source of infection for HCWs.  
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14 50 **Strengths and limitations of this study**

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17 51 • We assessed the prevalence of SARS-CoV-2 antibodies among healthcare workers,  
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19 52 strengthening the fact that working in COVID-19 frontline areas is not associated with  
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21 53 an increased risk of being infected which is more related to exposure within the  
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23 54 community.  
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26 55 • We performed our study on a large cohort of healthcare workers, from an area with a  
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28 56 high incidence of COVID-19.  
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32 57 • Our study was monocentric and performed in Italy, therefore the results may be  
33  
34 58 applicable only to similar scenario (e.g. Western countries with public health system).  
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40 60 **Keywords:** occupational exposure; screening; nosocomial transmission; SARS-COV-2;  
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42 61 COVID-19.  
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48 63 **Funding:** none related to the content of this manuscript.  
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51 64 **Conflict of interests:** none related to the content of this manuscript.  
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## 66 **Introduction**

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

77 In the current pandemic scenario, the optimal method to screen HCWs is still under debate.  
78 At present, the most frequently employed testing strategy is the detection of SARS-CoV-2  
79 RNA through reverse transcriptase–polymerase chain reaction (RT-PCR) on upper  
80 respiratory specimens in symptomatic individuals or in those exposed to confirmed cases of  
81 COVID-19. Unfortunately, the testing strategy based solely on upper respiratory specimens  
82 has significant limitations. In a large metanalysis, the rate of positive nasopharyngeal swabs  
83 (NPS) ranged from 25% to 80% and decreased with time and in asymptomatic or pauci-  
84 symptomatic cases [9]. Of note, no data on test sensitivity in asymptomatic infected  
85 individuals exists, and clinical symptoms of COVID-19 among infected HCWs are often  
86 relatively mild, with fever and dyspnoea reported in 38-60% and 13-47% of cases,  
87 respectively [2,3,7,8,10]. It is also not uncommon for HCWs to work with mild symptoms  
88 [8,11], which increases the hazard of nosocomial outbreaks.



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3 89 More recently, the serologic assessment of SARS-CoV-2 infection has been proposed as  
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5 90 screening strategy among both HCWs and the general population. Antibody sensitivity is  
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8 91 30% one week after symptoms onset and rises to 70% and >90% at 2 and 3 weeks,  
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10 92 respectively [12]. Hence, the most useful role for serology consists in detecting previous  
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12 93 SARS-CoV-2 infection as screening strategy in exposed or high-risk HCWs.

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15 94 Here we present the results of SARS-CoV-2 serology assessment performed on HCWs from  
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17 95 April 27, 2020 to June 12, 2020 at the Fondazione IRCCS Ca' Granda Ospedale Maggiore  
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19 96 Policlinico located in Milan, Lombardy, by far the Italian region mostly affected by COVID-  
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21 97 19. To cope with the COVID-19 emergency, the organization of our Hospital has been  
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23 98 modified, and different wards have been entirely dedicated to the management of COVID-19  
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25 99 patients to accommodate 350 of them [13]. We evaluated the association between positive  
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27 100 tests and demographic characteristics, occupation and working environment (frontline vs non-  
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29 101 frontline HCWs). In addition, we assessed the frequency of positive tests in HCWs with  
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31 102 previous symptoms of COVID-19 or who had been quarantined or in contact with suspected  
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33 103 or proven COVID-19 cases.

## 34 104 **Methods**

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39 105 We collected occupational and clinical characteristics of all the consecutive HCWs who  
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41 106 performed a serologic assay for SARS-CoV-2 at the Fondazione IRCCS Ca' Granda  
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43 107 Ospedale Maggiore Policlinico in Milan, Italy from April 27 to June 12, 2020. Policlinico  
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45 108 Hospital is one of the leading Italian hospitals in clinical and research activities located in  
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47 109 Milan, northern Italy, with more than 4,750 HCWs, 900 beds and 36,000 hospitalization per  
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49 110 year. From 21 February 2020, to cope with the COVID-19 emergency, the hospital  
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51 111 organization was quickly modified with the installation of four different pavilions entirely  
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53 112 dedicated to the management of COVID-19 patients to accommodate 350 patients, of which  
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3 113 50 in intensive care units (13). Specific clinical pathways for COVID-19 patients were  
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5 114 created for critical settings (*i.e.*, triage and emergency ward, operating rooms, radiology  
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7 115 department) and several internal guidelines were implemented and periodically updated.  
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9 116 Trainings on donning and doffing of personal protective equipment (PPE) were provided by  
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11 117 the infectious disease specialists and anaesthesiologists to the HCWs working in COVID-19  
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13 118 areas. Trainings were targeted to physicians, nurses and health assistants and consisted in  
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15 119 brief reviews on COVID-19 clinical and epidemiological issues, set-up of COVID-19 wards  
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17 120 in contaminated, buffer and clean areas, guidance on proper use of PPE in patient daily care  
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19 121 and in specific situations (*i.e.*, patient transportation, dialysis, surgical interventions including  
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21 122 childbirth).

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26 123 The serologic assay was offered freely to all hospital HCWs. At blood drawing, HCWs were  
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28 124 asked to complete a questionnaire containing demographics, occupational and clinical  
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30 125 characteristics. Information on age, gender, nationality, body mass index (BMI), smoking and  
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32 126 comorbidities (hypertension, diabetes, immunosuppressive therapies, cardiac, respiratory or  
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34 127 renal chronic diseases) was registered. HCWs were stratified by working environment in  
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36 128 frontline and non-frontline workers (whether they provided direct assistance to COVID-19  
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38 129 patients or not) and by job title in physicians (including residents), nurses and midwives,  
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40 130 healthcare assistants, health technicians, and clerical workers and technicians. The presence  
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42 131 of any of the following symptoms since the end of February 2020 was collected: fever,  
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44 132 cough, dyspnoea, diarrhoea, nausea or vomit, ageusia/dysgeusia or anosmia/parosmia,  
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46 133 rhinorrhoea, ocular symptoms, sore throat, headache, myalgia, asthenia. The presence of any  
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48 134 of the following risk factors for previous exposure to SARS-CoV-2 was investigated:  
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50 135 performance of NPS (date and results), prophylaxis for SARS-CoV-2 infection (day and type  
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52 136 of medication), home quarantine (period), contact with suspected or proven COVID-19 cases  
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54 137 (date and type of exposure).  
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3 138 The study was approved by the institutional review board (368\_2020bis) of our hospital and  
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5 139 was conducted in accordance with the Helsinki Declaration.  
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8 140 *SARS-CoV-2 serology*  
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11 141 SARS-CoV-2 serology was performed with LIAISON® SARS-CoV-2 S1/S2 IgG test on  
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13 142 LIAISON® XL (DiaSorin, Saluggia, Italy). The test is a chemiluminescent immunoassay  
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15 143 (CLIA) that detects quantitative anti-S1 and anti-S2 specific IgG antibodies against SARS-  
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17 144 CoV-2 in human serum. The test has, after >15 days from the infection, a declared sensitivity  
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19 145 of 97.4% and a specificity of 98.5%. A test was considered positive when the value observed  
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21 146 was equal to or above 15 AU/mL [14].  
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26 147 *Statistical analysis*  
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29 148 We calculated the adjusted seroprevalence using the formula: adjusted prevalence =  
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31 149 (observed prevalence + specificity – 1)/(sensitivity + specificity – 1) [15], where sensitivity  
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33 150 and specificity were those declared by the manufacturer.  
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36 151 We compared the prevalence of positive tests according to selected variables using chi-  
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38 152 squared tests. We then calculated odds ratios (OR) and 95% confidence intervals (CI) by  
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40 153 fitting a multivariable logistic regression model containing the following covariates: country  
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42 154 of origin, gender, age class, occupation, frontline work, BMI class, and cigarette smoking.  
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44 155 For other variables (quarantine, symptoms, contact with COVID-19 case,  
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46 156 prophylaxis/therapy, and NPS), we used univariate logistic models. We evaluated the  
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48 157 discriminating ability of the number of reported symptoms in a multivariable logistic  
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50 158 regression model containing all groups of symptoms. Area under the ROC curve (AUC) was  
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52 159 calculated after these models. To verify possible changes in IgG positivity over time, among  
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54 160 HCWs with a previous positive nasopharyngeal swab (NPS), we analysed the percentage of  
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3 161 subjects with elevated IgG levels according to the days elapsed since the first positive NPs  
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5 162 using logistic regression. Statistical analysis was performed with Stata 16 (StataCorp. 2019).  
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### 8 163 *Patient and Public Involvement*

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11 164 The serologic assessment was freely offered to all the healthcare workers of our hospital. The  
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13 165 majority of them (4,055/4,572, 88.7%) participated and autonomously completed a  
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15 166 questionnaire.  
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### 18 167 **Results**

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21 168 From April 27 to June 12, 2020, 4,055 HCWs with a mean age of 44.8 years, 2,823 women  
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23 169 (69.6%) and 1,232 men (30.4%), provided a blood sample and completed the questionnaire.  
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25 170 The majority were physicians/residents (1,292/4,055, 31.9%) and nurses/midwives  
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27 171 (1,230/4,055, 30.3%). The overall frequency of workers with a positive test was 309/4,055  
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29 172 (7.6%; 95% CI: 6.8-8.5%) (Table 1). The prevalence adjusted for declared test sensitivity and  
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31 173 specificity would be 6.4%. The frequency of positive tests was almost double among workers  
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33 174 from abroad (14.5%) compared to those of Italian ancestry (7.3%), whereas women and men  
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35 175 had a similar prevalence. The highest frequencies of a positive test were observed in the  
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37 176 lowest (<30 years) and highest ( $\geq 60$  years) age classes. Across HCWs' job titles, a significant  
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39 177 higher prevalence was detected among healthcare assistants (40/320, 12.5%), while weak  
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41 178 differences were found for the other occupations (6.0% to 8.0%). No difference was observed  
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43 179 between frontline and non-frontline HCWs (7.2% vs 7.9%). There was a positive trend of test  
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45 180 positivity according to BMI, while current smokers had less than half the prevalence of test  
46  
47 181 positivity than former and never smokers (4.0%, 8.9% and 8.5%, respectively). No  
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49 182 association was found between test results and comorbidities (hypertension, diabetes, cardiac,  
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51 183 respiratory, or renal chronic diseases) or being on immunosuppressive treatment (data not  
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53 184 shown). All findings of the univariate analyses were confirmed in the multivariable analysis.  
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**Table 1.** Association between selected variables and prevalence of positive tests (anti-SARS-CoV-2 IgG $\geq$ 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.

191 \*\*From a multivariable logistic regression model including country of origin, gender, age,  
192 occupation, frontline area, BMI, and smoking. Missing data not included in analyses.  
193 \*\*\*Includes biologists, radiology and laboratory technicians, psychologists, other health  
194 technicians  
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196 Serology results stratified according to risk factors for previous exposure to SARS-CoV-2 are  
197 reported in Table 2. A significant higher seropositivity was found among HCWs who had  
198 been quarantined (166/426=39.0%, OR=15.6 95% CI: 12.0-20.1), who had taken antiviral  
199 drugs as treatment or prophylaxis (44/135=32.3%, OR=6.59, 95%CI: 4.51-9.65) and who had  
200 reported any symptom of SARS-Cov-2 infection in the preceding four weeks  
201 (225/1,511=14.9%, OR=5.12, 95%CI: 3.95-6.64). We observed a clear monotonic increasing  
202 trend in test positivity with number of symptoms, from 56/608 (9.2%) among HCWs with  
203 just one symptom to 62/170 (36.5%) in those with five or more. Conversely, no symptom was  
204 reported in 84/309 HCWs with positive serological test (27.2%). The prevalence of positive  
205 tests was 5.6% (134/2,372) in HCWs who did not report contacts with a person with COVID-  
206 19 and 10.1% (154/1,525) in those who reported contacts with suspected or confirmed cases.  
207 Of note, prevalence of IgG positivity more than doubled if the reported contact was a family  
208 member (19/94=20.2%) compared to a patient or a colleague (78/888=8.8%). HCWs who had  
209 undergone SARS-CoV-2 NPS with negative result had a frequency of positive serology of  
210 7.4% (175/2,375), almost the same as the overall hospital seroprevalence. On the contrary,  
211 the percentage of IgG positivity was much higher (74.7%, 130/174) in those who had a  
212 positive NPS. In 162 subjects NPS had been performed before serology, while in 12 HCWs  
213 NPS was performed because of a positive serology. Only four workers among the 1,506 who  
214 had never performed NPS (0.3%) had elevated IgG levels.

215 **Table 2.** Association between quarantine, symptoms contact with COVID-19 patients, and  
216 prophylaxis and prevalence of positive tests (anti-SARS-CoV-2 IgG $\geq$ 15 AU/mL) among  
217 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

218 Abbreviations: CI, confidence interval; OR, odds ratio.

219 \*From chi-squared test. For number of symptoms: from chi-squared test for trend. Missing  
220 data not included in analysis.

221 \*\*From univariate logistic regression models. Missing data not included in analyses.

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223 There were 162 subjects with a positive NP swab before IgG testing. Among these, IgG  
224 testing was performed between 17 and 94 days (Figure 1, left panel), with a peak between 49  
225 and 63 days; the majority (159, 96.1%) were tested at least 21 days since the first positive  
226 swab. The percentage of positive IgG tests (N=121) increased linearly (in the logit scale) over

227 time (Figure 1, right panel); it was 50-60% between 17 and 28 days, reaching 80% only after  
 228 60 days since the first positive NP swab.

229 For every specific symptom, there was a positive association with elevated IgG levels (Table  
 230 3). Specifically, strong associations emerged with fever (19/374=31.8%) and with taste or  
 231 smell alterations (64/140=45.7%). In a multivariable model, these two symptoms were  
 232 confirmed as the strongest predictors of positive test (both ORs>4). Other symptoms  
 233 associated with positive SARS-CoV-2 serology were asthenia (OR=2.67), coryza (OR=1.90),  
 234 and cough (OR=1.65), while sore throat was negatively associated with test positivity  
 235 (OR=0.57). The AUC from the model containing all symptoms was 0.74 (95% CI: 0.74-  
 236 0.81).

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

	Workers	Positive test				
	N	N	%	p-value*	OR**	95% CI**
<b>Specific symptom</b>						
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

240 Abbreviations: CI, confidence interval; OR, odds ratio.

241 \*From chi-squared test.

242 \*\*From a multivariable logistic model including all symptoms.

## 243 Discussion

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

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3 257 Our results are in accordance with the data presented by Sandri and colleagues, who  
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5 258 described a rate of positive SARS-CoV-2 serologies (in their study defined as IgG>12  
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7 259 AU/mL) ranging from 6.4% to 9% among the HCWs of three different hospitals in Milan  
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9 260 [16]. In the same study the authors described a higher seroprevalence, between 35% and  
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11 261 43%, in HCWs from Bergamo district, one of the areas in northern Italy most affected by  
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13 262 COVID-19. These results are corroborated by the data provided by the Bergamo Health  
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15 263 Authority, which reported a SARS-CoV-2 seroprevalence of 30.6% among HCWs from the  
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17 264 Bergamo metropolitan area (15). Noteworthy is thus the fact that seroprevalence among  
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19 265 HCWs mirrors the levels encountered in the general population, ranging from 7.1% and  
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21 266 56.9% in the Milan and Bergamo metropolitan area, respectively [17,18]. Wide variations in  
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23 267 seroprevalence among HCWs are reported worldwide, reflecting the distinct epidemiologic  
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25 268 scenarios occurring in each Country: SARS-CoV-2 seroprevalence of 1.6%, 3.8%, 5.0%,  
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27 269 9.3%, 19.1%, 24.4% and 33% are reported from studies conducted among HCWs in  
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29 270 Germany, China, Netherlands, Spain, Sweden, United Kingdom and the USA, respectively  
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31 271 [6,19–24].  
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38 272 Contrasting findings exist regarding the role of direct assistance to COVID-19 patients on the  
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40 273 risk of SARS-CoV-2 infections in HCWs. Comparing frontline to non-frontline workers, we  
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42 274 observed no difference in seroprevalence rates, in line with the findings of Mani and  
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44 275 colleagues [7]. At the same time, we observed a significantly higher seroprevalence among  
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46 276 healthcare assistants (40/320, 12.5%), with all the other occupations (physician, nurses and  
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48 277 midwives, technicians) below 8%. A similar seroprevalence (11.8%) was observed among  
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50 278 healthcare assistants during the SARS pandemic in 2004 [25]. These results may suggest that,  
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52 279 when nosocomial transmission occurs, it mainly involves those workers who have the closest  
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54 280 contact with patients (e.g. healthcare assistants who take care of patients' primary needs) and  
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56 281 might therefore be at the highest risk. This condition may also reflect on the higher  
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3 282 seroprevalence detected among HCWs from abroad. Indeed, a large fraction of this group is  
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5 283 composed by healthcare assistants (46%). When looking at healthcare assistants only,  
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8 284 seroprevalence in workers from abroad was twice as high (20%) than in workers of Italian  
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10 285 ancestry (9.8%).  
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13 286 What appears from our results is that SARS-CoV-2 transmission largely occurred from close  
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15 287 contacts within the hospital in absolute terms (78 HCWs had contact with patients or  
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17 288 colleagues, against 19 at home). However, in relative terms the prevalence was higher outside  
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20 289 the hospital: in fact, HCWs who reported contacts with suspected or confirmed COVID-19  
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22 290 cases within the family had a prevalence of high IgG more than twice that of workers whose  
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24 291 contacts were patients or colleagues (20.2% vs 8.8%, respectively). Similar results of family  
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26 292 contacts as likely source of infection were reported by Sandri et al. with even higher  
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29 293 percentages (31.2%) [16] and were further corroborated by the molecular analyses performed  
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31 294 by Sikkema et al. [6].  
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34 295 Regarding the lower prevalence of positive serologies among smokers, a protective effect of  
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36 296 smoking on the risk of infection is unlikely. The lower seroprevalence we observed among  
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38 297 smokers might reflect the influence of smoking on major components of both innate and  
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41 298 adaptive immune cells [26]. Particularly, a decreased production of IgA, IgG and IgM has  
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43 299 been observed in smokers if compared to non-smokers [27].  
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46 300 In our study, the positivity rate of anti-SARS-CoV-2 IgG in HCWs who had a positive NPS  
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48 301 (130/174, 74.7%) is sensibly lower than the values reported by the manufacturer, which  
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50 302 reports a sensitivity of 90.7% and 97.9% at 5-15 and >15 days after infection, respectively  
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53 303 [14]. On the other hand, we found that 7.4% of workers with negative NPS (175/2,375) had  
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55 304  $\text{IgG} \geq 15$  AU/mL. Unfortunately, we are unable to ascertain what proportion is due to lack of  
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58 305 NPS sensitivity and what arises from imperfect specificity of IgG test. In fact, our study was  
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3 306 not designed to assess the sensitivity of the serologic test. Further reports of real-life data are  
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5 307 therefore needed.  
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8 308 Finally, positive serology was associated with a recent history of typical symptoms of SARS-  
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10 309 CoV-2 infection, especially taste and smell alterations and fever. These findings corroborate  
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12 310 previous observations made by our group who identified taste and smell alterations and fever  
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14 311 as the symptoms most frequently reported in HCWs with SARS-Cov-2 positivity on NPS  
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16 312 [10]. Other authors confirmed the same observations, suggesting that anosmia is the symptom  
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18 313 which better characterizes COVID-19 [16,21,22]. Notably, a large fraction of HCWs with  
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20 314 positive serology (84/309, 27.2%) did not report any symptom in the four weeks before the  
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22 315 test. This finding is also well-described in COVID-19 epidemiology, where the rate of  
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24 316 asymptomatic or pauci-symptomatic infected persons ranges from 1.6% to 56.5% depending  
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26 317 on subject characteristics and on the analysed country [28]. Unfortunately, in hospital settings  
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28 318 the absence of symptoms makes it difficult to identify infected HCWs and hampers many  
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30 319 strategies to control the infection.  
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36 320 The first limitation of our work has been noted above: this study was performed for health  
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38 321 surveillance purposes and thus not designed to evaluate serologic test performance  
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40 322 (sensitivity and specificity). Secondly, some degree of recall bias, i.e., under-reporting of  
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42 323 mild symptoms which occurred many weeks before serologic test, is a possibility. In this  
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44 324 case, we may have overestimated the proportion of asymptomatic workers with elevated IgG.  
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46 325 Yet, considering that the study started at the end of April 2020, and that the COVID-19  
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48 326 pandemic in Lombardy begun at the end of February, we probably missed only a small  
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50 327 percentage of subjects with clinical manifestations. Thirdly, the serologic assessment was not  
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52 328 mandatory and was therefore not performed on all HCWs. Nevertheless, considering that the  
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54 329 hospital employees are 4,572, our study has involved a large fraction of them (4,055/4,572,  
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56 330 88.7%) and thus provides a fair description of SARS-CoV-2 exposure in HCWs of our  
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3 331 Hospital. Finally, we could not evaluate the serologic status of all HCWs in a single day. As  
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5 332 the epidemic was still ongoing, even though on a much smaller scale (the zenith of the  
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8 333 infection was in March), we may have missed a few new infections.  
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11 334 What is suggested by our study, and by those similarly performed in the same area in the  
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13 335 context of the ongoing pandemic [16], is that the observed seroprevalence rate reflects the  
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15 336 spread of infection in the community served by the hospital. Assuming that PPE is provided  
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17 337 and correctly employed by all HCWs, hospitals do not seem to act as an epicentre of the  
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19 338 infection. In our study, healthcare assistants showed the highest seroprevalence rate. We do  
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21 339 believe that education and training of all HCWs should be strongly supported. Periodic  
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23 340 training of correct use of PPE and infection control procedures should be addressed not only  
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25 341 to physicians and nurses but also to other healthcare professionals.  
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30 342 The fact that more than one quarter of SARS-CoV-2 infections occurred unnoticed supports  
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32 343 the implementation of systematic testing strategies among HCWs without an ascertained  
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34 344 history of infection. Unfortunately, the best testing strategy as well as the timing and setting  
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36 345 in which these tests have the highest performance is still uncertain. Future studies should  
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38 346 address these gaps of knowledge. As of now, we deem it is important to monitor periodically  
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40 347 SARS-CoV-2 serology in HCWs to correlates the seroprevalence rates with those of general  
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42 348 population and detect any discrepancy. This will allow to implement timely and effective  
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44 349 infection control measures, thus preventing hospitals to become drivers of future COVID-19  
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46 350 outbreaks.  
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3 352 **Contributorship statement:** AL, DM, DC, AB and AG conceived the study. LC, PB, APC,  
4  
5 353 BT, MC, GL, ACP, LR and FC collected the data and performed the serologic survey. DC  
6  
7 354 performed the statistical analyses. AL, DM, DC wrote the first draft of the manuscript. All  
8  
9 355 authors revised the final version of the manuscript.  
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13 356 **Data sharing statement:** raw data will be provided on reasonable request contacting the  
14  
15 357 corresponding author.  
16  
17

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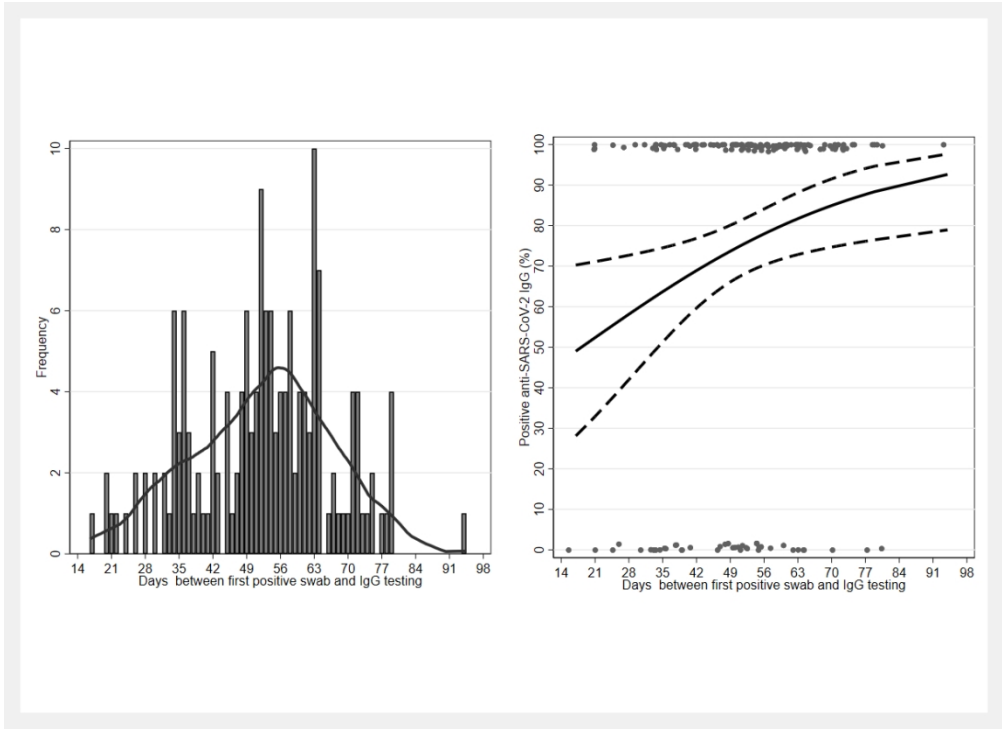
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3 445 **Figures**  
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6 446 **Figure 1**  
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9 447 Number of IgG tests (left panel) and percentage of positive IgG tests (right panel) in 162  
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11 448 subjects with a positive nasopharyngeal swab prior to serological testing, according to days  
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14 449 elapsed since day of first positive nasopharyngeal swab.  
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17 450 Left panel shows histogram and kernel density smoothing line. In right panel circles indicate  
18 451 subjects with negative (lower circles, N=41) or positive (upper circles, N=121) anti-SARS-  
19 452 CoV-2 IgG, solid and dashed lines are the predicted percentages calculated with a logistic  
20 453 regression model, and dashed lines are 95% bands around the predicted.  
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✓STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

	Item No	Recommendation
<b>Title and abstract</b>	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
<b>Introduction</b>		
Background/rationale	2✓	Explain the scientific background and rationale for the investigation being reported
Objectives	3✓	State specific objectives, including any prespecified hypotheses
<b>Methods</b>		
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
<b>Results</b>		
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

1	Other analyses	17✓	Report other analyses done—eg analyses of subgroups and interactions, and
2			sensitivity analyses
3			
4	<b>Discussion</b>		
5	Key results	18✓	Summarise key results with reference to study objectives
6	Limitations	19✓	Discuss limitations of the study, taking into account sources of potential bias or
7			imprecision. Discuss both direction and magnitude of any potential bias
8			
9	Interpretation	20✓	Give a cautious overall interpretation of results considering objectives, limitations,
10			multiplicity of analyses, results from similar studies, and other relevant evidence
11	Generalisability	21✓	Discuss the generalisability (external validity) of the study results
12			
13	<b>Other information</b>		
14	Funding	22✓	Give the source of funding and the role of the funders for the present study and, if
15			applicable, for the original study on which the present article is based
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18 \*Give information separately for exposed and unexposed groups.

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

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

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3 1 **Seroprevalence of anti-SARS-CoV-2 IgG among health-care workers of a large**  
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5 2 **university Hospital in Milan, Lombardy, Italy**  
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## 23 Abstract

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

26 **Design** Cross-sectional observational study

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

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

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

34 **Results** From April 27 to June 12, 2020, 4,055 HCWs were tested and 309 (7.6%) had a  
35 serologic positive test. No relevant difference was found between men and women (8.3% vs  
36 7.3%,  $p=0.3$ ), whereas a higher prevalence was observed among foreign-born workers  
37 (27/186, 14.5%,  $p<0.001$ ), employees younger than 30 (64/668, 9.6%,  $p=0.02$ ) or older than  
38 60 years (38/383, 9.9%,  $p=0.02$ ) and among healthcare assistants (40/320, 12.5%,  $p=0.06$ ).  
39 Working as frontline HCWs was not associated with an increased frequency of positive  
40 serology ( $p=0.42$ ). A positive association was found with presence and number of symptoms  
41 ( $p<0.001$ ). The symptoms most frequently associated with a positive serology were taste and  
42 smell alterations (OR 4.62, 95% CI 2.99-7.15) and fever (OR 4.37, 95% CI 3.11-6.13). No  
43 symptoms were reported in 84/309 (27.2%) HCWs with positive IgG levels. Declared  
44 exposure to a suspected/confirmed case was more frequently associated ( $p<0.001$ ) with  
45 positive serology when the contact was a family member (19/94, 20.2%) than a patient or  
46 colleague (78/888, 8.8%).

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3 47 **Conclusions** SARS-CoV-2 infection occurred undetected in a large fraction of HCWs and it  
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5 48 was not associated with working in COVID-19 frontline areas. Beyond the hospital setting,  
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7 49 exposure within the community represents an additional source of infection for HCWs.  
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14 51 **Strengths and limitations of this study**

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17 52 • The serologic test employed in our study has, after >15 days from the infection, a  
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19 53 declared sensitivity of 97.4% and a specificity of 98.5%.
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22 54 • We performed our study on a large cohort of healthcare workers, from an area with a  
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24 55 high incidence of COVID-19.
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27 56 • Our study was monocentric and performed in Italy, therefore the results may be  
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29 57 applicable only to similar scenarios (e.g. Western countries with public health  
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31 58 system).  
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38 60 **Keywords:** occupational exposure; screening; nosocomial transmission; SARS-COV-2;  
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40 61 COVID-19.  
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46 63 **Funding:** none related to the content of this manuscript.  
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49 64 **Conflict of interests:** none related to the content of this manuscript.  
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## 66 **Introduction**

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

77 In the current pandemic scenario, the optimal method to screen HCWs is still under debate.  
78 At present, the most frequently employed testing strategy is the detection of SARS-CoV-2  
79 RNA through reverse transcriptase–polymerase chain reaction (RT-PCR) on upper  
80 respiratory specimens in symptomatic individuals or in those exposed to confirmed cases of  
81 COVID-19. Unfortunately, the testing strategy based solely on upper respiratory specimens  
82 has significant limitations. In a large meta-analysis, the rate of positive nasopharyngeal swabs  
83 (NPS) ranged from 25% to 80% and decreased with time and in asymptomatic or pauci-  
84 symptomatic cases [9]. Of note, no data on test sensitivity in asymptomatic infected  
85 individuals exists, and clinical symptoms of COVID-19 among infected HCWs are often  
86 relatively mild, with fever and dyspnoea reported in 38-60% and 13-47% of cases,  
87 respectively [2,3,7,8,10]. It is also not uncommon for HCWs to work with mild symptoms  
88 [8,11], which increases the hazard of nosocomial outbreaks.

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3 89 More recently, the serologic assessment of SARS-CoV-2 infection has been proposed as  
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5 90 screening strategy among both HCWs and the general population. Antibody sensitivity is  
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8 91 30% one week after symptoms onset and rises to 70% and >90% at 2 and 3 weeks,  
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10 92 respectively [12]. Hence, the most useful role for serology consists in detecting previous  
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12 93 SARS-CoV-2 infection as screening strategy in exposed or high-risk HCWs. Little is known  
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14 94 about the duration of humoral immune response to SARS-CoV-2 infection. In some studies  
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16 95 antibody titers did not decline within 6 months after diagnosis [13–15]. Conversely, others  
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18 96 have reported a rapid waning over 3–4 months [16,17].

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22 97 Here we present the results of SARS-CoV-2 serology assessment performed on HCWs from  
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24 98 April 27, 2020 to June 12, 2020 at the Fondazione IRCCS Ca' Granda Ospedale Maggiore  
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26 99 Policlinico located in Milan, Lombardy, by far the Italian region mostly affected by COVID-  
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28 100 19. To cope with the COVID-19 emergency, the organization of our hospital has been  
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30 101 modified, and different wards have been entirely dedicated to the management of COVID-19  
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32 102 patients to accommodate 350 of them [18]. We evaluated the association between positive  
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34 103 tests and demographic characteristics, occupation and working environment (frontline vs non-  
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36 104 frontline HCWs). In addition, we assessed the frequency of positive tests in HCWs with  
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38 105 previous symptoms of COVID-19 or who had been quarantined or in contact with suspected  
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40 106 or proven COVID-19 cases.

## 41 42 43 44 45 107 **Methods**

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49 108 We collected occupational and clinical characteristics of all the consecutive HCWs who  
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51 109 performed a serologic assay for SARS-CoV-2 at the Fondazione IRCCS Ca' Granda  
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53 110 Ospedale Maggiore Policlinico in Milan, Italy from April 27 to June 12, 2020. Of note, the  
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55 111 first documented case of COVID-19 in our hospital occurred on February 23, 2020.  
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57 112 Policlinico hospital is one of the leading Italian hospitals in clinical and research activities  
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3 113 located in Milan, northern Italy, with more than 4,750 HCWs, 900 beds and 36,000  
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5 114 hospitalization per year. From 21 February 2020, to cope with the COVID-19 emergency, the  
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7 115 hospital organization was quickly modified with the installation of four different pavilions  
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9 116 entirely dedicated to the management of COVID-19 patients to accommodate 350 patients, of  
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11 117 which 50 in intensive care units (13). Specific clinical pathways for COVID-19 patients were  
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13 118 created for critical settings (*i.e.*, triage and emergency ward, operating rooms, radiology  
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15 119 department) and several internal guidelines were implemented and periodically updated.  
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17 120 Trainings on donning and doffing of personal protective equipment (PPE) were provided by  
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19 121 the infectious disease specialists and anaesthesiologists to the HCWs working in COVID-19  
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21 122 areas. Trainings were targeted to physicians, nurses and health assistants and consisted in  
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23 123 brief reviews on COVID-19 clinical and epidemiological issues, set-up of COVID-19 wards  
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25 124 in contaminated, buffer and clean areas, guidance on proper use of PPE in patient daily care  
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27 125 and in specific situations (*i.e.*, patient transportation, dialysis, surgical interventions including  
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29 126 childbirth).

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36 127 The serologic assay was offered freely to all hospital HCWs. At blood drawing, HCWs were  
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38 128 asked to complete a questionnaire containing demographics, occupational and clinical  
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40 129 characteristics. Information on age, gender, nationality, body mass index (BMI), smoking and  
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42 130 comorbidities (hypertension, diabetes, immunosuppressive therapies, cardiac, respiratory or  
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44 131 renal chronic diseases) was registered. HCWs were stratified by working environment in  
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46 132 frontline and non-frontline workers (whether they provided direct assistance to COVID-19  
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48 133 patients or not) and by job title in physicians (including residents), nurses and midwives,  
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50 134 healthcare assistants, health technicians, and clerical workers and technicians. The presence  
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52 135 of any of the following symptoms since the end of February 2020 was collected: fever,  
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54 136 cough, dyspnoea, diarrhoea, nausea or vomit, ageusia/dysgeusia or anosmia/parosmia,  
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58 137 rhinorrhoea, ocular symptoms, sore throat, headache, myalgia, and asthenia. The presence of  
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3 138 any of the following indicators of previous exposure to SARS-CoV-2 was investigated:  
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5 139 previous NPS (date and results), prophylaxis for SARS-CoV-2 infection (day and type of  
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7 140 medication), home quarantine (period), and contact with suspected or proven COVID-19  
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9 141 cases (date and type of exposure).  
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13 142 The study was approved by the institutional review board (368\_2020bis) of our hospital and  
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15 143 was conducted in accordance with the Helsinki Declaration.  
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#### 18 144 *SARS-CoV-2 serology*

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21 145 SARS-CoV-2 serology was performed with LIAISON® SARS-CoV-2 S1/S2 IgG test on  
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23 146 LIAISON® XL (DiaSorin, Saluggia, Italy). The test is a chemiluminescent immunoassay  
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25 147 (CLIA) that detects quantitative anti-S1 and anti-S2 specific IgG antibodies against SARS-  
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27 148 CoV-2 in human serum. The test has, after >15 days from the infection, a declared sensitivity  
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29 149 of 97.4%, and a specificity of 98.5%. A test was considered positive when the value observed  
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31 150 was equal to or above 15 AU/mL [19].  
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#### 36 151 *Statistical analysis*

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39 152 We calculated the adjusted seroprevalence using the formula: adjusted prevalence =  
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41 153 (observed prevalence + specificity – 1)/(sensitivity + specificity – 1) [20], where sensitivity  
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43 154 and specificity were those declared by the manufacturer.  
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46 155 We compared the prevalence of positive tests according to selected variables using chi-  
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48 156 squared tests. We then calculated odds ratios (OR) and 95% confidence intervals (CI) by  
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50 157 fitting a multivariable logistic regression model containing the following covariates: country  
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52 158 of origin, gender, age class, occupation, frontline work, BMI class, and cigarette smoking.  
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54 159 For other variables (quarantine, symptoms, contact with COVID-19 case,  
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56 160 prophylaxis/therapy, and NPS), we used univariate logistic models. We evaluated the  
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58 161 discriminating ability of the number of reported symptoms in a multivariable logistic  
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3 162 regression model containing all groups of symptoms. Area under the ROC curve (AUC) was  
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5 163 calculated after these models. To verify possible changes in IgG positivity over time, among  
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7 164 HCWs with a previous positive NPS, we analysed the percentage of subjects with elevated  
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9 165 IgG levels according to the days elapsed since the first positive NPS using logistic regression.  
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12 166 Statistical analysis was performed with Stata 16 (StataCorp. 2019).

### 15 167 *Patient and Public Involvement*

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18 168 The serologic assessment was freely offered to all the healthcare workers of our hospital. The  
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20 169 majority of them (4,055/4,572, 88.7%) participated and autonomously completed a  
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23 170 questionnaire.

### 26 171 **Results**

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29 172 From April 27 to June 12, 2020, 4,055 HCWs with a mean age of 44.8 years, 2,823 women  
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31 173 (69.6%) and 1,232 men (30.4%), provided a blood sample and completed the questionnaire.  
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33 174 The majority were physicians/residents (1,292/4,055, 31.9%) and nurses/midwives  
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35 175 (1,230/4,055, 30.3%). The overall frequency of workers with a positive test was 309/4,055  
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37 176 (7.6%; 95% CI: 6.8-8.5%) (Table 1). The prevalence adjusted for declared test sensitivity and  
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40 177 specificity would be 6.4%. The frequency of positive tests was almost double among workers  
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42 178 from abroad (14.5%) compared to those of Italian ancestry (7.3%), whereas women and men  
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44 179 had a similar prevalence. The highest frequencies of a positive test were observed in the  
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47 180 lowest (<30 years) and highest ( $\geq 60$  years) age classes. Across HCWs' job titles, a significant  
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49 181 higher prevalence was detected among healthcare assistants (40/320, 12.5%), while weak  
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51 182 differences were found for the other occupations (6.0% to 8.0%). No difference was observed  
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53 183 between frontline and non-frontline HCWs (7.2% vs 7.9%). There was a positive trend of test  
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55 184 positivity according to BMI, while current smokers had less than half the prevalence of test  
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57 185 positivity than former and never smokers (4.0%, 8.9% and 8.5%, respectively). No  
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186 association was found between test results and comorbidities (hypertension, diabetes, cardiac,  
187 respiratory, or renal chronic diseases) or being on immunosuppressive treatment (data not  
188 shown). All findings of the univariate analyses were confirmed in the multivariable analysis.

189 **Table 1.** Association between selected variables and prevalence of positive tests (anti-SARS-  
190 CoV-2 IgG $\geq$ 15 AU/mL) among healthcare workers in a large university hospital, Milan,  
191 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			

192 Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.

193 \*From chi-squared test. For BMI: from chi-squared test for trend. Missing data not included  
194 in analyses.

195 \*\*From a multivariable logistic regression model including country of origin, gender, age,  
196 occupation, frontline area, BMI, and smoking. Missing data not included in analyses.

197 \*\*\*Includes biologists, radiology and laboratory technicians, psychologists, other health  
198 technicians

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200 Serology results stratified according to risk factors for previous exposure to SARS-CoV-2 are

201 reported in Table 2. A significant higher seropositivity was found among HCWs who had

202 been quarantined (166/426=39.0%, OR=15.6 95% CI: 12.0-20.1), who had taken antiviral

203 drugs as treatment or prophylaxis (44/135=32.3%, OR=6.59, 95%CI: 4.51-9.65) and who had

204 reported any symptom of SARS-Cov-2 infection in the preceding four weeks

205 (225/1,511=14.9%, OR=5.12, 95%CI: 3.95-6.64). We observed a clear monotonic increasing

206 trend in test positivity with number of symptoms, from 56/608 (9.2%) among HCWs with

207 just one symptom to 62/170 (36.5%) in those with five or more. Conversely, no symptom was

208 reported in 84/309 HCWs with positive serologic test (27.2%). The prevalence of positive

209 tests was 5.6% (134/2,372) in HCWs who did not report contacts with a person with COVID-

210 19 and 10.1% (154/1,525) in those who reported contacts with suspected or confirmed cases.

211 Of note, prevalence of IgG positivity more than doubled if the reported contact was a family

212 member (19/94=20.2%) compared to a patient or a colleague (78/888=8.8%). HCWs who had

213 undergone SARS-CoV-2 NPS with negative result had a frequency of positive serology of

214 7.4% (175/2,375), almost the same as the overall hospital seroprevalence. On the contrary,

215 the percentage of IgG positivity was much higher (74.7%, 130/174) in those who had a

216 positive NPS. In 162 subjects NPS had been performed before serology, while in 12 HCWs

217 NPS was performed after the detection of a positive serology. Only four workers among the

218 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 $\geq$ 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

228 days; the majority (159, 96.1%) were tested at least 21 days since the first positive swab. The  
 229 percentage of positive IgG tests (N=121) increased linearly (in the logit scale) over time  
 230 (Figure 1, right panel); it was 50-60% between 17 and 28 days, reaching 80% only after 60  
 231 days since the first positive NPS.

232 For every specific symptom, there was a positive association with elevated IgG levels (Table  
 233 3). Specifically, strong associations emerged with fever (19/374=31.8%) and with taste or  
 234 smell alterations (64/140=45.7%). In a multivariable model, these two symptoms were  
 235 confirmed as the strongest predictors of positive test (both ORs>4). Other symptoms  
 236 associated with positive SARS-CoV-2 serology were asthenia (OR=2.67), coryza (OR=1.90),  
 237 and cough (OR=1.65), while sore throat was negatively associated with test positivity  
 238 (OR=0.57). The AUC from the model containing all symptoms was 0.74 (95% CI: 0.74-  
 239 0.81).

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

	Workers	Positive test				
	N	N	%	p-value*	OR**	95% CI**
<b>Specific symptom</b>						
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

243 Abbreviations: CI, confidence interval; OR, odds ratio.

244 \*From chi-squared test.

245 \*\*From a multivariable logistic model including all symptoms.

## 246 Discussion

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

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3 259 smokers. Finally, among symptoms, fever and smell and taste alteration were those more  
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5 260 frequently associated with IgG positivity.  
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8 261 Our results are in accordance with the data presented by Sandri and colleagues, who  
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10 262 described a rate of positive SARS-CoV-2 serologies (in their study defined as IgG>12  
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12 263 AU/mL) ranging from 6.4% to 9% among the HCWs of three different hospitals in Milan  
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14 264 [21]. In the same study the authors described a higher seroprevalence, between 35% and  
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16 265 43%, in HCWs from Bergamo district, one of the areas in northern Italy most affected by  
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18 266 COVID-19. These results are corroborated by the data provided by the Bergamo Health  
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20 267 Authority, which reported a SARS-CoV-2 seroprevalence of 30.6% among HCWs from the  
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22 268 Bergamo metropolitan area (15). Noteworthy is thus the fact that seroprevalence among  
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24 269 HCWs mirrors the levels encountered in the general population, ranging from 7.1% and  
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26 270 56.9% in the Milan and Bergamo metropolitan area, respectively [22,23]. Wide variations in  
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28 271 seroprevalence among HCWs are reported worldwide, reflecting the distinct epidemiologic  
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30 272 scenarios occurring in each Country: SARS-CoV-2 seroprevalence of 1.6%, 3.8%, 5.0%,  
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32 273 9.3%, 19.1%, 24.4% and 33% are reported from studies conducted among HCWs in  
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34 274 Germany, China, Netherlands, Spain, Sweden, United Kingdom and the USA, respectively  
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36 275 [6,24–29].  
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43 276 Contrasting findings exist regarding the role of direct assistance to COVID-19 patients on the  
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45 277 risk of SARS-CoV-2 infections in HCWs. Comparing frontline to non-frontline workers, we  
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47 278 observed no difference in seroprevalence rates, in line with the findings of Mani and  
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49 279 colleagues [7]. At the same time, we observed a significantly higher seroprevalence among  
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51 280 healthcare assistants (40/320, 12.5%), with all the other occupations (physician, nurses and  
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53 281 midwives, technicians) below 8%. A similar seroprevalence (11.8%) was observed among  
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55 282 healthcare assistants during the SARS pandemic in 2004 [30]. These results may suggest that,  
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57 283 when nosocomial transmission occurs, it mainly involves those workers who have the closest  
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3 284 contact with patients (e.g. healthcare assistants who take care of patients' primary needs) and  
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5 285 might therefore be at the highest risk. This condition may also reflect on the higher  
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7 286 seroprevalence detected among HCWs from abroad. Indeed, a large fraction of this group is  
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10 287 composed by healthcare assistants (46%). When looking at healthcare assistants only,  
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12 288 seroprevalence in workers from abroad was twice as high (20%) than in workers of Italian  
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15 289 ancestry (9.8%).

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18 290 What appears from our results is that SARS-CoV-2 transmission largely occurred from close  
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20 291 contacts within the hospital in absolute terms (78 HCWs had contact with patients or  
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22 292 colleagues, against 19 at home). However, in relative terms the prevalence was higher outside  
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24 293 the hospital: in fact, HCWs who reported contacts with suspected or confirmed COVID-19  
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26 294 cases within the family had a prevalence of high IgG more than twice that of workers whose  
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28 295 contacts were patients or colleagues (20.2% vs 8.8%, respectively). Similar results of family  
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30 296 contacts as likely source of infection were reported by Sandri et al. with even higher  
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33 297 percentages (31.2%) [21] and were further corroborated by the molecular analyses performed  
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36 298 by Sikkema et al. [6].

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39 299 Regarding the lower prevalence of positive serologies among smokers, a protective effect of  
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41 300 smoking on the risk of infection is unlikely. The lower seroprevalence we observed among  
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43 301 smokers might reflect the influence of smoking on major components of both innate and  
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45 302 adaptive immune cells [31]. Particularly, a decreased production of IgA, IgG and IgM has  
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48 303 been observed in smokers if compared to non-smokers [32].

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51 304 In our study, the positivity rate of anti-SARS-CoV-2 S1/S2 IgG in HCWs who had a positive  
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53 305 NPS (130/174, 74.7%) is sensibly lower than the values reported by the manufacturer, which  
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55 306 reports a sensitivity of 90.7% and 97.9% at 5-15 and >15 days after infection, respectively  
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58 307 [19]. Of note, 53/162 (32.7%) of the tested workers performed serology 2 or more months  
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3 308 after first NPS positivity (Figure 1, left panel), and it is currently unknown for how long  
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5 309 antibodies persist following SARS-CoV-2 infection. While in some studies antibody titres did  
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8 310 not decline within 6 months after diagnosis [13–15], others reported a rapid waning over 3–4  
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10 311 months [16,17]. In our cohort the percentage of positive IgG tests increased monotonically  
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12 312 over time (Figure 1, right panel), supporting the persistence of anti-SARS-CoV-2 S1/S2 IgG  
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14 313 up to 3 months from NPS positivity. On the other hand, we found that 7.4% of workers with  
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16 314 negative NPS (175/2,375) had  $\text{IgG} \geq 15$  AU/mL. Unfortunately, we are unable to ascertain  
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18 315 what proportion is due to lack of NPS sensitivity and what arises from imperfect specificity  
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20 316 of IgG test. In fact, our study was not designed to assess the accuracy of the serologic test.  
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23 317 Further reports of real-life data are therefore needed.

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26 318 Finally, positive serology was associated with a recent history of typical symptoms of SARS-  
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28 319 CoV-2 infection, especially taste and smell alterations and fever. These findings corroborate  
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30 320 previous observations made by our group who identified taste and smell alterations and fever  
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32 321 as the symptoms most frequently reported in HCWs with SARS-Cov-2 positivity on NPS  
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34 322 [10]. Other authors confirmed the same observations, suggesting that anosmia is the symptom  
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36 323 which better characterizes COVID-19 [21,26,27]. Notably, a large fraction of HCWs with  
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38 324 positive serology (84/309, 27.2%) did not report any symptom in the four weeks before the  
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41 325 test. This finding is also well-described in COVID-19 epidemiology, where the rate of  
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43 326 asymptomatic or pauci-symptomatic infected persons ranges from 1.6% to 56.5% depending  
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45 327 on subject characteristics and on the analysed country [33]. Unfortunately, in hospital settings  
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47 328 the absence of symptoms makes it difficult to identify infected HCWs and hampers many  
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50 329 strategies to control the infection.

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54 330 The first limitation of our work has been noted above: this study was performed for health  
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56 331 surveillance purposes and thus not designed to evaluate serologic test performance  
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59 332 (sensitivity and specificity). Secondly, some degree of recall bias, i.e., under-reporting of

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3 333 mild symptoms which occurred many weeks before serologic test, is a possibility. In this  
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5 334 case, we may have overestimated the proportion of asymptomatic workers with elevated IgG.  
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8 335 Yet, considering that the study started at the end of April 2020, and that the COVID-19  
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10 336 pandemic in Lombardy begun at the end of February, we probably missed only a small  
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12 337 percentage of subjects with clinical manifestations. Thirdly, the serologic assessment was not  
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14 338 mandatory and was therefore not performed on all HCWs. Nevertheless, considering that the  
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16 339 hospital employees are 4,572, our study has involved a large fraction of them (4,055/4,572,  
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18 340 88.7%) and thus provides a fair description of SARS-CoV-2 exposure in HCWs of our  
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20 341 hospital. Finally, we could not evaluate the serologic status of all HCWs in a single day. As  
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22 342 the epidemic was still ongoing, even though on a much smaller scale (the zenith of the  
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24 343 infection was in March), we may have missed a few new infections.

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29 344 What is suggested by our study, and by those similarly performed in the same area in the  
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31 345 context of the ongoing pandemic [21], is that the observed seroprevalence rate reflects the  
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33 346 spread of infection in the community served by the hospital. Assuming that PPE is provided  
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35 347 and correctly employed by all HCWs, hospitals do not seem to act as an epicentre of the  
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37 348 infection. In our study, healthcare assistants showed the highest seroprevalence rate. We do  
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39 349 believe that education and training of all HCWs should be strongly supported. Periodic  
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41 350 training of correct use of PPE and infection control procedures should be addressed not only  
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43 351 to physicians and nurses but also to other healthcare professionals.

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48 352 The fact that more than one quarter of SARS-CoV-2 infections occurred unnoticed supports  
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50 353 the implementation of systematic testing strategies among HCWs without an ascertained  
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52 354 history of infection. Unfortunately, the best testing strategy as well as the timing and setting  
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54 355 in which these tests have the highest performance is still uncertain. Future studies should  
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56 356 address these gaps of knowledge. As of now, we deem it is important to monitor periodically  
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58 357 SARS-CoV-2 serology in HCWs to correlates the seroprevalence rates with those of general

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3 358 population and detect any discrepancy. This will allow to implement timely and effective  
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5 359 infection control measures, thus preventing hospitals to become drivers of future COVID-19  
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8 360 outbreaks.  
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For peer review only

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3 362 **Contributorship statement:** AL, DM, DC, AB and AG conceived the study. LC, PB, APC,  
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5 363 BT, MC, GL, ACP, LR, AM and FC collected the data and performed the serologic survey.  
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7 364 DC performed the statistical analyses. AL, DM, DC wrote the first draft of the manuscript.  
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10 365 All authors revised the final version of the manuscript.  
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15 367 corresponding author.  
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17  
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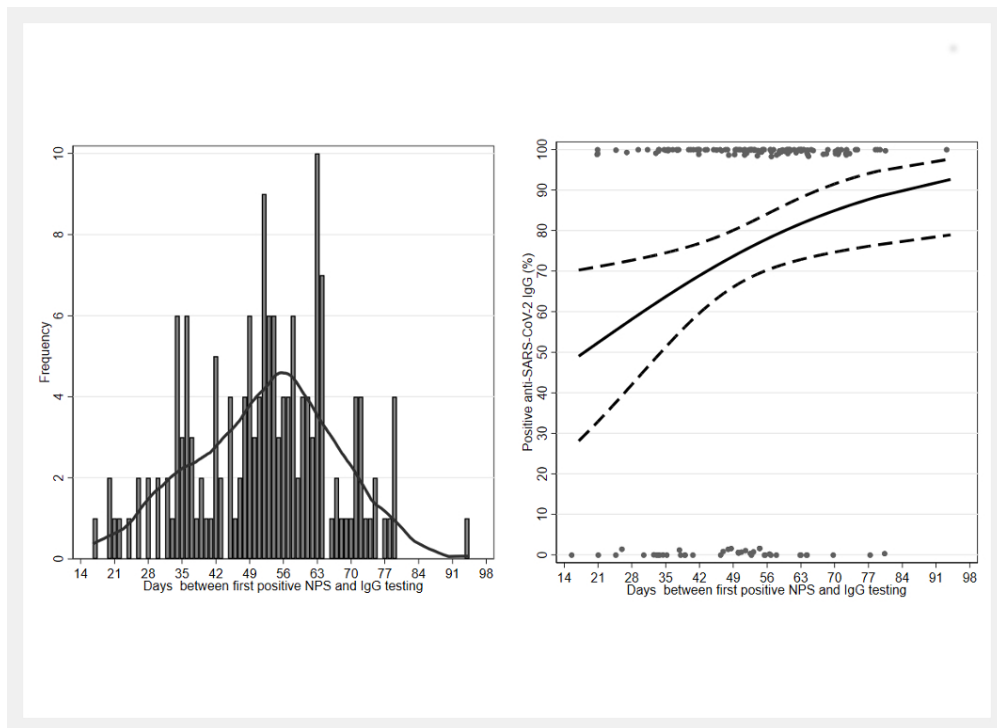
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3 472 **Figures**  
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6 473 **Figure 1**  
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9 474 Number of IgG tests (left panel) and percentage of positive IgG tests (right panel) in 162  
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11 475 subjects with a positive nasopharyngeal swab prior to serologic testing, according to days  
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13 476 elapsed since day of first positive nasopharyngeal swab.  
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17 477 Left panel shows histogram and kernel density smoothing line. In right panel circles indicate  
18 478 subjects with negative (lower circles, N=41) or positive (upper circles, N=121) anti-SARS-  
19 479 CoV-2 IgG, solid and dashed lines are the predicted percentages calculated with a logistic  
20 480 regression model, and dashed lines are 95% bands around the predicted.  
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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.

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

	Item No	Recommendation
<b>Title and abstract</b>	1	(a) Indicate the study's design with a commonly used term in the title or the abstract <b>p. 2</b> (b) Provide in the abstract an informative and balanced summary of what was done and what was found <b>p. 2-3</b>
<b>Introduction</b>		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported <b>p. 4-5</b>
Objectives	3	State specific objectives, including any prespecified hypotheses <b>p. 5</b>
<b>Methods</b>		
Study design	4✓	Present key elements of study design early in the paper <b>p. 5-6</b>
Setting	5✓	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection <b>p. 5-6</b>
Participants	6✓	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <b>p. 5-6</b> (b) For matched studies, give matching criteria and number of exposed and unexposed <b>n/a</b>
Variables	7✓	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable <b>p. 6-8</b>
Data sources/ measurement	8*✓	For each variable of interest, give sources of data and details of methods of assessment (measurement). <b>p. 6-8</b> Describe comparability of assessment methods if there is more than one group <b>n/a</b>
Bias	9✓	Describe any efforts to address potential sources of bias <b>p. 16</b>
Study size	10✓	Explain how the study size was arrived at <b>p. 5-6</b>
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why <b>p. 7-8</b>
Statistical methods	12✓	(a) Describe all statistical methods, including those used to control for confounding <b>p. 7-8</b> (b) Describe any methods used to examine subgroups and interactions <b>p. 7-8</b> (c) Explain how missing data were addressed <b>n/a</b> (d) If applicable, explain how loss to follow-up was addressed <b>n/a</b> (e) Describe any sensitivity analyses <b>p. 7-8</b>
<b>Results</b>		
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>p. 8</b> (b) Give reasons for non-participation at each stage <b>n/a</b> (c) Consider use of a flow diagram <b>n/a</b>
Descriptive data	14*✓	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders <b>p. 8-13</b> (b) Indicate number of participants with missing data for each variable of interest <b>n/a</b> (c) Summarise follow-up time (eg, average and total amount) <b>p. 8-13</b>
Outcome data	15*✓	Report numbers of outcome events or summary measures over time <b>p. 8-13</b>
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>p. 8-13</b>
		(b) Report category boundaries when continuous variables were categorized <b>p. 8-13</b>
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period <b>p. 8-13</b>
Other analyses	17✓	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses <b>p. 8-13</b>
<b>Discussion</b>		
Key results	18✓	Summarise key results with reference to study objectives <b>p. 13</b>
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 <b>p. 16-17</b>
Interpretation	20✓	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence <b>p. 14-16</b>
Generalisability	21✓	Discuss the generalisability (external validity) of the study results <b>p. 17</b>
<b>Other information</b>		
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 <b>p. 3</b>

\*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>.