SARS-CoV-2-specific neutralizing antibody responses in Norwegian healthcare workers after the first wave of COVID-19 pandemic: a prospective cohort study

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Summary: Low numbers of SARS-CoV-2 seropositive HCW were found in Norway and 1.8% of HCW seroconverted with neutralizing antibodies. Seropositivity was higher than RT-PCR positivity in HCW with 36% asymptomatic representing a risk of infection within the healthcare setting.

Abstract

Background: During the coronavirus disease 2019 (COVID-19) pandemic, many countries experienced infection in healthcare workers (HCW) due to overburdened healthcare systems. However, whether infected HCW acquire protective immunity against SARS-CoV-2 is unclear. Here, we characterized SARS-CoV-2-specific antibody responses in Norwegian HCW in a prospective cohort study.

Methods: We enrolled 607 HCW pre- and post-the first COVID-19-pandemic wave. Exposure history, COVID-19-like symptoms and serum samples were collected. SARS-CoV-2-specific antibodies were characterized by spike-protein IgG/IgM/IgA enzyme-linked immunosorbent and live-virus neutralization assays.

Results: Spike-specific IgG, IgM, and IgA antibodies increased after the first pandemic wave in HCW with COVID-19-patient exposure, but not in HCW without patient exposure. Thirty-two HCW (5.3%) had spike-specific antibodies (11 seroconverted with ≥4-fold increase, 21 were seropositive at baseline). Neutralizing antibodies were found in 11 HCW that seroconverted, of whom 4 (36.4%) were asymptomatic. Ninety-seven HCW were tested by reverse-transcriptase-polymerase chain reaction (RT-PCR) during follow-up, 8 were positive (7 seroconverted and 1 had undetectable antibodies).

Conclusions: We found increases in SARS-CoV-2-neutralizing antibodies in infected HCW, especially after COVID-19-patient exposure. Our data show a low number of SARS-CoV-2-seropositive HCW in a low prevalence setting, however, the proportion of seropositivity was higher than RT-PCR positivity, highlighting the importance of antibody testing.

Keywords: Healthcare workers; COVID-19; SARS-CoV-2; spike protein; antibody characterization; IgG; IgM; IgA; neutralizing antibody; seroconversion

Background

The novel severe acute respiratory-syndrome coronavirus 2 (SARS-CoV-2) causing coronavirus disease 2019 (COVID-19) emerged in Wuhan, China in late December 2019 [1]. Cases increased dramatically in January 2020, before lockdown was implemented, which brought the outbreak under control. However, the virus had spread to other countries and Europe became the epicenter of the pandemic in March 2020. The high numbers of cases and associated mortality, overwhelmed healthcare services in Italy[2,3], leading many countries to implement lockdown to reduce the spread of SARS-CoV-2 and protect their citizens.

Healthcare workers (HCW) in contact with COVID-19 patients are at higher risk of occupational infection, up to 11.6-fold, compared to other HCW and the community [4-6]. The overwhelming hospital admission rates of severely ill patients, exhausting the healthcare resources, have globally resulted in thousands of infected HCW [5-9] and hundreds of deaths [10-12].

Current testing for SARS-CoV-2 relies on amplification of the viral genome using reverse transcriptase-polymerase chain reaction (RT-PCR) during the acute infection, whereas serological assays can determine infection over a longer period. In China, 17% of HCW were seropositive after exposure to COVID-19 patients, despite testing negative by RT-PCR [13]. Serological assays detecting antibodies to the SARS-CoV-2 spike protein and its receptor-binding domain (RBD) provide an important tool for examining infection in healthcare settings [14].

COVID-19 cases in Norway were first detected on February 26th [15] and increased rapidly before a lockdown was implemented on March 12th, with the pandemic peaking on March 26th. The local epidemic period is well defined with early and prioritized RT-PCR testing of HCW with COVID-19 patient-exposure or COVID-19-like symptoms. This enabled detailed follow-up of infected HCW and determination of the extent of undiagnosed asymptomatic or mild illness, which can fuel the spread of virus in hospitals and the community. In this study, we enrolled 607 HCW before their exposure to COVID-19 patients to investigate antibody responses to SARS-CoV-2, infection rates and associated risk factors during the first COVID-19 pandemic wave in Bergen, Norway.

Methods

Study design

We conducted a prospective cohort study of HCW working in healthcare facilities testing and treating COVID-19 patients (Bergen Municipality Emergency Room, Haukeland University and Haraldsplass Deaconess Hospitals), which was approved by the Western Norway Ethics committee (#118664). Inclusion criteria were HCW with or without present/potential future exposure to COVID-19 patients, and working during March 6th to April 9th. Exclusion criteria were HCW in quarantine or RT-PCR-confirmed SARS-CoV-2 infection at recruitment. All HCW provided written informed consent before inclusion and serum samples were collected at baseline and 6-10 weeks later (before and after the peak of the first COVID-19 pandemic wave) (supplementary-Figure 1). Sera were coded with a unique identification number, aliquoted and stored at -80^{o}C, and heat-inactivated for 1 hour at 56°C before use.

Case report form (CRF)

A cloud-based CRF was developed using REDCap electronic data capture tools [16] to collect relevant clinical and demographic data, such as recent travel history, contact with suspected or confirmed COVID-19 patients, use of personal protective equipment (PPE), intercurrent illnesses including respiratory disease (fever, dry cough, difficulty breathing, sore throat, myalgia, malaise and any other relevant symptoms) and RT-PCR results (Table 1).

Antigens and viruses

The SARS-CoV-2 RBD and spike proteins were produced and purified as previously described [14]. The live SARS-CoV-2/Human/NOR/Bergen1/2020 virus was isolated *in-house* from the throat swabs of a Norwegian RT-PCR-confirmed patient and propagated in Vero cells before use in neutralization assays.

Enzyme-linked immunosorbent assay (ELISA)

Sera were tested in a 2-step ELISA process: screening ELISA for high-throughput detection of RBD-reactive samples followed by a confirmatory spike protein ELISA, with minor modifications [14]. Paired baseline and follow-up sera (diluted 1:100) were tested in duplicates in 96-well plates to detect total immunoglobulins (Sigma-Aldrich) binding to the RBD protein using 3,3´,5,5´-tetramethylbenzidine (TMB) (BDbiosciences). A negative control panel of pre-pandemic sera (n=128) and a positive control panel of RT-PCR-confirmed COVID-19 patient sera (n=43) were used to define the negative and positive cutoffs, respectively, based upon the optical density (OD) at 450/620nm (Supplementary-Figure 2). Positive (OD >0.708) or intermediate (OD >0.430) sera by screening ELISA were titrated, starting from 1:100, to detect IgG (Sigma-Aldrich) binding to the RBD and spike proteins. A pre-pandemic sera pool, a hospitalized patient serum, and the human monoclonal antibody reactive to both SARS-CoV-1 and 2 (CR3022) were used as controls. The mean endpoint titer was calculated for each sample. Positive sera with spike-specific IgG endpoint titers above 3 standard deviations of the mean of pre-pandemic negative control pool (>485) were further titrated to detect spike-specific IgM and IgA (Sigma-Aldrich). Samples with undetectable antibodies were assigned an endpoint titer of 50 for calculation purpose. HCW were defined as seroconverters if they had ≥4-fold increase in antibody titres from baseline to follow up.

Neutralization assays

Paired baseline and follow-up sera with positive or intermediate results by RBD screening ELISA were tested in the microneutralization (MN) assay and positive MN samples were further tested in the virus neutralization (VN) assay, performed in a certified Biosafety Level-3 Laboratory using the live hCoV-19/Norway/Bergen-01/2020 (GISAID accession ID EPI_ISL_541970) virus. ***MN assay***: Briefly, sera were serially diluted, starting from 1:20, and mixed with 100 tissue-culture infectious dose 50% ($TCID_{50}$) virus in 96-well plates. The mixtures were incubated for 1 hour at 37^{o}C before transferring to 96-well plates pre-seeded with Vero cells for 24-hour incubation at 37^{o}C. Cells were fixed and permeabilized with methanol and 0.6% H_2O_2 and incubated with the anti-

SARS-CoV-2-nucleoprotein rabbit-monoclonal IgG (Sino Biological), then anti-rabbit biotinylated goat IgG (H+L) (Southern Biotech), extravidin-peroxidase (Sigma-Aldrich) and substrate o-Phenylenediamine dihydrochloride (OPD, Sigma-Aldrich). The MN titer was the reciprocal of the serum dilution giving 50% inhibition of virus infectivity. ***VN assay***: As above, except that the serum/virus/cells were incubated for 4-5 days at 37^{o}C. All wells were examined under a microscope for cytopathic effect. The VN titer was determined as the reciprocal of the highest serum dilution giving 100% inhibition of virus infectivity (no cytopathic effect). Titers <20 were assigned a value of 10 for calculation purpose.

Statistics

ELISA endpoint titers and MN titers were calculated using Prism-v.8.4.2 (GraphPad). Demographic, clinical characteristics and serological data of occupationally exposed groups were examined using Chi-squared tests and adjusted for confounding variables in generalized mixed-effect models. The odds ratio (OR) with 95% confidence interval (CI) was calculated for HCW having SARS-CoV-2-specific antibodies. Serological data were log-transformed and compared between time points in mixed-effects models with adjustment for subject variance and confounding variables. All statistical analyses were performed in R-v.4.0.2 and visualized in Prism-v.8.4.2. P-values <0.05 were considered statistically significant.

Results

The pandemic period was well defined in Bergen, Norway due to early rigorous centralized RT-PCR testing of suspected COVID-19 cases, with the first detection of confirmed cases on February 28th, providing a unique opportunity to study the impact of the pandemic on HCW.

Study population

HCW (n=607, 77.1% female and median age 39, range 20-78 years old) were enrolled from Bergen's main healthcare institutions testing and treating COVID-19 patients, including 286 nurses (47.1%) and 174 physicians (28.7%) (Table 1). Recruitment started from March 6th before the first

hospitalizations (March 9th) and the first death (March 23rd) from COVID-19 (Figure 1A). HCW were followed up throughout the first pandemic wave in Bergen (6-10 weeks).

HCW were grouped by their occupational exposure: high-risk (n=383, 63.1%) working at the testing facility or on COVID-19-designated wards, and low-risk (n=224, 36.9%) with no COVID-19 patient-exposure (Table 1). We found no significant differences in age, sex or recent travel history between the two groups, although there were more doctors and nurses in the high-risk (85.1%) than the low-risk group (59.8%) (p=0.035). HCW with COVID-19-like illness were prioritized for RT-PCR testing and 97 (16%) HCW were tested during the follow-up period. Only 8 HCW tested positive for SARS-CoV-2 (5 high-risk and 3 low-risk HCW).

Serology results

The SARS-CoV-2 virus attaches to the host cells through RBD on its spike protein, therefore, antibodies binding to these proteins have the potential to block viral entry. Spike- and RBD-specific antibodies were used in this study to define infection, as they do not cross-react with other human coronaviruses [14].

RBD- and spike-specific antibodies

Using screening ELISA, we found that the majority of HCW had undetectable antibodies to RBD before and after the first pandemic wave (90.6% at baseline and 89.3% at follow-up) (Table 1, Figure 1a), although the RBD-binding antibody OD values were significantly higher at follow-up than at baseline in both high-risk (p=0.027) and low-risk (p=0.034) groups (Figure 1B). The RBD-specific IgG levels were measured for HCW with positive or intermediate results by screening ELISA (n=76, 12.5%), which were also confirmed by spike protein IgG ELISA (supplementary-Figure 3A, B). We observed a significant increase in RBD-specific IgG geometric mean endpoint titers (GMT) after the first pandemic period (p<0.001), from 336 at baseline to 637 at follow-up (Figure 2A). This increase was significant in the high-risk group (p=0.002), but not in the low-risk group (Figure 2B). In agreement, a significant increase in spike-specific IgG GMT was observed after the first pandemic wave (p=0.002), from 277 at baseline to 518 at follow-up, which was only significant in the high-risk

group (p=0.012) (Figure 3A,B supplementary-Figure 3B). Forty-four HCW (7.2%) had RBD-specific IgG above the positive cutoff of 400; 14 were positive at follow-up (11 seroconverted with ≥4-fold increase and 3 had <2.5-fold increase in titers) and 30 were positive at both baseline and follow-up (Figure 2C). Of these, 32 HCW (5.3%) were confirmed seropositive in the spike IgG ELISA with endpoint titers above the positive cutoff of 485; 11 seroconverted at follow-up (9/11 in the high-risk group) and 21 were positive at baseline (Figure 3C). Notably, 5 HCW (4/5 in the high-risk group) had >2-fold increase in IgG titers at follow-up but remained below the positive cutoff.

The spike-specific IgM and IgA antibodies were measured in the 32 IgG-seropositive HCW (Figure 4A-D, supplementary-Figure 4A-D). An increase in both spike-specific IgM and IgA GMT was observed in HCW after the pandemic period, although only significant for IgA and high-risk HCW (Figure 3C,D). The IgM GMT increased from 87 to 159 (p=0.068) and the IgA GMT increased from 58 to 101 (p=0.005) from baseline to follow-up in the high-risk group (Figure 3B,D). Nine HCW had IgM antibodies above the positive cutoff of 300; 7 seroconverted (≥4-fold increase) at follow-up (6/7 were high-risk HCW) and 2 were seropositive at baseline (supplementary-Figure 4C). Six HCW had IgA antibodies above the positive cutoff of 200; 4 seroconverted (3/4 were high-risk HCW) and 2 had increases in titre but did not seroconvert (<4-fold increase) at follow-up from a negative titer at baseline (supplementary-Figure 4D). Four HCW in the high-risk group who IgG seroconverted had >2-fold increase in either IgM or IgA titers at follow-up but remained below the positive cutoff.

Neutralizing antibodies

Virus neutralizing antibodies can potentially prevent re-infection with SARS-CoV-2 [17-19]. Therefore, we further assessed *in-vitro* protective immunity against SARS-CoV-2 in HCW with positive or intermediate results by screening RBD ELISA (n=76) using the microneutralization assay to confirm infection. The MN titers increased significantly from baseline to follow-up (p=0.002) (Figure 4A). MN antibodies were found in the 11 HCW who IgG-spike seroconverted (range 35-291), of whom 10 seroconverted and 1 had 2.3-fold increase in MN titers (Figure 4B).

We further extended our work to investigate the presence of sterilizing immunity that provides complete protection against SARS-CoV-2 infection *in-vitro* in the 11 MN-positive HCW. The VN titers increased significantly from baseline to follow-up (p=0.003), of which 6 HCW had VN antibodies (range 20-40) and 3 seroconverted with ≥4-fold increase in titer at follow-up (Figure 4C,D).

Infection rates and risk factors

The overall SARS-CoV-2 seropositive rate was 5.3% (32/607) by using our 2-step ELISAs. Twenty-one HCW were seropositive at baseline, suggesting previous exposure to SARS-CoV-2 before the study, of which 6 had travelled internationally or treated confirmed/suspected COVID-19 patients before recruitment and were not tested or RT-PCR negative. The remaining 15 HCW did not recall any potential source of infection. Eleven HCW seroconverted during follow-up, indicating recent SARS-CoV-2 infection. Of these, 7 HCW with COVID-19-like symptoms were confirmed by RT-PCR, while one was RT-PCR-negative and 3 HCW were not tested as they were asymptomatic (supplementary-Table 1). Interestingly, one RT-PCR-positive asymptomatic HCW did not develop anti-RBD antibodies. The total infection rate identified by either SARS-CoV-2 RT-PCR or serology testing was 2.0% (12/586, excluding 21 IgG-seropositive HCW at baseline). The infection rates by occupational exposure were 2.4% (9/370) in high-risk and 1.4% (3/216) in low-risk departments (p=0.4). The majority of infected HCW in high-risk departments (7/9, 77.8%) were young nurses, aged 23-31 years. Three infected HCW (3/7, 42.9%) reported partial uses of PPE when treating COVID-19 patients. Among 3 infected HCW in low-risk departments, 2 had travelled internationally and 1 had community exposure.

Risk factors for SARS-CoV-2 seropositivity at baseline were recent travel history (OR 1.8, 95% CI 0.5-7.1), contact with confirmed or suspected patients (OR 1.7, 95% CI 0.7-4.3), having COVID-19-like symptoms i.e. sore throat (OR 1.9, 95% CI 0.8-4.3) and myalgia (OR 1.6, 95% CI 0.6-4.3), young age (OR 1.5, 95% CI 0.7-3.4) and nursing occupation (OR 1.3, 95% CI 0.7-2.7) (Supplementary-Table 2). At follow-up, HCW using partial PPE when treating COVID-19 patients

had 2.5-fold higher odds (95% CI 0.5-12.2) of being seropositive than HCW with no COVID-19 patient-exposure.

Discussion

During the COVID-19 pandemic, many countries experienced unprecedented increases in hospitalizations, overwhelming their healthcare systems, and rapidly depleting supplies of PPE, resulting in thousands of HCW infections and deaths [4,20,21]. In the UK, Spain and the Netherlands, 6-38% of HCW tested positive by RT-PCR [20,22,23], some requiring hospitalization and intensive care unit treatment [22], although most infections were community-acquired. Here, we show a considerably lower SARS-CoV-2 infection rate among frontline HCW during the first wave of the pandemic in Bergen, Norway by RT-PCR and serological testing (5.3% overall and 2.0% during follow-up), probably due to low levels of community infection and strict occupational use of PPE. None of the HCW in our study died or were hospitalized. There was little overcrowding of emergency rooms and good compliance with infection prevention and control (IPC) measures. The early lockdown in Norway, implemented on March 12th before the first national deaths on March 16th, contributed to the low number of community cases and hospitalizations, thus, protecting the healthcare system and its most vital asset, the HCW. Recent modeling studies have shown that the unprecedented lockdowns have dramatically reduced mortality in many countries, preventing 12,000 deaths in Norway [24,25]. No excess deaths were reported during the first pandemic wave in Norway, highlighting the success of the rapid deployment of effective public health responses. The Norwegian experience with early lockdown is especially important when compared to countries without or with

delayed lockdown, which report higher infection and mortality rates in the community and HCW [4,20,21].

Several countries reported higher rates of SARS-CoV-2 infection among HCW treating COVID-19 patients, up to 11.6-fold increased risk, compared to other HCW or the community [5,8,26,27]. In agreement, we found that the infection rate was 1.7-fold higher in HCW with COVID-19 patient-exposure (2.4%) than in HCW with no exposure (1.4%), although not statistically significant. Unlike many other countries, Norway did not experience shortages of PPE, although the stockpile was alarmingly low. Only 1.7% (10/605) of our HCW reported occupational exposure without PPE to 1-2 COVID-19 patients and/or contact with an infected colleague; none of whom were positive by SARS-CoV-2 RT-PCR or serological assays. However, 42.9% (3/7) of seroconverted HCW reported having used partial PPE when treating ≥1 COVID-19 patients. HCW using partial PPE had 2.5-fold higher odds of being SARS-CoV-2 seropositive than HCW without patient contact. Nurses have a greater occupational exposure than other medical staff. Although we cannot exclude community infection, Norway had low levels of community spread and one of the highest levels of RT-PCR testing in Europe. In the UK, 48% of RT-PCR positive HCW were nurses [23], while we found 77.8% (7/9) of seroconverted HCW treating COVID-19 patients were young nurses (23-31 years old). Indeed, working as a young nurse had higher odds of being SARS-CoV-2 seropositive, perhaps due to less experience in IPC measures. Better training in IPC for junior staff and ensuring adequate availability of PPE in the future will help to prevent the spread of SARS-CoV-2 in healthcare settings.

Serological assays can determine SARS-CoV-2 infection over a longer time period than RT-PCR, which can only detect acute infection. In our study, only 8 HCW tested positive by RT-PCR, one of whom had no symptoms but was tested due to recent international travel. In contrast, serological assays identified infection in 32 HCW, of whom 11 HCW seroconverted after the first COVID-19 pandemic wave, indicating recent infection. Seven seroconverted HCW had COVID-19-like symptoms and infection confirmed by RT-PCR, however 4 seroconverted HCW (36.4%) were asymptomatic and

either were RT-PCR negative or not tested. Similarly, other seroprevalence studies also reported asymptomatic and/or not RT-PCR tested individuals who were positive by SARS-CoV-2 serology testing [6,13,28,29]. In asymptomatic cases, T cell immunity may control SARS-CoV-2 infection in the absence of antibodies. The role of asymptomatic HCW in SARS-CoV-2 transmission is not clear, particularly in nosocomial transmission to vulnerable patients and other HCW, with some studies reporting similar viral loads [30] and others reporting less infectious virus [31] found in asymptomatic than in symptomatic cases. Combined RT-PCR and serology testing is crucial to determine infection spread in the healthcare settings and improve IPC.

Importantly, virus neutralizing antibodies can potentially prevent re-infection with SARS-CoV-2 [17-19]. We developed neutralization assays to investigate protective antibodies against the live SARS-CoV-2 virus in HCW, which most studies have not [3-11,13,20-22,26,32-41]. We showed that 11 recently-infected HCW had neutralizing antibodies, six of them had sterilizing immunity *in-vitro*, which could provide protection from reinfection by SARS-CoV-2 perhaps during the second wave of the pandemic. Neutralizing antibodies were not detected in 21 HCW who were infected before the study with unknown time of infection. One explanation could be the change in the spike protein of SARS-CoV-2 viruses. In March 2020, a variant strain has replaced the previous SARS-CoV-2 originally isolated in Wuhan, China [42]. The 21 HCW infected before the study recruitment in March probably were exposed to the strain closer to the Wuhan isolate, which may not be recognized by our neutralization assays using the more recently isolated Norwegian strain. Recent reinfection of a previously confirmed SARS-CoV-2 infection highlights the importance of measuring neutralizing antibodies [43]. Future studies should investigate the correlates of protection of SARS-CoV-2 neutralizing antibodies against reinfection and its longevity to aid future COVID-19 vaccine development.

Home quarantine was introduced in Norway in late February for people with mild COVID-19 infection and their family members, HCW with close contact to COVID-19 patients without proper PPE, and travelers returning from countries with SARS-CoV-2 infection. Norway had good adherence with household quarantine, which was covered by statutory sick pay. Limitations in initial testing capacity led to 18% of our HCW being quarantined during the study, which reduced the available workforce and increased the workload for the remaining HCW. As increased RT-PCR testing became available, there was a lower threshold for testing of HCW with COVID-19-like symptoms and consequently reduced staff absenteeism.

The advantages of our prospective cohort study are the early recruitment of HCW before the first pandemic wave and prior to COVID-19 patient-exposure, the use of stringent serological assays to define infection and the investigation of neutralizing antibodies to assess protection from reinfection. Our moderate patient burden may have resulted in limited exposure but ensured correct characterization of HCW exposure as patients were only hospitalized on COVID-19-designated wards. The low infection rates in Norwegian HCW limited the statistical power of our study, thus, the odds ratio estimates should be interpreted with caution. Although the relevance of neutralizing antibodies using Vero cell based in vitro neutralization assays for correlates of protection remains to be determined, neutralizing antibody titres correlated with in vivo protection after SARS-CoV-2 challenge of DNA vaccinated rhesus macaque [43] . Our findings emphasize the importance of good IPC systems in healthcare facilities to isolate patients with suspected infection to reduce risk of exposure, ensure HCW have suitable training and access to PPE.

Ensuring the safety of HCW and protecting them from infection, reinfection and further transmission, is one of the most important measures to sustain healthcare services during a pandemic. RT-PCR and serological testing of HCW are crucial to prevent infection

within the hospital, as the tests complement each other. Our data document low infection rates in Norwegian frontline HCW, where the moderate burden of patients enabled rational patient management and compliance with IPC measures. Neutralizing antibodies were found in all infected HCW who seroconverted, which may provide protection against reinfection perhaps during the future waves of the pandemic. HCW are vital for managing the ongoing outbreak and can be protected through timely national or local control of the outbreak, access to PPE and through training in IPC. There is an urgent need to understand the most effective measures to protect HCW during this pandemic and our study highlights the importance of early control measures to protect society and the HCW.

Footnotes

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Conflict of interest: An ELISA assay used to screen for seroconversion was developed in Florian Krammer´ laboratory. Mount Sinai has filed patent applications to protect that assay and has licensed its use to several companies. Mount Sinai is also commercializing the assay. All authors declare no conflict of interest.

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Table 1: Demographics, clinical and serological characteristics of healthcare workers

Characteristic*	Total (n=607)	High-risk** (n = 383)	Low-risk** (n = 224)	Crude p[a]	Adjusted p[b]
Age median ± SD	39 ± 12.6	36 ± 12.6	42.5 ± 12.7		
Age group				**0.0096**	0.8382
20-50 years	446 (73.5%)	295 (77.0%)	151 (67.4%)		
51-78 years	161 (26.5%)	88 (23.0%)	73 (32.6%)		
Sex				**0.0138**	0.6269
Female	468 (77.1%)	283 (73.9%)	185 (82.3%)		
Male	139 (22.9%)	100 (26.1%)	39 (17.4%)		
Workplace				**<0.0001**	**0.0010**
Haukeland University Hospital	471 (77.6%)	247 (64.5%)	224 (100%)		
Haraldsplass Deaconess Hospital	54 (8.9%)	54 (14.1%)	-		
Bergen Municipality Emergency	82 (13.5%)	82 (21.4%)	-		
Profession				**<0.0001**	**0.0347**
Physician	174 (28.7%)	126 (32.9%)	48 (21.4%)		
Nurse	286 (47.1%)	200 (52.2%)	86 (38.4%)		
Other	147 (24.2%)	57 (14.9%)	90 (40.2%)		
Travel history in 2020 at baseline				0.2271	0.9452
International	133/606 (21.9%)	91/382 (23.8%)	42/224 (18.7%)		
Domestic	38/606 (6.3%)	26/382 (6.8%)	12/224 (5.3%)		
None	435/606 (71.8%)	265/382 (69.2%)	170/224 (75.9%)		
Occupational exposure at baseline				**<0.0001**	**0.0094**
Confirmed patient contact	37/597 (6.2%)	37/379 (9.8%)	0/218 (0%)		
Suspected patient contact	74/597 (12.4%)	74/379 (19.5%)	0/218 (0%)		
No patient contact	486/597 (81.4%)	268/379 (70.7%)	218/218 (100%)		
Occupational exposure at follow-up				**<0.0001**	**<0.0001**
With PPE	263/605 (43.5%)	263/381 (69.0%)	0/224 (0%)		
Without PPE	10/605 (1.7%)	10/381(2.6%)	0/224 (0%)		

No contact	332/605 (54.9%)	108/381 (28.3%)	224/224 (100%)		
Community exposure at follow-up	17/604 (2.8%)	15/380 (3.9%)	2/224 (0.9%)	**0.0283**	0.4295
Symptoms at follow-up				0.0920	0.3229
Any symptom	116/606 (19.1%)	81/382 (21.1%)	35/224 (15.6%)		
Dry cough	59/113 (52.2%)	40/78 (51.2%)	19/35 (54.3%)		
Fever	29/113 (25.7%)	22/79(27.9%)	7/34 (20.1%)		
Dyspnea	22/113 (19.5%)	20/79 (25.3%)	2/34 (5.9%)		
SARS-CoV-2 RT-PCR test at follow-up				0.9719	0.7291
Positive	8 (1.3%)	5 (1.3%)	3 (1.3%)		
Negative	89 (14.7%)	62 (16.2%)	27 (12.1%)		
Been in quarantine at follow-up	108/598 (18.1%)	74/377 (19.6%)	34/221 (15.4%)	0.1928	0.1808
Screening anti-RBD SARS-CoV-2 antibodies at baseline				0.4987	0.2401
Negative	550 (90.6%)	343 (89.6%)	207 (92.4%)		
Intermediate	46 (7.6%)	32 (8.3%)	14 (6.2%)		
Positive	11 (1.8%)	8 (2.1%)	3 (1.4%)		
Screening anti-RBD SARS-CoV-2 antibodies at follow-up				0.5362	0.2400
Negative	542 (89.3%)	338 (88.3%)	204 (91.1%)		
Intermediate	47 (7.7%)	33 (8.6%)	14 (6.2%)		
Positive	18 (3.0%)	12 (3.1%)	6 (2.7%)		

*Data are presented as number (%), unless otherwise specified;

**High-risk group tested and treated COVID-19 patients includes emergency, infectious diseases and intensive care unit departments at Haukeland University Hospital, Haraldsplass Deaconess Hospital and Bergen Municipality Emergency Room; and low-risk group did not treat COVID-19 patients includes other clinical departments and laboratories;

[a]Crude p-value was determined by Chi-square test (associations between exposure groups and characteristics) in R;

[b]Adjusted p-value was calculated by generalized linear mixed-effects model including all demographics, clinical and serological characteristics in R.

Figure Legends

Figure 1: Screening for SARS-CoV-2 receptor-binding domain (RBD)-specific antibodies in healthcare workers (HCW) before and after COVID-19 patient admissions. (**a**) HCW (n=607) were recruited between March 6th and April 9th, and followed up after 6-10 weeks. Each circle represents one HCW (grey baseline and purple follow-up) and their anti-RBD antibodies measured in the screening ELISA as optical density (OD) at 450/620nm (left y-axis). The numbers of hospitalized COVID-19 patients (blue line) and cumulative deaths (orange line) in Bergen, Norway are plotted on the right y-axis. Lockdown was initiated in Norway on March 12th, 2020, and a gradual reopening starting on April 20th, 2020. (**b**) HCW were grouped into high risk (testing facility, COVID-19-designated wards and intensive care unit wards) and low risk (no known exposure to COVID-19 patients) of occupational exposure to SARS-CoV-2 according to their working department and information in their case report forms. Dotted lines are cutoffs for negative screening results (OD <0.430) and positive screening results (OD ≥ 0.708) (see Supplementary Figure 1 for further information). Horizontal lines represent mean with standard deviation. OD values were log-transformed and compared between time points in mixed-effects models with adjustment for subject variation, age, sex, and other relevant demographic factors. *p<0.05.

Figure 2: SARS-CoV-2 receptor-binding domain (RBD)-specific IgG antibodies in healthcare workers (HCW) before and after COVID-19 patient admissions. The RBD-specific IgG levels were measured for HCW with positive or intermediate RBD screening results (n=76) were titrated for endpoint titres (**A**) by enzyme-linked immunosorbent assay **(**ELISA). RBD- specific IgG endpoint titers **(B)** in high-risk and low-risk HCW groups. HCWs were divided into seroconverters (blue circle) who were seropositive and had ≥4-fold increase in IgG titers at follow-up and non-seroconverters (grey circle) who were either seronegative or had <4-fold increase in IgG titers at follow-up (**C)**. The fold changes are

plotted on the right y-axis with horizontal lines representing the mean with standard error of the mean. Dotted lines represent cutoffs for positive results, calculated as 3 standard deviations above the mean of the pre-pandemic negative sera (RBD IgG endpoint titer ≥400). Individuals with undetectable antibodies were assigned an endpoint titer of 50 for plotting and calculation purposes. Endpoint titers were log-transformed and compared between time points in mixed-effects models with adjustment for subject variation, age, sex, and other relevant demographic factors. **p<0.01. ***p<0.001.

Figure 3: SARS-CoV-2 spike-specific IgG antibodies in healthcare workers (HCW) before and after COVID-19 patient admissions. The RBD-specific IgG levels were measured for HCW with positive or intermediate RBD screening results (n=76), which were confirmed in a confirmatory spike IgG enzyme-linked immunosorbent assay **(**ELISA). Spike-specific IgG endpoint titers **(A)** in high-risk and low-risk HCW groups. (**B**) HCWs were divided into seroconverters (blue circle) who were seropositive and had ≥4-fold increase in IgG titers at follow-up and non-seroconverters (grey circle) who were either seronegative or had <4-fold increase in IgG titers at follow-up (**C)**. The fold changes are plotted on the right y-axis with horizontal lines representing the mean with standard error of the mean. Dotted lines represent cutoffs for positive results, calculated as 3 standard deviations above the mean of the pre-pandemic negative sera (spike IgG endpoint titer ≥485). Individuals with undetectable antibodies were assigned an endpoint titer of 50 for plotting and calculation purposes. Endpoint titers were log-transformed and compared between time points in mixed-effects models with adjustment for subject variation, age, sex, and other relevant demographic factors. *p<0.05. **p<0.01.

Figure 4: SARS-CoV-2 spike-specific IgM and IgA antibodies in healthcare workers (HCW) before and after COVID-19 patient admissions. HCW with positive spike IgG results (n=32) were further analyzed in spike IgM and IgA enzyme-linked immunosorbent assay **(**ELISA). Spike-specific IgM and IgA endpoint titers **(A,C)** were calculated and each circle represents one HCW (grey baseline and purple follow-up). Horizontal lines represent geometric mean with 95% confidence interval. The spike-specific

IgM and IgA endpoint titers in HCW in high-risk and low-risk groups (**B, D**). Dotted lines represent cutoffs for positive results, calculated as 3 standard deviations above the mean of the pre-pandemic negative sera (IgM endpoint titer ≥300, IgA endpoint tire ≥200). Individuals with undetectable antibodies were assigned an endpoint titer of 50 for plotting and calculation purposes. Endpoint titers were log-transformed and compared between time points in mixed-effects models with adjustment for subject variation, age, sex, and other relevant demographic factors. *p<0.05. **p<0.01.

Figure 5: SARS-CoV-2 neutralizing antibodies in healthcare workers (HCW). (**A**) The microneutralization (MN) titers of HCW with positive or intermediate SARS-CoV-2 receptor-binding domain (RBD) screening results (n=76). (**C**) The live virus neutralization (VN) titers of HCW positive with MN antibodies (n=11). Each circle represents one HCW (grey baseline and purple follow-up). Horizontal lines represent geometric mean with 95% confidence interval. (**B, D**) HCW were divided into seroconverters (blue circle) who had ≥4-fold increase in MN and VN titers, respectively, and non-seroconverters (grey circle) who had <4-fold increase in titers. Their respective fold changes in MN and VN titers are plotted on the right y-axis with horizontal lines representing the mean with standard error of the mean. Dotted lines represent positive neutralizing antibody titers of 20. Individuals with undetectable antibodies were assigned a titer of 10 for plotting and calculation purposes. MN and VN titers were log-transformed and compared between time points in mixed-effects models with adjustment for subject variation, age, sex, and other relevant demographic factors. **p<0.01.