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Characterization of risk factors and preventive measures for COVID-19

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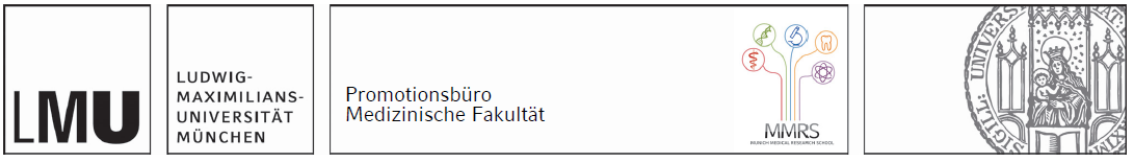

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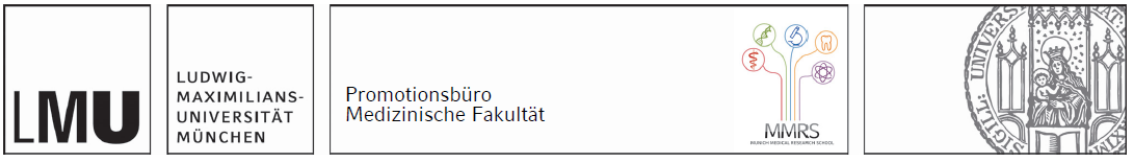

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Table of content

List of abbreviations

COVID-19	-	coronavirus disease 2019
HCoV	-	seasonal endemic coronavirus
HCW	-	healthcare worker
ICU	-	intensive care unit
PPE	-	personal protective equipment
SARS-CoV-2	-	severe acute respiratory syndrome coronavirus type 2
VoC	-	variant of concern

List of publications

Publications used for this cumulative dissertation:

Wratil PR[*,#], Schmacke NA[*], Osterman A[*], Weinberger T, Rech J, Karakoc B, Zeilberger M, Steffen J, Mueller TT, Spaeth PM, Stern M, Albanese M, Thun H, Reinbold J, Sandmeyer B, Kressirer P, Grabein B, Falkai P, Adorjan K, Hornung V, Kaderali L, Klein M[†], Keppler OT[#,†]. In-depth profiling of COVID-19 risk factors and preventive measures in healthcare workers. ***Infection***. 2022 Apr;50(2):381-394. doi: 10.1007/s15010-021-01672-z. Epub 2021 Aug 11.

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Your contribution to the publications

1.1 Contribution to paper I

In the publication “In-depth profiling of COVID-19 risk factors and preventive measures in healthcare workers” published in *Infection* in 2022, Niklas A. Schmacke, Andreas Osterman, Tobias Weinberger, Jochen Rech, Kristina Adorjan, Matthias Klein, Oliver T. Keppler and I conceived the study. I contributed to the design of the study questionnaire, and together with Niklas Schmacke and Tobias Weinberger I developed the data safety concept and drafted the data safety application. Niklas Schmacke, Jochen Rech and I developed the logistics to collect, process and archive blood samples, store test result data and report them to the participants as well as connect participants’ test result data with their answers from the study questionnaire. Niklas Schmacke, Jochen Rech, Burak Karakoc and I set up the machines, established the laboratory workflow, and obtained the consumables for the analysis and storage of all study specimens.
In part assisted by Victoria Anetsberger, Manouk Feinendegen, Ann-Kathrin Friedl, and Anna Trebo, Burak Karakoc and I extracted the sera from all 7,554 blood samples donated in the study, processed them for further analysis, and archived them. Together with Burak Karakoc, I measured the levels of anti-SARS-CoV-2 nucleocapsid antibodies in all study samples using a commercially available assay. Niklas Schmacke, Jochen Rech, Burak Karakoc and I performed a self-developed assay to detect nucleocapsid-specific anti-SARS-CoV-2 IgG antibodies in all sera. Burak Karakoc and I measured all sera that scored positive or indeterminate in the assays mentioned above or originated from participants reporting a previous SARS-CoV-2 infection employing a commercially available anti-SARS-CoV-2 spike S1 domain detection assay. Niklas Schmacke and I prepared the titrations of all anti-SARS-CoV-2 antibody-positive sera and aliquoted these samples into the respective plate formats for the live-virus neutralization assays, which were performed in the P3 laboratory by Marcel Stern.
Niklas Schmacke, Lars Kaderali, and I evaluated and interpreted all data obtained, and performed subgroup analyses.
I assisted Niklas Schmacke in preparing all figures depicted in the publication.
Niklas Schmacke, Matthias Klein, Oliver T. Keppler and I wrote the original draft of the manuscript. All authors reviewed and edited the manuscript.
Prior to submission of this publication, the senior and first authors agreed on the shared first-authorship of Niklas Schmacke, Andreas Osterman, and myself (Wratil, Schmacke, Osterman et al.). Niklas Schmacke and Andreas Osterman largely contributed to the planning, realization and evaluation of the study as well as the preparation of the publication, altogether justifying a shared first-authorship between these two and myself.
Due to my involvement in all parts of the study from planning to publishing, Oliver T. Keppler and I agreed that we are both listed as corresponding authors in this publication.

1.2 Contribution to paper II

Oliver T. Keppler and I conceived and planned the study entitled “Evidence for increased SARS-CoV-2 susceptibility and COVID-19 severity related to pre-existing immunity to seasonal coronaviruses” published in *Cell Reports* in 2021.

I selected and processed all 1,202 serum samples for further analyses. I assisted Burak Karakoc in performing the commercially available line blot assay for determining antibody responses against SARS-CoV-2 and seasonal, endemic coronaviruses.
Assisted by Niklas Schmacke, Burak Karakoc, and Alex Dulovic, I performed the evaluation of all primary assay results, and linked obtained data to the patients' medical records.
I performed all statistical analyses in the publication, except for the calculation of Kendall's correlation coefficients (τ) and multivariate analyses, which was performed by Lars Kaderali.
I prepared all figures, tables, supplementary figures, and supplementary tables depicted in the publication and drafted the respective figure and table legends.
Oliver T. Keppler and I drafted the first version of the manuscript. All authors contributed to the interpretation of data as well as reviewed and edited the manuscript.
I generated the source data file of the publication.

1.3 Contribution to paper III

In the publication "Three exposures to the spike protein of SARS-CoV-2 by either infection or vaccination elicit superior neutralizing immunity to all variants of concern" published in Nature Medicine in 2022, I performed all laboratory assays for determining IgG-type antibody responses to the S1 domain of SARS-CoV-2 in the participants' serum specimens. I measured the binding strength of SARS-CoV-2 IgG-type antibodies in a large portion of the sera, in part together with Alina Priller and Annika Willmann. I prepared the titrations of all sera tested and aliquoted these samples into the respective plate formats for being measured in the live virus neutralization assay. Marcel Stern performed the great majority of live-virus neutralization assays in the P3 laboratory. Using the raw data obtained from the assays measuring IgG-type anti-SARS-CoV-2 spike antibody responses and antibody-mediated virus neutralization, I calculated the binding antibody units per milliliter as well as the half-maximal serum concentrations for neutralizing the different SARS-CoV-2 variants in all specimens tested. Moreover, I evaluated a portion of the raw data to uncover the binding strength of serum IgG-type antibodies to SARS-CoV-2 spike protein.
I performed all statistical analyses in the publication.
I prepared all main Figures (4 in total) and Extended Data Figures (8 in total) depicted in the publication except for Extended Data Figure 8. In case of Figures 1g, 2d, 2e and Extended Data Figure 5, Alina Priller assisted me. Furthermore, I drafted the figure legends of all Figures and Extended Data Figures except for the legend of Extended Data Figure 8.
Alina Priller, Marcel Stern, Percy Knolle, Oliver T. Keppler, Ulrike Protzer, and I wrote the original draft of the manuscript. All authors reviewed and edited the manuscript.
In cooperation with Alina Priller, Marcel Stern and Percy Knolle, I wrote the reporting summary of the publication, and with the help of Alina Priller I generated the source data files.
Prior to submission of this publication, the corresponding and first authors agreed on the shared first-authorship of Marcel Stern, Alina Priller and myself (Wratil, Stern, Priller et al.). Among others and besides their contributions to this publication listed above, Marcel Stern was heavily involved in developing and performing the live-virus neutralization assay with different SARS-CoV-2 variants as well as expanding and characterizing the clinical SARS-CoV-2 isolates, and Alina Priller played a major role in recruiting the study participants, collecting their serum samples and archiving these, altogether justifying a shared first-authorship between these two and myself.

2. Introductory summary

2.1 Abstract

In my dissertation, I present three studies that my colleagues and I conducted to discover risk factors for the coronavirus disease 2019 (COVID-19) and evaluate different preventive measures against acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) infection.
In a cross-sectional study, we discovered multiple occupational COVID-19 risk factors in healthcare workers, including high-risk exposures, working as a nurse and in other patient-facing occupations, as well as treating COVID-19 patients. Working remotely had no effect on the risk of COVID-19. A testing strategy focusing on symptoms and disclosure of high-risk exposures, however, was sufficient to detect most COVID-19 cases among hospital employees.
Next, we investigated the immunological interplay between SARS-CoV-2 and seasonal endemic coronaviruses (HCoVs) in a longitudinal study. We found evidence that pre-existing humoral immune responses to HCoVs increased susceptibility to SARS-CoV-2 and severity of COVID-19. Apparently, high levels of IgG-type antibodies against the nucleocapsid of seasonal α-coronaviruses and the spike S2 domain of HCoV-OC43 play a crucial role in this process.
Finally, in a longitudinal study, we discovered that SARS-CoV-2 variant Omicron evades humoral immune responses in both vaccinated naïve and convalescent individuals after two encounters with viral spike antigen. A third exposure either by an additional vaccination or a breakthrough infection, however, elicited superior neutralizing immunity against all SARS-CoV-2 variants, including Omicron. The broadening neutralization capacities observed after every encounter with the viral spike antigen were likely due to antibody maturation.
Collectively, our findings on COVID-19 risk factors and preventive measures may help pave the way for the development and refinement of future approaches to combat SARS-CoV-2 and alleviate the burden of the pandemic to global health.

2.2 Introduction

The coronavirus disease 2019 (COVID-19) caused by the recently emerged human viral pathogen severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) rapidly evolved into a pandemic in early 2020 with approximately 640 million diagnosed cases and 6.6 million deaths by November 2022 [1].
SARS-CoV-2, a β-coronavirus belonging to the taxonomic family of coronaviridae, is a positive-sense single stranded RNA virus [2]. It spreads from an infected person's mouth or nose via viral particles that pass through the air and are inhaled (aerosol/airborne transmission) or via droplets that come into direct contact with the eyes, nose, or mouth (droplet transmission) [3]. Upon transmission, SARS-CoV-2 infects epithelial cells in the host's respiratory tract that express the angiotensin-converting enzyme 2 (ACE2) [4, 5]. Symptomatic COVID-19 patients most commonly show fever, fatigue, and dry cough [6, 7]. Moreover, anosmia and dysgeusia are often reported symptoms [8-11]. Severe disease characterized by signs of viral pneumonia with hypoxia as well as severe respiratory distress can occur in over 15 % of non-vaccinated patients, and 5 % of all cases suffer from critical disease defined as acute respiratory distress syndrome, septic shock, or other conditions requiring life-sustaining therapies [12, 13]. The overall case fatality rate in non-vaccinated individuals is above 1 % [14]. Numbers of severe and critical illness as well as fatality in COVID-19 patients are influenced by several risk factors, including age, sex, as well as

comorbidities, and are declining since the beginning of vaccination campaigns and since the SARS-CoV-2 variant Omicron has been dominating the pandemic [15, 16]. However, due to rapid viral evolution, novel variants of SARS-CoV-2 with enhanced pathogenicity and immune escape might emerge in the future. Depending on the applied definitions and cohorts examined, between 2.3 % and 80 % of patients develop a post COVID-19 condition [17, 18], i.e., symptoms that occur usually three months after the onset of COVID-19, last for at least two months and cannot be explained by an alternative diagnosis [19]. Symptoms associated to a post COVID-19 condition are often episodic and multisystem, including respiratory, neurological, cardiovascular, metabolic as well as mental health symptoms [20].

Collectively, COVID-19 is a fundamental threat to global health. Unfortunately, effective treatments for the disease are still under development or unavailable for broad application. Hence, it is crucial to obtain better understanding on the etiology, epidemiology, and pathology of COVID-19 as well as on how SARS-CoV-2 interacts with the host immune system, to effectively prevent infections and contain the pandemic. Risk factors that increase individuals' susceptibility to infection or severe disease need to be identified. Furthermore, the effectiveness of containment and protective measures should be closely evaluated. This includes not only behavioral measures (e.g., social distancing), the use of personal protective equipment (PPE), and screening for infections, but also COVID-19 vaccination. Moreover, the immunological interplay between SARS-CoV-2 and seasonal endemic coronaviruses (HCoVs) should be investigated to understand whether previous HCoV infections influence SARS-CoV-2 susceptibility and the course of COVID-19. In this dissertation, I present a part of my work tackling these important research objectives.

2.3 COVID-19 risk factors and preventive measures in healthcare workers

The characterization of COVID-19 risk factors is highly important to identify vulnerable individuals and to ease their access to COVID-19 screening and preventive measures. A relevant occupational group at increased risk for COVID-19 are healthcare workers (HCWs) [21, 22]. HCWs experience frequent exposures to SARS-CoV-2 while treating COVID-19 patients; this was especially true during the first wave of the pandemic in early 2020 when, due to the high pathogenicity of early SARS-CoV-2 variants and absence of vaccines, 5 – 20 % of all COVID-19 patients required hospitalization [23, 24], and up to 35 % of those hospitalized needed accelerated, life-sustaining therapies in intensive care units (ICUs) [25, 26]. However, even two and a half years later, COVID-19-related hospital and ICU occupancies in Germany are similar compared to early 2020 [27], likely caused by a drastic increase in case numbers. This highlights the ongoing threat that work-related SARS-CoV-2 exposures pose to healthcare professionals and the burden of COVID-19 on the healthcare system. HCWs are key to ensure adaptable and adequate hospital capacities and to battle the COVID-19 pandemic. Protecting this occupational group appropriately from SARS-CoV-2 is, therefore, of utmost priority and requires the characterization of HCW-specific COVID-19 risk factors. Simultaneously, the effectiveness of protective measures in hospitals should be evaluated, such as the use of PPE, screening for infections and allowing HCWs who are not directly involved in patient care to work remotely.

To address these objectives, my colleagues and I performed a cross-sectional study at a multi-center quaternary care hospital in Munich, Germany (LMU Klinikum). After the primary wave of the pandemic, we measured the prevalence of humoral immune responses to SARS-CoV-2 to detect COVID-19 in HCWs and linked obtained results to data from a questionnaire that assessed

epidemiological, occupational, and COVID-19-specific information. 7,554 of all 11,580 hospital staff members (65.2 %) donated a blood sample and completed the questionnaire. In 2.2 % of these participants, we identified antibodies specific to the nucleocapsid antigen of SARS-CoV-2, which was evidence for subacute or resolved COVID-19.

Our statistical analyses revealed multiple occupational COVID-19 risk factors: One of them was high-risk exposures, not only to SARS-CoV-2-infected persons outside of work (in the community), but also in the hospital to COVID-19 patients and, to lesser extent, infected co-workers. Such high-risk exposures are defined, for example, as > 15 min face-to-face contact with infected individuals without protective gear, or direct contact with infectious secretions [28]. The participants reported that the great majority of all high-risk exposures they experienced were to COVID-19 patients and colleagues and did not occur in the community, underlining the importance of such high-risk exposures as an occupation-specific risk factor for HCWs. The high predictive value that high-risk exposures had for SARS-CoV-2 infections in employees simultaneously highlights the pivotal role of appropriate PPE as a preventive measure to limit virus spread in hospitals.

Healthcare professionals working in occupations with frequent patient contacts had an elevated risk for COVID-19. Among these patient-facing HCWs, nurses were at increased risk for SARS-CoV-2 infection likely due to their daily responsibilities involving closer and longer patient contacts. Additionally, we observed a trend towards higher risk with increasing numbers of patient contacts per day. Comparing patient-facing HCWs from different departments within the hospital, we found an increased risk for HCWs working in internal medicine. Staff in non-clinical departments, and without contact to patients, in contrast, had a decreased COVID-19 risk. Analyzing the different types of clinical units in which contacts to patients were reported, we found that HCWs treating COVID-19 patients both in ICUs and non-ICUs had an increased risk, whereas employees facing patients in the emergency room, operating theaters, and outpatient units had a similar risk compared to colleagues without patient contacts. Collectively, these results indicate not only that patient contacts are an important COVID-19 risk factor in HCWs, but also that the frequency and intensity of these contacts have an impact on the risk of infection. Treating COVID-19 patients elevated the risk, even though the hospital had precautions and safety measures to protect its employees when working with SARS-CoV-2-infected individuals, including advanced PPE.

A study conducted after the first pandemic wave in the metropolitan area of New York, USA, discovered no hospital-associated COVID-19 risk factors in HCWs [29]. Interestingly, New York had an approximately 4 – 8 times higher prevalence of individuals who went through a SARS-CoV-2 infection, at that time, compared the site of our study [30-32]. This indicates that the discovery of hospital-associated risk factors in HCWs might be impeded under circumstances of high prevalence by frequent high-risk exposures in the community – a relevant COVID-19 risk factor reported here and by others [33-36].

Other studies identified, in part, similar HCW-specific risk factors compared to ours for SARS-CoV-2 infection: patient-facing occupations [22], treating COVID-19 patients [21, 22], and working in internal medicine [22, 37]. However, our study identified, for the first time, being a nurse as well as work-related high-risk exposures as important COVID-19 risk factors in healthcare professionals and highlights the importance and significance of certain risk factors in its complex multivariate analysis.

Surprisingly, non-smokers had a significantly higher risk to become infected with the novel coronavirus compared participants who reported active smoking behavior. How active smoking can lower the COVID-19 risk might, on one hand, be explained by behavioral factors, such as being obligated to smoke outdoors and, thus, potentially preventing high-risk exposure to

colleagues during breaks. On the other hand, exposure to cigarette smoke was shown to have direct antiviral effects [38, 39]. Supporting our findings, more recent studies and meta-analyses have shown a reduced risk for SARS-CoV-2 infections in active smokers [40-42].

Our study took place immediately after the primary wave of the COVID-19 pandemic. During first weeks of the pandemic, PPE as well as capacities for PCR testing were limited, entire units of the hospital were rapidly restructured, and HCWs were redeployed to patient-facing positions [43]. Therefore, risk and protective factors reported in our study may not be applicable to the same extent to subsequent phases of the pandemic. In addition, COVID-19 vaccination campaigns might have influenced HCW-specific risk factors [44]. However, there is evidence that experiencing high-risk exposures to patients and being a nurse are risk factors for SARS-CoV-2 breakthrough infections also in fully vaccinated HCWs [45], indicating that the risk factors identified by us are still relevant today.

Dysgeusia was the symptom that was the most predictive of SARS-CoV-2 infection among the participants in our study, which is in line with other reports that indicate an association between taste disorder and COVID-19 [8, 9, 22]. We found the symptom complex dysgeusia, headache, fatigue, fever to characterize COVID-19 most specifically. More than a fourth of all COVID-19 cases among the staff were asymptomatic, which is comparable to the rates of asymptomatic SARS-CoV-2-infected HCWs identified in other cross-sectional studies after the first pandemic wave [46, 47].

Next, we aimed to evaluate the effect of working remotely to prevent the spread of SARS-CoV-2 infections. Of note, only those HCWs whose absence on-site would not have impacted patient care were given the possibility to work from home. Surprisingly, working remotely did not mitigate the risk for COVID-19 in these employees, despite the great majority of homestays having continued for at least three weeks – a significant duration in the first three months of the COVID-19 pandemic.

Finally, we evaluated the hospital's PCR testing strategy. Utilizing PCR testing to screen for acute SARS-CoV-2 infection can help identify COVID-19 cases and contain virus spread. Hence, it is a crucial surveillance strategy. At the time of the study, HCWs were mainly tested for two reasons: displaying COVID-19-associated symptoms and self-reporting high-risk exposures. Indeed, more than two-thirds of all employees who reported high-risk exposures, and more than a third of all staff who reported one or more symptoms, were screened in the study hospital. Among those HCWs who had a SARS-CoV-2 infection, approximately two-thirds received a PCR test, and no clusters of more than two SARS-CoV-2-infected employees remained undetected at the study site. Taken together, these results indicate that a simple and focused testing procedure was sufficient to detect the majority of HCWs suffering from COVID-19 and avert undetected disease outbreaks in the hospital.

In summary, our study uncovered both protective and risk factors for COVID-19 in healthcare professionals and identified several disease-associated symptoms. Furthermore, we analyzed specific preventive strategies against SASR-CoV-2 spread among HCWs. Working remotely had no effect, but most COVID-19 among the hospital employees were identified via an unsophisticated surveillance approach. Studies like ours may help enable risk factor-driven application of preventive measures protecting healthcare professionals from SARS-CoV-2 infection and curtail disease spread in hospitals in the future.

2.4 Influence of pre-existing immunity to seasonal coronaviruses on SARS-CoV-2 susceptibility and COVID-19 severity

The individual's susceptibility to SARS-CoV-2 infection as well as the course and severity of COVID-19 might be influenced by pre-existing immune responses to other viral pathogens. Herein, the immunological interplay between SARS-CoV-2 and seasonal, endemic coronaviruses (HCoVs) is especially relevant to be investigated, not only because HCoVs are closely related to the novel coronavirus, but also because HCoV infections are highly prevalent in humans.

Two of the four HCoV species, HCoV-229E and HCoV-NL63, are α-coronaviruses, whereas the others, HCoV-HKU1 and HCoV-OC43, are β-coronaviruses – similar to SARS-CoV-2. The genome organization and the life cycle of SARS-CoV-2 and HCoVs are generally analogous but, among others, they can differ in the number of accessory genes and their host cell tropism [48, 49]. HCoV infections usually lead to mildly symptomatic, self-limiting illness of the respiratory tract [50-53]. Seasonal endemic coronavirus infections are highly common [54-56], especially in the winter, accounting for approximately 15 – 30 % of all common cold cases [57]. Consequently, humoral immune responses to HCoVs can be detected in the great majority of the population [56, 58, 59]. Protective immunity to HCoVs, however, seems to be short-lived, and re-infections frequently occur [60].

Due to structural and antigenic similarities between the proteins of HCoVs and SARS-CoV-2 [61], it is conceivable that immune responses to previous HCoV infections could have an influence on the susceptibility to SARS-CoV-2 and the severity of COVID-19. Indeed, former exposure to seasonal coronaviruses was hypothesized to induce cross-protective immunity to SARS-CoV-2 [62]. Corroborating this hypothesis, a study showed that previous HCoV infections are related to lower disease severity in SARS-CoV-2-infected individuals [63]. However, adaptive immunity to distinct seasonal coronaviruses was not evaluated in this work, but only health record data. In other studies, reactivity of pre-existing T cells to HCoV antigens were suggested to play a protective role in SARS-CoV-2 infection [64, 65].

Regarding humoral immune responses to endemic coronaviruses and their potential influence on SARS-CoV-2 susceptibility and the COVID-19 disease course, a recent study found levels of IgG-type antibodies against the spike antigen of HCoV-OC43 to be indifferent in COVID-19 patients prior to SARS-CoV-2 infection compared to persons who avoided infection [66]. Moreover, no correlation was observed between the titers of anti-HCoV-OC43 spike antibodies prior to infection and COVID-19 severity [66]. In conclusion, there appeared to be no association between humoral immune responses to HCoVs and protection against SARS-CoV-2 infection or severe COVID-19. As a limitation of this study, nucleocapsid specific-antibody levels were not evaluated and, furthermore, the collection of the pre-infection sera from COVID-19 patients, in many instances, dated back several years. However, in the course of months after infection, humoral immune responses to HCoVs were reported to wane and be highly unsteady [60]. This finding challenges the meaningfulness of evaluating the matched sera in the aforementioned study [66].

In a more comprehensive methodological approach, my colleagues and I quantified IgG-type antibodies specific to the nucleocapsid and spike antigens of the novel coronavirus and all four HCoVs in sera collected from 888 healthy adults before the pandemic as well as in 314 longitudinally sampled sera from 96 patients with COVID-19. As expected, we found antibodies against the nucleocapsid and spike proteins of SARS-CoV-2 to be highly elevated in COVID-19 patients compared to healthy adults. Surprisingly, anti-nucleocapsid antibodies specific to the two α-coronaviruses and, to lesser extent, HCoV-HKU1 were also significantly increased in SARS-CoV-2-infected individuals. Moreover, we observed stronger responses to the nucleocapsid protein of HCoV-229E, HCoV-NL63 and HCoV-HKU1, comparing critically ill COVID-19 patients

and less severely affected cases (disease severity definitions according to WHO guidelines [12]). Regarding anti-spike antibodies, we found higher levels of antibodies specific to the S2 domain of HCoV-OC43 spike antigen in COVID-19 patients than in pre-pandemic donors. Critically ill patients, in contrast, showed reduced antibody reactivity to the spike S1 domain of HCoV-OC43 compared to healthy adults.

The elevated anti-HCoV antibody concentrations observed in COVID-19 patients could be explained by two possible confounders: First, anti-SARS-CoV-2 antibodies in patients might be cross-reactive to HCoV antigen components in the detection assays utilized. Second, it is conceivable that clones of already existing plasma cells specific to HCoV antigens are stimulated upon SARS-CoV-2 infection. To tackle these confounders, we analyzed longitudinal alterations in the antibody concentration of 28 COVID-19 patients who had donated serum specimens both in the first two weeks post symptom onset and thereafter. While titers of SARS-CoV-2-specific antibodies were dramatically rising after the development of symptoms in these patients, antibody concentrations against the four HCoVs stayed mostly indifferent. Next, mean antibody titers in COVID-19 patients measured during the first two weeks after the development of symptoms were tested for statistically significant differences to antibody responses quantified thereafter. We detected elevated antibody titers against SARS-CoV-2, distinctive of the newly mounting immune response to the pandemic coronavirus. In the same comparison, however, we observed mostly insignificant changes for anti-HCoV antibody responses. Compared to healthy adults, on the contrary, antibody responses to the nucleocapsid of the two α-coronaviruses and the spike S2 domain of HCoV-OC43 were noticeably elevated in these 28 COVID-19 patients, whereas anti-spike S1 domain antibody titers against HCoV-OC43 were significantly decreased. Taken together, our results largely exclude cross-reactive anti-SARS-CoV-2 antibodies and activation of HCoV-specific plasma cells upon infection with the novel coronavirus as confounders.

To control for other potential confounders, antibody responses measured in each patient were compared to additional health record data. Anti-SARS-CoV-2 and anti-HCoV antibody levels showed only weak correlations to the age of patients, comorbidities, their interleukin-6 concentration, and the duration they spend in the hospital or the ICU. There was, however, a significant correlation between male gender and anti-nucleocapsid antibodies targeting seasonal coronaviruses. Additionally, multivariate analysis revealed a significant correlation between interleukin-6 responses at hospital admission and COVID-19 disease severity, in line with the results from a recent study [67].

We conclude that elevated concentrations of antibodies against the nucleocapsid of seasonal endemic α-coronaviruses and the spike S2 domain of HCoV-OC43 were highly likely pre-existing in COVID-19 patients. This is evidence that pre-existing adaptive, humoral immunity to seasonal endemic coronaviruses is connected to increased susceptibility to infection with SARS-CoV-2 and adverse disease outcome.

During the validation of one of the assays utilized in the study, similar, albeit less pronounced, trends for increased IgG-type antibody responses against the nucleocapsid antigen of HCoV-229E and HCoV-NL63 were observed in COVID-19 patients [68]. Conversely, reduced levels of nucleocapsid-specific anti-HCoV antibodies were found in a cohort of symptomatic, SARS-CoV-2 infected healthcare professionals compared to asymptomatic cases [69]. Another study found evidence that high titers of anti-nucleocapsid antibodies against HCoV-OC43 prevented individuals from SARS-CoV-2 infection [70]. Of note, the cohorts analyzed in these three studies consisted mainly [68, 69], or entirely [70], of asymptomatic or mildly ill patients, while our study contained a drastically lower proportion of mildly affected COVID-19 cases.

Multiple studies described elevated concentrations of antibodies targeting the spike antigen of HCoV-OC43 in COVID-19 patients and vaccinees compared to non-vaccinated, naïve individuals,

in line with our findings [66, 71, 72]. However, their data analyses indicated that these elevated anti-HCoV-OC43 spike antibody concentrations were dependent on the course of COVID-19 or vaccination [66, 71, 72], and largely mediated by antibodies specific to the spike S2 domain [66]. Corroborating these observations, our data show elevated anti-HCoV-OC43 spike S2 domain antibody concentrations. However, our longitudinal evaluation revealed that these antibody concentrations remained stable during the course of COVID-19, indicating that increased antibody responses were, in fact, pre-existing. Both, differences in the specificities of the assays used to detect HCoV antigens, and dissimilarities in the patient cohorts investigated may account for the discrepant results. The described studies comprised of less severely and critically affected COVID-19 patients compared to ours. Collectively, our findings are presumably better applicable to more severe COVID-19. Supporting this notion, two recent studies found elevated anti-HCoV-OC43 spike S2 domain antibodies to be associated with critical disease severity in COVID-19 patients [73, 74].

In summary, our study indicates that pre-existing, humoral immunity to HCoVs, namely IgG-type antibodies against both the nucleocapsid of seasonal α-coronaviruses and the spike protein of HCoV-OC43, increase susceptibility to SARS-CoV-2 and COVID-19 severity. Anti-HCoV antibody levels might serve as markers for clinical COVID-19 risk stratification. Furthermore, we propose that advanced preventive measures against COVID-19 may be beneficial for individuals who recently recovered from seasonal coronavirus infections. The development of a universal vaccine against SARS-CoV-2 and HCoVs could mitigate the immunological crosstalk between the different species of human pathogenic coronaviruses and its antagonistic effect on subsequent, potentially life-threatening coronavirus infections.

2.5 Immune evasion of SARS-CoV-2 variant Omicron in convalescent and vaccinated individuals

In response to the pandemic, different types of COVID-19 vaccines were developed [75]. The most commonly administered among these vaccines in Europe and North America are mRNA vaccines [76, 77], because they were shown to be safe and effective [78-81], as well as rapidly manufactured at relatively low cost [82]. In simplified terms, the mRNA in these vaccines is packaged into lipid nanoparticles, taken up and translated by host cells after administration leading to the endogenous expression of SARS-CoV-2 spike antigen that is, ultimately, eliciting adaptive immune responses in recipients [83]. The basic immunization with COVID-19 mRNA vaccines requires two doses of the respective vaccines given in short succession of three to six weeks [78, 79, 81]. Unfortunately, the immunity after these first two vaccinations wanes in the following months [84, 85]. Thus, an additional dose of mRNA vaccines is often administered more than six months after basic immunization [86-88]. Until mid-2022, all approved mRNA vaccines were encoding for the spike antigen of early SARS-CoV-2 variant Wuhan-Hu1. Overall, these vaccines do not prevent SARS-CoV-2 transmission entirely, but they can prevent symptomatic and severe disease after infection with earlier virus variants at high efficacy [85].

Since the beginning of the pandemic, however, SARS-CoV-2 underwent considerable evolution. Several new virus variants emerged that showed enhanced pathogenicity, increased transmissibility, or partial immune escape [89]. Five of these so-called variants of concern (VoCs) have emerged thus far: Alpha, Beta, Gamma, Delta, and Omicron [90]. As of early 2022, VoC Omicron is the dominant SARS-CoV-2 variant globally [91]. Omicron exhibits the ability to spread faster and more efficiently than previous virus variants and, thus, caused a drastic increase in COVID-19 cases since its appearance [1]. This enhanced fitness of SARS-CoV-2 VoC Omicron

is, among others, likely due to a large number of amino acid substitutions, insertions and deletions in the viral spike protein compared to the original Wuhan-Hu1 virus strain [92, 93]. Many of these mutations affect spike antigen epitopes that are suspected to be relevant for neutralization by polyclonal antibodies [93-95]. Therefore, Omicron was anticipated to escape humoral immune responses in convalescent and vaccinated naïve individuals [96, 97].

Neutralizing antibody responses were discovered to be highly predictive of immune protection from symptomatic SARS-CoV-2 infection [98]. The breadth and efficiency of neutralizing antibodies can be expanded by affinity maturation [99]. In this process, somatic hypermutations in variable regions of antibodies increase their binding affinity to the respective antigen [94, 100]. The quality of affinity maturation, however, depends on the type and duration of antigen exposure [94, 100]. In case of COVID-19, it was shown that the capacity of neutralizing antibodies to control newly emerging SARS-CoV-2 variants was enhanced by affinity maturation [101]. Conceivably, this process might enable the neutralization of emerging virus variants that have evolved to escape neutralization by ancestral antibodies, including SARS-CoV-2 VoC Omicron.

In a longitudinal cohort study, my colleagues and I aimed to characterize the ability of Omicron and previous VoCs to escape humoral immune responses and review the hypothesis that this immune evasion can be counteracted by antibody affinity maturation. We characterized anti-spike IgG antibody titers, IgG antibody avidity, and infection neutralizing capacities in a cohort of 98 convalescent individuals infected with early SARS-CoV-2 variants contributing 412 longitudinally sampled sera, and 73 infection-naïve individuals matched for sex, age, working conditions, and risk factors donating 305 sera [102]. To analyze the dynamics of infection neutralization against SARS-CoV-2 and its VoCs after different, timely spaced infection events and vaccinations, the participants were continuously followed since the first wave of the COVID-19 pandemic, through their initial COVID-19 vaccinations with mRNA BNT162b2, and after a third application of the vaccine. The first and second vaccinations were administered three weeks apart and the third dose was applied nine months later.

Utilizing a newly developed high-throughput live-virus neutralization assay, we assessed serum-neutralizing activities against an early SARS-CoV-2 isolate i.e., EU1, and all five VoCs: Alpha, Beta, Gamma, Delta, and Omicron (sublineage BA.1). We found low-level infection neutralization capacities against all SARS-CoV-2 variants in sera collected from convalescent individuals approximately nine months after infection. After the first vaccination, serum-neutralization levels in these convalescents showed a drastic increase, whereas those in infection-naïve vaccinees remained close to background. Neutralization titers in naïve individuals were considerably boosted after the second vaccination but, still, remained significantly lower compared to those in convalescents. Giving a second vaccination to convalescents three weeks after the first had an insignificant effect on their neutralization capacity compared to administration of a single dose even when measured four and seven months later. Overall, the capacities to neutralize Omicron and, albeit less pronounced, VoC Beta were drastically lower than for the other SARS-CoV-2 variants. These results provide evidence for the prominent immune escape of Omicron after two exposures with spike antigen from early SARS-CoV-2 variants. Corroboration this finding, several other studies reported Omicron to evade neutralizing immunity in convalescent and twice-vaccinated naïve individuals [96, 103-107].

Applying an additional dose of BNT162b2 to our cohort nine months after the initial vaccinations, however, induced high infection-neutralization titers against all VoCs, including Omicron, in both naïve and convalescent individuals. Apparently, a third exposure with spike antigen from an early SARS-CoV-2 variant was, at least partly, sufficient in overcoming the humoral immune evasion of VoC Omicron, in line with results from other studies [96, 103].

Next, we quantified the dynamics of antibody responses against the spike S1 domain of the original SARS-CoV-2 variant Wuhan-Hu1. Antibody levels of IgG-type antibodies reached their maximum in convalescents after one vaccine dose, and in naïve individuals after two vaccinations. Subsequently, antibody concentrations gradually declined in both groups at four and seven months after the initial vaccinations. After the third exposure to viral spike antigen, serum antibody titers increased again significantly in both convalescent and naïve individuals.

The striking decline of anti-spike IgG antibody concentrations in both groups following the initial vaccinations was not observed in the individuals' virus neutralization activities. In contrast, the infection neutralization capacities remained largely stable even at four and seven months after the second exposure to spike antigen. In sera of convalescents, we found neutralization capacities per antibody unit that increased slightly after every vaccination. In naïve individuals, this ratio was low shortly after the first and second vaccinations but increased over time and was boosted further after the third vaccination.

Taken together, this lack of a direct association between anti-spike antibody concentrations and virus neutralization indicates a maturation of the antibody response over time and after each encounter with the SARS-CoV-2 spike antigen. This could be due either to an increased breadth of the polyclonal neutralizing antibody repertoire or an increase of the antibody binding to the spike protein. To investigate the latter, we measured the avidity of serum IgG-type antibodies to the spike protein ectodomain of the original SARS-CoV-2 strain Wuhan-Hu1. A striking increase in antibody avidity was observed in convalescent individuals after a single mRNA vaccine dose. Antibody avidities remained generally stable in the following seven months and were not further elevated by another dose of the vaccine nine months after the initial vaccination. Consequently, a single vaccination appeared to be sufficient to reach maximal avidity in convalescents, corroborating reports of anti-spike antibody maturation after SARS-CoV-2 infection [108, 109]. In naïve individuals, in contrast, anti-spike antibody avidity only increased seven months after the second vaccination, and the third vaccination was necessary to reach avidity levels comparable to those in vaccinated convalescents. Collectively, these results suggest that increasing antibody avidities play a pivotal role in achieving highly potent infection-neutralization capacities. In line with these findings, a study reported affinity-matured memory B cells up to six months after SARS-CoV-2 infection [110]. We provide evidence that two timely spaced vaccinations in convalescents and three vaccinations in infection-naïve individuals are highly beneficial to counteract immune evasion of SARS-CoV-2 VoCs such as Omicron.

Finally, we investigated whether three timely spaced exposures to SARS-CoV-2 spike protein also induced high-neutralizing immunity in twice-vaccinated individuals suffering from a SARS-CoV-2 breakthrough infection. We determined infection neutralization capacities in a cohort of 31 participants, who received their initial vaccinations, on average, five months before breakthrough infection (16 with Delta and 15 with Omicron). One week after PCR-based diagnosis of COVID-19, neutralization titers in these individuals were markedly higher compared to twice-vaccinated naïve study participants from the other cohort and, furthermore, comparable to those measured in twice vaccinated convalescent and triple-vaccinated naïve individuals. There were no significant differences in the infection neutralization capacities against the different SARS-CoV-2 variants tested, including Omicron, between participants with either Delta or Omicron breakthrough infections. In line with these results, we observed increasing antibody avidities to the Wuhan-Hu1 spike antigen in this cohort after Delta or Omicron breakthrough infection.

In summary, our study reports four key findings: First, SARS-CoV-2 VoC Omicron displays a strong immune escape evading antibody neutralization in vaccinated individuals. This immune evasion can be, at least partly, overcome by an additional timely spaced vaccination several months after the initial immunization. Second, convalescents do not benefit from a second

vaccination given within three weeks after the first. Third, there is no strong association between anti-spike IgG levels and infection neutralization capacity in longitudinal analysis. However, a drastic increase in anti-spike antibody avidity was detected after first vaccination in convalescents and after the second and third vaccinations in naïve individuals. This highlights that the quality of antibodies rather than their mere quantity is important, and that antibody maturation is, indeed, crucial to achieve a broad neutralizing immunity against different SARS-CoV-2 VoCs. Fourth, triple-vaccinated naïve individuals reach almost the same levels of antibody-mediated virus neutralization compared to those who acquired a 'hybrid immunity' i.e., vaccinated convalescents and individuals after a breakthrough infection. A single infection with SARS-CoV-2 alone, however, does not achieve a similar level of protection as the combination of infection and vaccination.

Our study was among the first to report the immune evasion of VoC Omicron that can possibly be overcome by repeated exposure to viral spike antigen fueling the maturation of neutralizing antibodies. Together with the findings from similar studies it may have helped to refine strategies and guidelines for vaccination in different countries. Since the emergence of SARS-CoV-2 VoC Omicron, however, the immunological landscape among the population became far more complex: the number of convalescent individuals increased drastically [1]. Simultaneously, several novel sublineages of Omicron emerged, some of which were reported to show an even stronger immune escape than the original sublineage BA.1 [111, 112]. Furthermore, novel mRNA vaccines modified to achieve better protection against Omicron and its sublineages were developed [113-115], and a fraction of the population have thus far received a fourth vaccination [76]. Thus, evaluating the protective capacities of vaccinations and past infections is becoming increasingly difficult, but it remains an important aim for future research.

2.6 Outlook

Since early 2020, the COVID-19 pandemic has posed substantial economic, social and, moreover, health-related challenges to our society. Thanks to great efforts, health authorities, scientist and healthcare professionals swiftly installed containment measures, identified risk factors, and evaluated, developed as well as refined methods to limit SARS-CoV-2 spread and prevent severe COVID-19. Taken together, these endeavors undoubtedly saved countless lives and helped coping with the pandemic.

However, COVID-19 remains a substantial threat to global health. The emergence of novel SARS-CoV-2 variants with increased pathogenicity and more pronounced immune escape could thwart our accomplishments in fighting this pandemic.

Therefore, it remains crucial to research SARS-CoV-2 and COVID-19: among others, risk groups as well as risk factors should be monitored and evaluated. The effectiveness of protective measures, such as vaccination, to prevent or mitigate infections with novel virus variants requires continuous monitoring. Furthermore, alternative approaches need to be explored, including the development of effective and safe antiviral drugs and universal coronavirus vaccines.

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ORIGINAL PAPER

In-depth profiling of COVID-19 risk factors and preventive measures in healthcare workers

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Abstract

Purpose To determine risk factors for coronavirus disease 2019 (COVID-19) in healthcare workers (HCWs), characterize symptoms, and evaluate preventive measures against SARS-CoV-2 spread in hospitals.

Methods In a cross-sectional study conducted between May 27 and August 12, 2020, after the first wave of the COVID-19 pandemic, we obtained serological, epidemiological, occupational as well as COVID-19-related data at a quaternary care, multicenter hospital in Munich, Germany.

Results 7554 HCWs participated, 2.2% of whom tested positive for anti-SARS-CoV-2 antibodies. Multivariate analysis revealed increased COVID-19 risk for nurses (3.1% seropositivity, 95% CI 2.5–3.9%, $p = 0.012$), staff working on COVID-19 units (4.6% seropositivity, 95% CI 3.2–6.5%, $p = 0.032$), males (2.4% seropositivity, 95% CI 1.8–3.2%, $p = 0.019$), and HCWs reporting high-risk exposures to infected patients (5.5% seropositivity, 95% CI 4.0–7.5%, $p = 0.0022$) or outside of work (12.0% seropositivity, 95% CI 8.0–17.4%, $p < 0.0001$). Smoking was a protective factor (1.1% seropositivity, 95% CI 0.7–1.8% $p = 0.00018$) and the symptom taste disorder was strongly associated with COVID-19 (29.8% seropositivity, 95% CI 24.3–35.8%, $p < 0.0001$). An unbiased decision tree identified subgroups with different risk profiles. Working from home as a preventive measure did not protect against SARS-CoV-2 infection. A PCR-testing strategy focused on symptoms and high-risk exposures detected all larger COVID-19 outbreaks.

Conclusion Awareness of the identified COVID-19 risk factors and successful surveillance strategies are key to protecting HCWs against SARS-CoV-2, especially in settings with limited vaccination capacities or reduced vaccine efficacy.

Keywords SARS-CoV-2 · COVID-19 · Healthcare workers · Risk factors · Prevention

Introduction

The coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) rapidly evolved to a pandemic in early 2020 with more than 173.4 million confirmed cases and 3.73 million deaths by June 7th, 2021 [1]. Effective treatment options for COVID-19 have not been discovered and vaccination programs are not yet available at scale in many countries, potentially weakened by the emergence of variants of concern (VOCs) [2, 3], or not well-accepted by parts of the population [4]. To this date, COVID-19 remains a major threat to global health and continues to dictate policymaking around the world.

With 5–20% of confirmed COVID-19 cases being hospitalized [5, 6], and approximately 20% subsequently requiring intensive care [7], uncontrolled SARS-CoV-2 transmission threatens to overwhelm healthcare systems [8, 9]. Ensuring adaptable and adequate hospital capacities depends heavily on the availability of skilled healthcare workers (HCWs). Given that frontline HCWs are particularly at risk

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of infection due to their increased exposure to SARS-CoV-2, protecting them appropriately is of high priority. Indeed, several reports of larger COVID-19 outbreaks within hospitals highlight the threat that nosocomial infections pose to both patients and HCWs [10–14]. The importance of identifying HCW-specific risk factors is underscored by the recent emergence of SARS-CoV-2 VOCs with substantially increased transmissibility, possibly elevated case fatality rates, and reduced vaccine efficacy for some [2–4, 15, 16].

Here, we report the findings from a cross-sectional study assessing SARS-CoV-2 seroprevalence as an indicator of COVID-19 in HCWs at a multicenter, quaternary care hospital in Munich, Germany. Using a questionnaire covering epidemiological and COVID-19-specific items, we identified risk groups and risk factors, characterized symptoms of SARS-CoV-2 infection, and evaluated measures to identify and prevent SARS-CoV-2 infections among employees.

Materials and methods

Study design, setting and participants

Between May 27th and August 12th, 2020, we invited all 11,580 employees of the LMU Klinikum, a quaternary care university hospital complex with two centers in Munich, Germany, to enroll in this cross-sectional study.

Data collection

Participants donated a blood sample to determine the seroprevalence of antibodies against SARS-CoV-2. Furthermore, they answered an online-questionnaire assessing epidemiological, occupational, and COVID-19-specific data e.g., occurrence of symptoms, self-quarantining, or high-risk exposure to SARS-CoV-2-infected individuals (Supplementary Tables 1, 2). High-risk exposure was defined according to the criteria of the European Centre for Disease Prevention and Control [17]. The occupational health office and the HR department of the LMU Klinikum provided time-resolved numbers of hospitalized COVID-19 patients, and SARS-CoV-2-infected or quarantined HCWs, respectively.

Anti-SARS-CoV-2 antibody detection assays

The following four commercial tests were used according to the manufacturers' instructions to determine the presence of SARS-CoV-2-specific antibodies in serum specimens: Architect SARS-CoV-2 IgG (Abbott, Illinois, USA), Anti-SARS-CoV-2-ELISA IgG (EuroImmun, Lübeck, Germany), Elecsys® Anti-SARS-CoV-2 (Roche, Basel, Switzerland), and recomLine SARS-CoV-2 IgG (Mikrogen, Neuried, Germany). We included a threshold for indeterminate test results in the Elecsys® assay at 0.8 COI value. Additionally, a self-developed assay was utilized. Herein, 96-well high-binding plates were coated overnight at 4 °C with purified, trimeric SARS-CoV-2 spike protein (1 µg/mL, 50 µL/well) in 0.1 M sodium carbonate pH = 9.57, and blocked with 3% milk in 0.05% Tween-20 in PBS (PBST, 100 µL/well) for 1 h at RT. After blocking, plates were incubated for 1 h at RT with 50 µL/well heat-inactivated patient serum samples diluted 1:150 in PBS containing 1% milk. Subsequently, horseradish peroxidase (HRP) conjugated goat anti-human IgG antibody (Sigma-Aldrich A0293, 50 µL/well, diluted 1:3000 in 1% milk in PBST) was added and samples were incubated for 1 h at RT. After all steps mentioned above, plates were washed with PBST. For the HRP-catalyzed reaction, samples were incubated with 50 µL/well BD OptEIA™ TMB substrate (BD Biosciences, New Jeresey, USA) and the reaction was stopped after 10 min by addition of 50 µL/well 5% H_2SO_4. Finally, absorption was recorded at 450 nm. Samples were called indeterminate or positive with a background-subtracted absorption of more than 15% (indeterminate) and 45% (positive) of the absorption of a uniform plate-wise positive control that consisted of several pooled sera from hospitalized COVID-19 patients.

The performance of the anti-SARS-CoV-2 antibody detection assays was determined on a set of 1152 pre-pandemic serum samples from adults and children, as well as 332 specimens from 99 COVID-19 patients (Supplementary Tables 3, 4).

Sera from all participants were tested using both the Elecsys® assay, and the self-developed ELISA. Samples that were tested negative in both screening assays, but either scored indeterminate in at least one of the two assays or originated from a participant who reported a positive SARS-CoV-2 rRT-PCR result in the study questionnaire, were further analyzed via the other assays (Supplementary Fig. 1a). As COVID-19 vaccines were not administered to HCWs at the LMU Klinikum before or during study sampling, the detection of anti-SARS-CoV-2 antibodies in participants' sera was indicative of (sub-)acute or resolved SARS-CoV-2 infection and therefore, according to the case definition of the European Centre for Disease Prevention and Control (ECDC), these HCWs were classified as COVID-19 cases [18].

SARS-CoV-2 neutralization assay

CaCo-2 cells (American Type Culture Collection, ATCC, Virginia, USA) in cell culture medium (Dulbecco's Modified Eagle's Medium containing 2% fetal bovine serum) were challenged for 2 h with a clinical isolate (GISAID EPI ISL 4,66,888) previously obtained from a nasopharyngeal swab of a COVID-19 patient. Subsequently, cell culture medium was exchanged, and three days post infection supernatants

were passaged on Vero-E6 cells (ATCC). After three additional days, cell culture supernatants were harvested and stored at −80 °C. The virus stock was characterized by rRT-PCR and by titration on human lung epithelial A549 cells (ATCC), overexpressing the human angiotensin-converting enzyme 2 receptor, ACE2 (A549-hACE2 cells).

A volume of this virus stock, which results in a 90% cytopathic effect three days post infection, was incubated for 2 h with patient sera at different dilutions. Subsequently, 10 µL of the virus-serum mixtures were added to 20 µL A549-hACE2 cells cultured in 384-well plates (7500 cells/well). Three days post infection, 10 µL of CellTiter-Glo® 2.0 reagent (Promega, Wisconsin, USA) were added to each well and the luminescence recorded (0.5 s integration time, no filter). The half-maximal inhibitory concentrations (IC_{50}) for inhibiting virus-mediated cell death were computed via normalized sigmoidal dose–response curve approximation with variable slopes. Neutralizing activities were categorized via the obtained IC_{50} values: none (IC_{50} < tenfold serum dilution), low (IC_{50} < 90-fold serum dilution), intermediate (IC_{50} < 270-fold serum dilution), high (IC_{50} < 2430-fold serum dilution), very high (IC_{50} ≥ 2430-fold serum dilution).

Statistical analysis

Data were analyzed in R version 4.0.3 (www.r-project.org) using the R package epitools. Parameters of multivariate significance are the result of a logistic regression, using recursive elimination of the least significant remaining factor. *p* values on pair-wise comparisons were calculated using Fisher's exact test with Holm's multiple testing correction as indicated. Decision trees were computed using the party package in R with default parameters [19]. Confidence intervals for absolute risks were calculated with Wilson's method using the binconf function from the Hmisc R package.

Results

Pandemic situation and study population

Until August 12th, 2020, the Munich Metropolitan region was among the areas most severely affected by the COVID-19 pandemic in Germany (Fig. 1a, blue), accounting for 12.8% (28,010/2,18,519) of all cases registered [20]. Quarantining (Fig. 1b, green) was mandatory for SARS-CoV-2 PCR-positive HCWs (Fig. 1b, red), those who returned from designated high-risk areas [21], and for HCWs non-essential for patient care reporting high-risk exposures to infected individuals. Until August 12th, 2020, 231 COVID-19 patients were hospitalized at the quaternary care hospital surveyed here, at peak times 70 per day (Fig. 1b, blue),

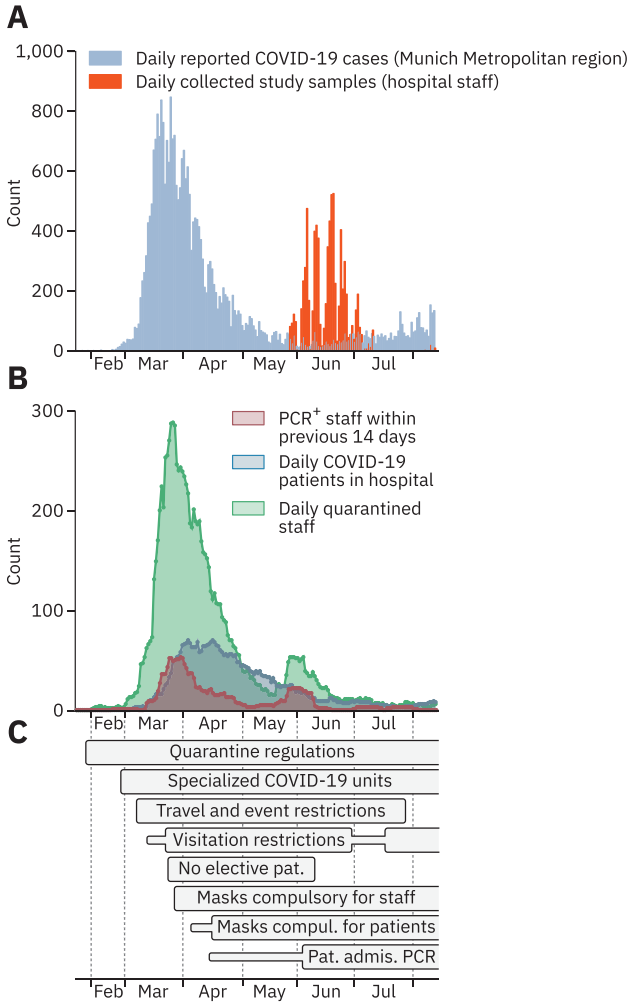


Fig. 1 Dynamics of the COVID-19 pandemic and implementation of preventive measures. **a** COVID-19 cases officially reported for the Munich metropolitan region until August 12th, 2020 (blue) and the number of blood samples collected from staff members (orange) are depicted as one bar per day. **b** Number of HCWs who tested positive for SARS-CoV-2 by PCR within a two-week window preceding the reported date (red), number of COVID-19 patients treated in the hospital (blue), and number of hospital staff in quarantine (green). **c** Time-resolved depiction of state-imposed and institutional measures taken to prevent SARS-CoV-2 spread at the multicenter hospital. Thinner, horizontal bars represent less strict measures of the same type. Measures that were still in effect by August 12th, 2020 are depicted as bars with open endings. Pat. Admis. PCR – Mandatory PCR test for newly admitted patients

and several COVID-19 countermeasures were implemented (Fig. 1c, Supplementary Table 5).

Between May 27th and August 12th, 2020, after the first wave of the COVID-19 pandemic had largely subsided, we invited all 11,580 staff members of the multicenter hospital to submit a blood sample for analysis of anti-SARS-CoV-2 antibodies (Fig. 1a, orange), and to complete a questionnaire. 7554 employees (65.2% of all staff) participated, 2.2% (166/7554) of whom tested positive for anti-SARS-CoV-2

Table 1 Epidemiological information and anti-SARS-CoV-2 antibody status of 7554 healthcare workers participating in the study

	Anti-SARS-CoV-2 Ab		95% CI
	Positive/total	%	
Total	166/7554	2.20	1.89–2.55
Age group (*Y*)			
≤30	64/2170	2.95	2.32–3.75
31–40	39/1951	2.00	1.47–2.72
41–50	29/1430	2.03	1.42–2.90
51–60	23/1467	1.57	1.05–2.34
>60	11/536	2.05	1.15–3.64
Gender			
Female	115/5431	2.12	1.77–2.54
Male	51/2118	2.41	1.84–3.15
3rd gender	0/5	0.00	
Patient care occupations			
Nurse	68/2185	3.11	2.46–3.93
Physician	38/1345	2.83	2.07–3.85
Other	17/1199	1.42	0.88–2.26
Total	123/4729	2.60	2.18–3.10
Non-patient care occupations			
Administration/IT	15/822	1.82	1.11–2.99
Research	12/977	1.23	0.70–2.14
Transportation	1/28	3.57	0.63–17.71
Cleaning personnel	4/119	3.36	1.32–8.33
Other	11/879	1.25	0.70–2.23
Total	43/2825	1.52	1.13–2.04

Binominal 95% confidence intervals (95% CI) were calculated using the Wilson score interval

antibodies (Supplementary Fig. 1a). Results from the two screening assays agreed in 98.1% (7349/7491) of cases (Supplementary Fig. 1b). Seropositivity was most frequent among HCWs under 30 years of age (2.95%, Table 1). More participants were female (5431/7553, 71.9%), and male gender was a COVID-19 risk factor in multivariate analysis (2.41% seropositivity, 95% CI 1.8–3.2, *p* value for multivariate analysis (p_m) = 0.019, Table 2). 88.2% (164/186) of serum samples from anti-SARS-CoV-2 antibody positive (Ab^+) HCWs or those reporting positive SARS-CoV-2 PCR results exhibited neutralizing activity (Supplementary Fig. 2a, b). This neutralizing activity correlated with antibody titers, but not with the time elapsed since a positive PCR test (Supplementary Fig. 2c, d).

High-risk exposure to infected individuals

Participants were asked to report high-risk exposures (defined according to the criteria of the ECDC [17]) to either patients, co-workers, or individuals in their non-work-related environment ("community") with acute COVID-19. High-risk exposures within a HCW's community or to COVID-19 patients were risk factors for SARS-CoV-2 infection in multivariate analysis (12.0% seropositivity, 95% CI 8.0–17.4, $p_m < 0.0001$, and 5.5% seropositivity, 95% CI 4.0–7.5, $p_m = 0.0022$) (Table 2). Moreover, compared to staff members without high-risk exposure, HCWs' exposures in the hospital to either infected co-workers (risk ratio (RR) 3.76, 95% CI 2.32–6.10) or COVID-19 patients (RR 3.65, 95% CI 2.33–5.71), and especially to infected individuals in the community (RR 9.84, 95% CI 5.98–16.19) resulted in increased risk for seropositivity ($p < 0.0001$ for all three comparisons)

Table 2 Significant risk and protective factors for SARS-CoV-2 seropositivity among participants in multivariate analysis

Parameter	Anti-SARS-CoV-2 Ab			p_m value	*Z* value
	Positive/total	%	95% CI		
All participants	166/7554	2.2	1.9−2.6		
Male gender	51/2067	2.4	1.8–3.2	0.019	2.35
Active smoking behavior	16/1407	1.1	0.7–1.8	0.00018	−3.74
Works in non-clinical department	9/1149	0.8	0.4–1.6	0.017	−2.55
Working on COVID-19 unit	28/583	4.6	3.2–6.5	0.032	2.14
High-risk exposure to infected patients	38/651	5.5	4.0–7.5	0.0022	3.06
High-risk exposure in community	22/162	12.0	8.0–17.4	<0.0001	5.04
Occupation: nurse	68/2117	3.1	2.5–3.9	0.012	2.52
Symptom: taste disorder	72/170	29.8	24.3–35.8	<0.0001	14.81
Symptom: sore throat	53/1853	2.8	2.1–3.6	<0.0001	−4.35
Symptom: fatigue	86/1413	5.7	4.7–7.0	<0.0001	4.76
Patient contacts primarily in operating theaters	9/896	1.0	0.5–1.9	<0.0001	−4.06

Binominal 95% confidence intervals (95% CI) were calculated using the Wilson score interval

Logistic regression followed by recursive feature elimination up to a threshold of $p = 0.05$. p_m value−*p* value for multivariate analysis

(Fig. 2a, Supplementary Fig. 3a). Dual high-risk exposures to either co-workers or patients in combination with an exposure in the community led to greater COVID-19 risk than exposures in the hospital alone (Fig. 2b). However, markedly more HCWs reported high-risk exposures in the hospital than in their community (Fig. 2a). 55% (91/166) of seropositive HCWs did not report any high-risk exposure, underscoring the importance of unrecognized exposure for infection.

Occupation-specific risk factors

Nurses, doctors, cleaning- and transport personnel had the highest risk for seropositivity (Table 1) and working as a nurse was a risk factor of multivariate significance (3.1% seropositivity, 95% CI 2.5–3.9, $p_m = 0.011$, Table 2). HCWs with low risk included researchers and medical technicians. Generally, patient-facing HCWs were more at risk for SARS-CoV-2 infection than non-patient-facing HCWs (RR 1.77, 95% CI 1.25–2.50, $p = 0.002$, Table 1). Frequent patient contacts increased the COVID-19 risk across all patient-facing occupations (Fig. 2c). Nurses reporting six to ten patient contacts per day had a noticeably low risk (Fig. 2c, blue line). 36.7% (218/594) of nurses in this group worked in operating theaters (Supplementary Fig. 4a), where few COVID-19 patients were treated, and nurses' overall risk was lowest (Supplementary Fig. 4b). Nurses reporting between one and five patient contacts per day were, in turn, highly at risk for SARS-CoV-2 infection. Analysis of this subgroup revealed that 75.1% (511/680) worked on intensive care units (ICUs, Supplementary Fig. 4a), where, despite few patient contacts, nurses were highly at risk (Supplementary Fig. 4b).

Department- and unit-specific risk factors

The majority of departments deployed staff members to COVID-19 units (Supplementary Table 6). Among HCWs from these "COVID-19 response departments" who did not work on COVID-19 units, only personnel from conservative departments showed an increased rate of seropositivity compared to personnel without patient contact (RR 2.27, 95% CI 1.54–3.34, $p = 0.0004$). Within this group, HCWs in departments of internal medicine had a markedly increased COVID-19 risk (RR 3.74, 95% CI 2.40–5.81, $p < 0.0001$, Fig. 2d). Working on COVID-19 units was associated with an overall increased risk for seropositivity in a multivariate model (4.6% seropositivity; 95% CI 3.2–6.5, $p_m = 0.032$, Table 2). Among personnel working on COVID-19 units, staff members from internal medicine departments were highly at risk compared to non-patient-facing HCWs (RR 7.80, 95% CI 4.39–13.84, $p < 0.0001$), and even compared to employees on COVID-19 units from other departments (RR 3.47, 95% CI 1.65–7.32, $p = 0.006$, Fig. 2d). Staff working in non-clinical departments, including those without patient contact, had a significantly decreased risk for SARS-CoV-2 infection in a multivariate model (0.78% seropositivity, 95% CI 0.41–1.46, $p_m = 0.0179$, Table 2).

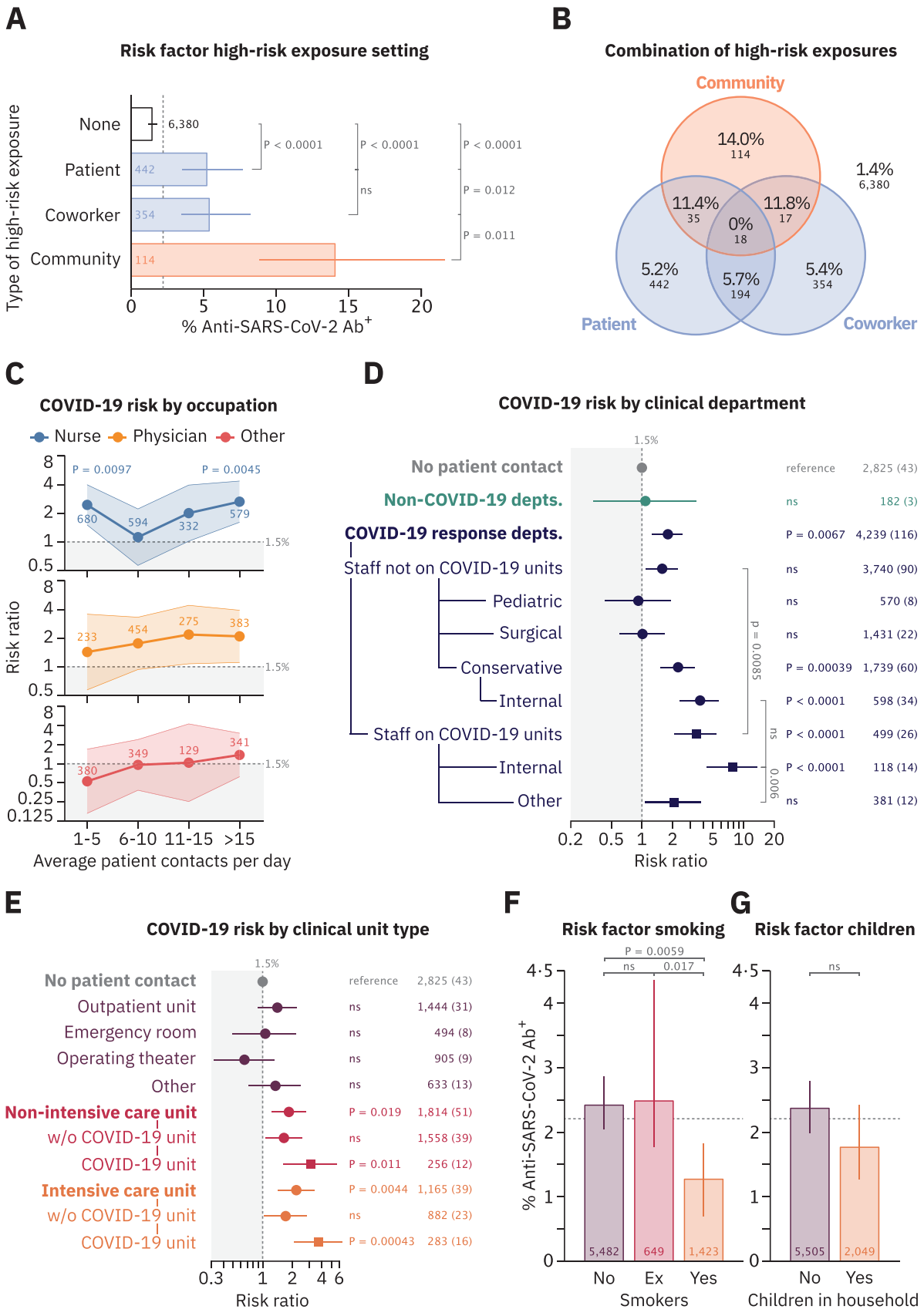
Regarding COVID-19 risk in relation to patient contacts on different types of clinical units, HCWs both on ICUs and non-ICUs treating COVID-19 patients had an increased risk (RR 3.08, 95% CI 1.65–5.76, $p = 0.011$, and RR 3.71, 95% CI 2.12–6.51, $p = 0.00043$), whereas HCWs in outpatient units, operating theaters, and in the emergency room (ER) had a largely unaltered risk compared to non-patient-facing employees (Fig. 2e). Notably, of the 28 Ab^+ staff members working on COVID-19 units, none reported high-risk exposures in the community, while 18 (64.3%) reported high-risk exposures in the hospital (Supplementary Fig. 5a). There were no significant differences in the risks for SARS-CoV-2 infection for HCWs being deployed to COVID-19 units or those not working on COVID-19 units comparing employees from the two different study centers i.e., Central Munich and Großhadern (Supplementary Fig. 5b).

Smoking behavior, children in household and medical preconditions

Interestingly, self-reported smoking behavior was associated with decreased COVID-19 risk compared to non-smokers (RR 0.47, 95% CI 0.28–0.78, $p = 0.0059$) or employees that stopped smoking within the last ten years (ex-smoker, RR 0.41, 95% CI 0.21–0.79, $p = 0.017$) (Fig. 2f) and in multivariate analysis ($p_m = 0.00018$, Table 2). HCWs with children in their households and those reporting medical preconditions were not at increased risk for SARS-CoV-2 infection (Fig. 2g, Supplementary Fig. 5c). Of note, schools and kindergartens in the area were closed between March 16th and May 11th, 2020.

Symptoms

HCWs were asked to report symptoms they had experienced within the previous three months. 72.2% (120/166) of Ab^+ HCWs noted at least one of nine symptoms given, while 27.7% (46/166) were asymptomatic (Fig. 3a). Taste disorder was the symptom with the highest predictive value for SARS-CoV-2 infection ($p_m < 0.0001$, Table 2, with 43.4% (72/120) of symptomatic Ab^+ HCWs experiencing taste disorder compared to only 5.9% (170/2866) of symptomatic anti-SARS-CoV-2 antibody negative (Ab^-) HCWs (Fig. 3b). Cold-like symptoms, such as sore throat, running nose or cough, in contrast, had low predictive value for COVID-19, sometimes even being more frequent among Ab^- HCWs (Fig. 3b). Overall, symptomatic Ab^+ staff members experienced more symptoms compared to their symptomatic


A
Risk factor high-risk exposure setting
Type of high-risk exposure
None
Patient
Coworker
Community
6,380
442
354
114
P < 0.0001
P < 0.0001
ns
P < 0.0001
P = 0.012
P = 0.011
0
5
10
15
20
% Anti-SARS-CoV-2 Ab+
B
Combination of high-risk exposures
Community
Patient
Coworker
14.0%
114
1.4%
6,380
11.4%
35
11.8%
17
0%
18
5.2%
442
5.7%
194
5.4%
354
C
COVID-19 risk by occupation
Nurse
Physician
Other
P = 0.0097
P = 0.0045
680
594
332
579
233
454
275
383
380
349
129
341
1.5%
Risk ratio
1-5
6-10
11-15
>15
Average patient contacts per day
D
COVID-19 risk by clinical department
No patient contact
Non-COVID-19 depts.
COVID-19 response depts.
Staff not on COVID-19 units
Pediatric
Surgical
Conservative
Internal
Staff on COVID-19 units
Internal
Other
reference 2,825 (43)
ns 182 (3)
P = 0.0067 4,239 (116)
ns 3,740 (90)
ns 570 (8)
ns 1,431 (22)
P = 0.00039 1,739 (60)
P < 0.0001 598 (34)
P < 0.0001 499 (26)
P < 0.0001 118 (14)
ns 381 (12)
p = 0.0085
ns
0.006
0.2
0.5
1
2
5
10
20
Risk ratio
E
COVID-19 risk by clinical unit type
No patient contact
Outpatient unit
Emergency room
Operating theater
Other
Non-intensive care unit
w/o COVID-19 unit
COVID-19 unit
Intensive care unit
w/o COVID-19 unit
COVID-19 unit
reference 2,825 (43)
ns 1,444 (31)
ns 494 (8)
ns 905 (9)
ns 633 (13)
P = 0.019 1,814 (51)
ns 1,558 (39)
P = 0.011 256 (12)
P = 0.0044 1,165 (39)
ns 882 (23)
P = 0.00043 283 (16)
0.3
1
2
4
6
Risk ratio
F
Risk factor smoking
P = 0.0059
ns
0.017
% Anti-SARS-CoV-2 Ab+
4·5
4
3
2
1
0
5,482
649
1,423
No
Ex
Yes
Smokers
G
Risk factor children
ns
5,505
2,049
No
Yes
Children in household

◂**Fig. 2** Risk factors for SARS-CoV-2 seropositivity among healthcare workers. **a** Percentage of SARS-CoV-2 seropositive HCWs by self-reported instances of different types of high-risk exposure. Only staff reporting exposures of a single type is shown. **b** Percentages and absolute numbers of SARS-CoV-2 Ab^+ staff members self-reporting combinations of high-risk exposures in different settings. Numbers outside the diagram correspond to staff members in none of the depicted groups. **c** SARS-CoV-2 seropositivity risk ratio (RR) of nurses, physicians and other patient-facing HCWs and average self-reported patient contacts per day relative to staff without patient contact (RR set to 1). Shaded areas depict 95% confidence intervals (CIs). *p* values from Fisher's exact test are reported where $p < 0.05$. **d** SARS-CoV-2 seropositivity RRs for HCWs originating from different departments relative to staff without patient contact (RR set to 1). Departments that deployed staff members to COVID-19 units are termed "COVID-19 response depts.", all others are grouped under "non-COVID-19 depts.". Staff from COVID-19 response departments were further stratified according to their deployment to COVID-19 units and to the medical specialty of their department. Dots represent risk ratios, while lines indicate 95% CIs. **e** SARS-CoV-2 seropositivity RRs for HCWs self-reporting patient contact on different types of clinical units. Multiple selections were possible. **f** Self-reported smoking behavior and risk for SARS-CoV-2 seropositivity. Bars represent percentages of anti-SARS-CoV-2 Ab^+ staff. Error bars represent 95% CIs. **g** Self-reported number of children living in the same household with HCWs as a risk factor for SARS-CoV-2 seropositivity. *p* values in **a**, **d**–**g** were calculated using Fisher's exact test and are reported as adjusted *p* values after Holm's multiple testing correction. Numbers next to datapoints indicate number of staff members per group and numbers in braces indicate number of Ab^+ staff members (**c**–**e**). Dotted lines correspond to the risk of staff without patient contact (**c**–**e**, 1.5%) or number of SARS-CoV-2 Ab^+ staff from the entire dataset (**a**, **f**, **g**, 2.2%)

Ab^- counterparts (Fig. 3c). No symptom combination provided a predictive signature for COVID-19 in HCWs (Fig. 3d). The most specific symptom complex for COVID-19 was taste disorder, headache, fatigue and fever, with 46.9% (23/49) of all HCWs reporting this complex being Ab^+ (Supplementary Fig. 6a). However, this combination of symptoms was reported by only 13.9% (23/166) of all Ab^+ HCWs.

Risk stratification in an unbiased decision tree

We built a decision tree based on all parameters with multivariate significance (Table 2) to identify classifiers for high- and low-risk subgroups among HCWs (Fig. 3e). Nodes in the tree represent the parameters that most significantly bisect the respective subgroup of HCWs into seropositive and negative. For example, of these classifying parameters, high-risk exposures in the community most significantly identified seropositive HCWs in the subgroup of those who did not experience taste disorder. Taste disorder had the highest predictive value for seropositivity on the entire dataset and smoking as well as working as a nurse were strong predictors of an Ab^- or Ab^+ outcome in the indicated subgroups, respectively. Interestingly, working in a clinical department can significantly identify both a higher and a lower-risk population in different subgroups. Having a sore throat predicted a lower COVID-19 risk in two separate subgroups (Fig. 3e).

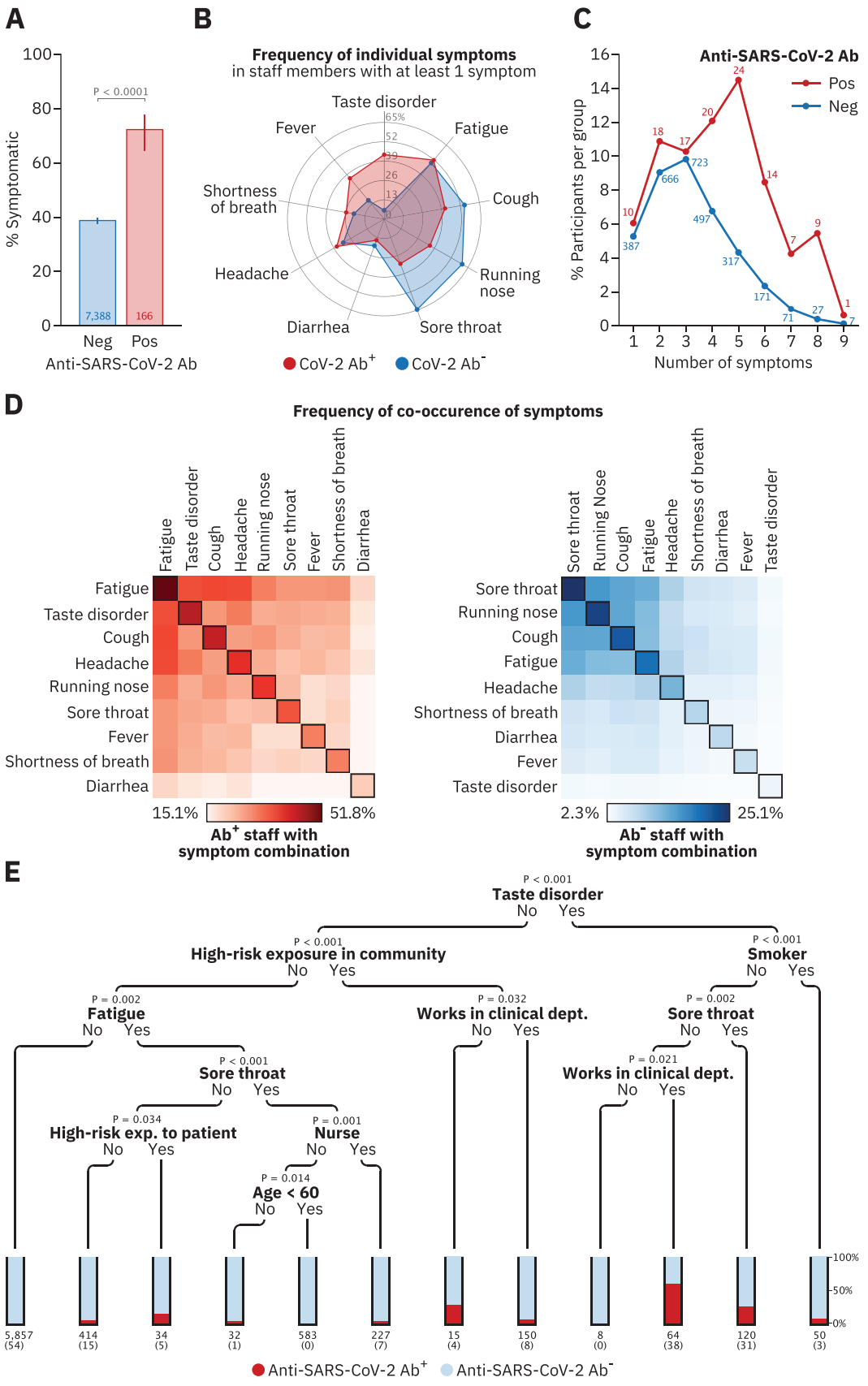
Quarantining and working from home

Participants were asked to report whether they self-quarantined or worked from home as a preventive measure. Since HCWs self-quarantined upon confirmed or suspected SARS-CoV-2 infection, the rate of Ab^+ individuals in this group was high (23.9%, Fig. 4a). While working from home reduced high-risk exposures to infected co-workers, it did not reduce such exposures within the HCW's community and, surprisingly, did not lower the overall COVID-19 risk (RR 1.06, 95% CI 0.63–1.77) (Fig. 4a, Supplementary Fig. 7a, b), despite 76.6% (837/1093) of these homestays continuing for at least three weeks (Fig. 4b). Of note, working from home as a precaution was only possible for those employees whose presence in the hospital was not essential for patient care.

Evaluation of the PCR-testing strategy

Major indications for SARS-CoV-2 testing by PCR in HCWs were presentation with COVID-19-associated symptoms and reporting high-risk exposures. The seropositivity rate among the group who reported neither testing indication nor having been PCR-tested was four-fold lower (0.55%) than the average seropositivity rate observed in this study (2.20%, Fig. 4c). 72.1% (846/1174) of HCWs who reported a high-risk exposure in the questionnaire were also tested by PCR. Of the remaining 27.9% (328/1174), 64.9% (213/328) were asymptomatic. Among staff members reporting high-risk exposures in the hospital that were not tested by PCR, 66.5% (189/284) reported not having notified the occupational health office about this perceived risk, despite being obligated to do so. Overall, 75.8% (964/1272) of all high-risk exposures to SARS-CoV-2-infected individuals in the hospital (to patients or co-workers) were reported to the occupational health office, with no difference between occupations (Supplementary Fig. 7c).

34.8% (1038/2986) of all staff members reporting at least one symptom were tested by PCR, and symptomatic HCWs who were tested by PCR were more likely to seroconvert compared to non-PCR-tested, symptomatic HCWs indicating that not all symptoms listed in the study questionnaire urged employees to get PCR-tested (Fig. 4c). Indeed, three of the four symptoms that constitute the symptom combination with the highest predictive value for an Ab^+ status i.e., taste disorder, fever and headache, were more abundant among symptomatic staff members who got PCR-tested, irrespective of whether participants had additionally reported high-risk exposures to individuals with COVID-19


A
% Symptomatic
P < 0.0001
7,388
166
Neg
Pos
Anti-SARS-CoV-2 Ab
B
Frequency of individual symptoms
in staff members with at least 1 symptom
Taste disorder
Fatigue
Cough
Running nose
Sore throat
Diarrhea
Headache
Shortness of breath
Fever
CoV-2 Ab+
CoV-2 Ab-
C
Anti-SARS-CoV-2 Ab
Pos
Neg
% Participants per group
Number of symptoms
D
Frequency of co-occurence of symptoms
15.1%
51.8%
Ab+ staff with symptom combination
2.3%
25.1%
Ab- staff with symptom combination
E
Taste disorder
High-risk exposure in community
Smoker
Fatigue
Works in clinical dept.
Sore throat
Works in clinical dept.
Sore throat
High-risk exp. to patient
Nurse
Age < 60
Anti-SARS-CoV-2 Ab+
Anti-SARS-CoV-2 Ab-

◂**Fig. 3** COVID-19 associated symptoms in healthcare workers and risk stratification in an unbiased decision tree. **a** Percentage of SARS-CoV-2 Ab^+ and Ab^- HCWs who reported having experienced at least one of nine symptoms shown in **b**. *P*-value was calculated using Fisher's exact test. **b** Frequency of individual symptoms in SARS-CoV-2 Ab^+ and Ab^- staff members with at least one self-reported symptom as a percentage of the respective group. **c** Percentage of SARS-CoV-2 Ab^+- and Ab^- staff reporting the indicated number of symptoms. Numbers beside data points indicate number of staff members per group. **d** Frequency of co-occurrence of pairs of symptoms in Ab^+ (red) and Ab^- (blue) staff members. Squares on the diagonal represent the frequency of single symptoms. **e** A conditional inference tree (decision tree) was trained in R using the *ctree* function implemented in the party package, using default parameters. All significant parameters from the logistic regression were included in the training dataset. Depicted is the resulting decision tree with the stop-criterium for tree splits set at a significance level of $\alpha = 0.05$. Numbers underneath bars represent the total number of HCWs in the respective group, numbers in braces those of Ab^+ staff members

(Supplementary Fig. 8a). 66.9% (111/166) of Ab^+, compared to 24.8% (1832/7388) of Ab^- HCWs, had been tested by PCR at least once (Fig. 4d). Focusing on the group of Ab^+ participants, we found that 92.0% (69/75) of those indicating a high-risk exposure had been tested by PCR (Fig. 4e). Among Ab^+ HCWs without high-risk exposures, 46.2% (42/91) had been PCR-tested (Fig. 4e). Of the 55 seroconverted HCWs who reported not having been tested by PCR, 40.0% (22/55) were asymptomatic.

Combining data on PCR testing of HCWs provided by the occupational health office and pseudonymized data from study participants, we investigated the occurrence of potentially unrecognized COVID-19 clusters. No cluster of more than two HCWs participating in this study remained undetected in individual organizational units (Fig. 4f). In all COVID-19 clusters among Ab^+ HCWs involving more than 10 individuals, $\geq 75\%$ of the cluster size had been detected by PCR (Fig. 4f, outer grey circles), with higher rates of unrecognized cases in those clusters that also contained more HCWs who did not report any high-risk exposure (Fig. 4f, white areas in pie charts).

Discussion

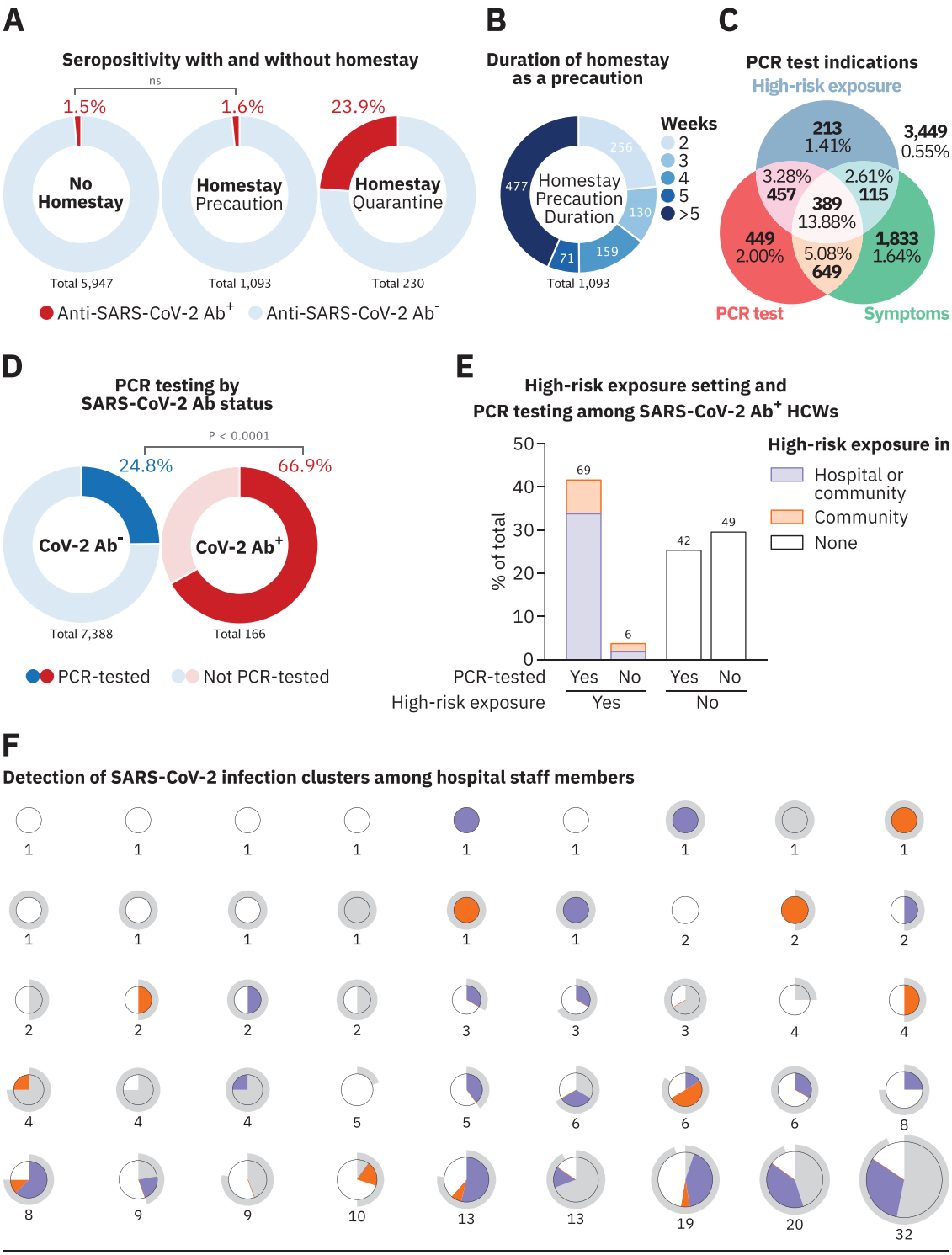
In this cross-sectional study conducted at a multicenter quaternary care hospital at the end of the first pandemic wave we identified several occupation-specific COVID-19 risk factors for HCWs, including high-risk exposures in the hospital and the community, working in patient-facing occupations, particularly as nurses, in departments of internal medicine, and on COVID-19 units, as well as being of male gender. Surprisingly, we found smoking behavior to be protective against SARS-CoV-2 infection. Among the symptoms analyzed, especially taste disorder was highly associated with COVID-19.

A common strategy to cope with hospital-associated COVID-19 is vaccinating HCWs against SARS-CoV-2. In many countries, however, vaccination programs are not yet available at scale. Furthermore, for some of the recently emerged VOCs that are spreading rapidly, reduced vaccine efficiencies have been reported [2, 3]. New VOCs escaping current vaccine responses may develop over the next months [22] resulting in an increased risk of infection at a population level irrespective of the vaccination status. Moreover, in certain countries, a considerable fraction of citizens, among them HCWs, are reluctant to become vaccinated against SARS-CoV-2 [4]. Consequently, the identification of occupation-specific risk factors in HCWs and the evaluation of surveillance strategies as well as preventive measures remain crucial to ensure adequate hospital capacities in the COVID-19 pandemic.

A study conducted in the New York Metropolitan region, USA, found no hospital-specific risk factors for SARS-CoV-2 infection in HCWs [23]. However, the overall prevalence of Ab^+ individuals in New York State was estimated to be 6.9–14.0% by the end of April 2020 [24, 25]. In contrast, data from Munich, Germany, the city in which our study was conducted, indicate a seroprevalence of only 1.8%, by the end of April 2020 [26]. Conceivably, high prevalence concomitant with a high risk of transmission in the community may overshadow the identification of hospital-specific risk factors for HCWs. This is underscored by the relevance of high-risk exposures in the community for HCWs reported here and by others [27–30]. We hypothesize that private high-risk exposures might overall be longer and more intense than professional exposures in the hospital setting, and the former thus more contagious. Congruently, we discovered that working from home as a preventive measure did not reduce the risk of seropositivity in HCWs. However, at the hospital complex surveyed here, only those employees were eligible for working from home whose presence at the hospital was not crucial to ensure adequate patient care i.e., mainly those individuals working in non-patient-facing occupations. Whether working from home may have been protective for patient-facing HCWs, therefore, cannot be answered by our study.

The aforementioned overshadowing effect of SARS-CoV-2 transmission in the community could also explain why studies conducted in high prevalence areas did not identify working on ICUs to be associated with increased risk for seropositivity [27, 31]. We observed the contrary, especially for nurses, even though ICU nurses reported fewer patient contacts per day compared to their colleagues working on other wards.

Other studies identified, in part, similar COVID-19 risk factors in HCWs compared to ours, including male gender [32], working in patient-facing occupations [32, 33], on COVID-19 units and in departments of internal medicine [31, 32], as well as taste disorder [32]. However, several


A
Seropositivity with and without homestay
ns
1.5%
1.6%
23.9%
No Homestay
Homestay Precaution
Homestay Quarantine
Total 5,947
Total 1,093
Total 230
Anti-SARS-CoV-2 Ab+
Anti-SARS-CoV-2 Ab-
B
Duration of homestay as a precaution
Weeks
2
3
4
5
>5
Homestay Precaution Duration
256
130
159
71
477
Total 1,093
C
PCR test indications
High-risk exposure
213
1.41%
3,449
0.55%
3.28%
457
2.61%
115
389
13.88%
449
2.00%
5.08%
649
1,833
1.64%
PCR test
Symptoms
D
PCR testing by SARS-CoV-2 Ab status
P < 0.0001
24.8%
66.9%
CoV-2 Ab-
CoV-2 Ab+
Total 7,388
Total 166
PCR-tested
Not PCR-tested
E
High-risk exposure setting and PCR testing among SARS-CoV-2 Ab+ HCWs
% of total
50
40
30
20
10
0
69
6
42
49
High-risk exposure in
Hospital or community
Community
None
PCR-tested
Yes
No
Yes
No
High-risk exposure
Yes
No
F
Detection of SARS-CoV-2 infection clusters among hospital staff members
1 1 1 1 1 1 1 1 1
1 1 1 1 1 1 2 2 2
2 2 2 2 3 3 3 4 4
4 4 4 5 5 6 6 6 8
8 9 9 10 13 13 19 20 32
Infection cluster in a single department
Cases in cluster previously identified by PCR test
x
Size of cluster
Cases in cluster by high-risk exposure
CoV-2 Ab+ Exposure in hospital or hospital and community
CoV-2 Ab+ Exposure only in community
CoV-2 Ab+ No exposure
PCR+, CoV-2 AbN/A Exposure N/A

◂**Fig. 4** Effectiveness of measures to track and prevent SARS-CoV-2 transmission in hospital staff. **a** SARS-CoV-2 serostatus among staff reporting to have stayed at home for at least two weeks either as a precaution (middle circle) or quarantined (right circle) in comparison to staff members not staying at home (left circle). Participants who indicated to have been quarantined or stayed at home for at least two weeks without reporting to have worked from home were considered quarantined. **b** HCWs who stayed home as a precaution for at least two weeks grouped by the duration of their homestay. **c** Total numbers and percentages of anti-SARS-CoV-2 Ab^+ HCWs who self-reported on (1) having been tested by PCR, (2) experienced at least one symptom depicted in Fig. 3B, or (3) had a high-risk exposure. **d** Numbers and percentages of anti-SARS-CoV-2 Ab^+ and Ab^- staff who were tested for SARS-CoV-2 infection by PCR. **e** Percentages of anti-SARS-CoV-2 Ab^+ HCWs who were tested for SARS-CoV-2 infection by PCR or reported a high-risk exposure in (1) the hospital or the hospital and their community (blue) or (2) their community only (orange). **f** Analysis of SARS-CoV-2 infection clusters and their detection among HCWs in the hospital. Each pie chart represents one infection cluster and clusters are separated by departments. Inner pie charts represent high-risk exposure types reported by Ab^+ study participants in each cluster (blue, orange and white). Grey areas in inner pie charts represent individuals who were PCR-tested at the hospital but did not participate in this study. Grey circles around each pie chart represent the cluster's fraction of COVID-19 cases previously identified by PCR testing. Numbers below the pie charts indicate the amount of SARS-CoV-2-infected HCWs in each cluster. Study participants reporting a positive PCR test in the study questionnaire were assumed to be identical to those registered at the occupational health office. HCWs who were PCR-tested at the hospital complex but did not participate in the study were added to the respective clusters as recognized cases (grey areas in inner pie charts). *p* values in **a**, **e** were calculated using Fisher's exact test

risk and protective factors described here, such as working as a nurse and high-risk exposure in the hospital were thus far unknown. Moreover, we show in this study for the first time that certain COVID-19 risk factors among HCWs are statistically significant in multivariate analysis, thus underlining their importance.

High-risk exposures in hospitals can be minimized by strictly enforcing patients and staff to wear appropriate personal protective equipment (PPE), testing patients for acute SARS-CoV-2 infection upon admission and rapid isolation of suspected COVID-19 cases in separate rooms. In the hospital complex surveyed here, the ER implemented these measures early on, possibly explaining the low seropositivity among these HCWs, despite the ER being a common entry point for symptomatic COVID-19 patients into hospitals [34].

The increased COVID-19 risk for HCWs working on ICUs, especially for nurses, indicates that patients with critical COVID-19 being treated on ICUs may pose a higher risk of contagion possibly due to individual patient contacts being more intense compared to other wards. Also, working as a nurse requires closer and longer patient contacts, which could serve as an explanation for the elevated COVID-19 risk ratio in this occupational group. In addition, specific characteristics in their work environment or socioeconomic factors may put nurses at higher risk.

HCWs reporting smoking behavior had a lower risk for seropositivity in multivariate analysis. A fraction of active smokers might have deliberately not reported their smoking behavior. This reporting bias could have lead to an underestimation of the protective effect of active smoking on the risk of SARS-CoV-2 infection in our analysis. Behavioral factors might explain the preventative effect of active smoking in HCWs, including the requirement to smoke outside the hospital that may have avoided high-risk exposures to colleagues in designated break areas and lunchrooms. However, direct antiviral effects related to smoking have also been reported [35, 36].

We showed that in resource-limited settings, a PCR-testing strategy for HCWs that focused on the presentation of symptoms and reporting of high-risk exposure, was sufficient to identify the majority of COVID-19 cases and prevent larger unrecognized outbreaks in the study population. However, if testing capacities are higher this strategy can be complemented by interval screening for acute SARS-CoV-2 infection, especially in the identified risk groups. Risk stratification in an unbiased decision tree, as shown in this study, may help refine screening efforts and enable more effective, personalized application of preventive measures.

This study was conducted directly after the first wave of the pandemic had subsided in the region. HCWs' risk of SARS-CoV-2 infection was potentially increased during the early weeks of the pandemic due to limited PPE and PCR testing capacities, the need for rapid restructuring of units within the hospital and redeployment of HCWs to frontline positions [37]. Thus, risk factors reported here might not directly apply to later stages of the pandemic to the same extent. In turn, the COVID-19 seroprevalence at the start of the pandemic was generally low enabling a well-defined identification of hospital-specific rather than risk factors in the general population [26]. Participation rates were high among nurses (91.2%), and physicians (72.6%), but lower among other occupations such as cleaning personnel (18.3%) leading to risk assessments with limited confidence in the latter groups.

Of note, 19.2% (32/166) of seroconverted participants in our study reported having received only negative PCR results. We assume this represents the group of HCWs either returning from quarantine after COVID-19 or who had been tested PCR-negative during the incubation period [38]. The high specificities of the two anti-SARS-CoV-2 antibody detection assays used for screening (Elecsys® 100%, and self-developed assay 99.9%) make false-positive antibody testing unlikely to explain this observation. Conversely, 21.8% (22/101) of participating HCWs did not seroconvert despite self-reporting a positive PCR test. Among others, this observation may be explained by reduced sensitivity of anti-SARS-CoV-2 antibody detection assays in

asymptomatic and mild COVID-19 cases during the first weeks after infection.

54.8% of seropositive participants reported no high-risk contacts, suggesting that even professionals in the healthcare sector can be unaware of relevant exposures to SARS-CoV-2. Alternatively, deliberate underreporting of high-risk exposures may have occurred despite pseudonymized data collection. Moreover, HCWs returning from early COVID-19 hotspots in late February 2020 [39, 40], after the winter break in Southern Germany, may not have been aware of SARS-CoV-2 exposures during their vacation.

In summary, we identified several risk and protective factors for SARS-CoV-2 infection in HCWs related to high-risk exposures, profession, department, work unit, gender and behavior, as well as COVID-19-associated symptoms. Multivariate analysis underlined the importance of these factors, and risk stratification in an unbiased decision tree revealed subgroups within HCWs with distinct risk profiles. For the first time, we evaluated protective measures against SARS-CoV-2 spread and revealed that working from home was not effective, while a simple PCR-testing strategy was sufficient to detect the majority of COVID-19 cases among employees. Our findings suggest that future efforts to protect HCWs from COVID-19, including, training programs, screening for acute infection, quarantining, and vaccination, should be risk factor-driven.

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Author contributions PRW, NAS, AO, TW, JR, KA, MK, and OTK conceived the study. PRW, NAS, MK and OTK drafted the first version of the manuscript. AO, TW, JS, VH and LK contributed to drafting sections of the manuscript. PRW, NAS, and LK analyzed the data. PRW, NAS, AO, JR, BK, PMS, MS, and MA conducted laboratory experiments. MZ, TTM, HT, JR, BS, PK, BG, and PF participated in designing the study. All authors contributed to the interpretation of data and approved the final manuscript. The corresponding authors attest that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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Data availability and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Conflicts of interest The authors declare that they have no competing interests.

Ethical approval The study was approved by the ethics committee of the Faculty of Medicine at the Ludwig Maximilian University of Munich (study-No.: 20–247).

Consent to participate All participants were of legal age and gave written informed consent before entering the study.

Consent for publication Not applicable.

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Supplementary Information:

In-depth profiling of COVID-19 risk factors and preventive measures in healthcare workers

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Supplementary figure legends

Supplementary Fig. 1. Testing and calling strategy used to determine anti-SARS-CoV-2 antibody status. (a) All samples were screened with both a commercially available and a self-developed SARS-CoV-2 antibody test. Three samples were determined positive with only positive Elecsys results. Five indeterminate samples were unavailable for additional testing and were called negative. (b) Agreement of the Elecsys and self-developed IgG assay across all study samples. Two samples were positive in the self-developed IgG assay but called negative by the Elecsys test (red).

Supplementary Fig. 2. Neutralizing activity in SARS-CoV-2 PCR$^+$ or Ab$^+$ sera. (a) Overview of neutralization assay procedure. (b) Number of PCR$^+$ or Ab$^+$ HCWs by their SARS-CoV-2 neutralizing activity categorized as follows: “none”: $IC_{50} < 10$; “weak”: $IC_{50} < 90$; “medium”: $IC_{50} < 270$; “strong”: $IC_{50} < 2430$; “very strong”: $IC_{50} > 2430$. (c) SARS-CoV-2 neutralizing activity of serum from PCR$^+$ or Ab$^+$ participants by how long ago they were first PCR-tested. Black triangles represent the strength of neutralizing activity from “none” to “very strong” as in (b). (d) SARS-CoV-2 neutralizing ability by antibody titer in serum as measured by the Elecsys assay. *P*-values throughout the figure

were calculated using Kendall's τ statistic.

Supplementary Fig. 3. High-risk exposures in HCWs including multiple exposure types. (a) Related to Fig. 2a, risk of SARS-CoV-2 seroconversion of staff members by self-reported instances of different types of high-risk exposure. Multiple answers are included in each respective group. Lines indicate 95% CIs.

Supplementary Fig. 4. Work environments and associated risks in study participants. (a) Numbers of nurses, physicians and other patient-facing HCWs who reported patient contacts on the indicated clinical units compared to how many patient contacts per day they reported on average. Multiple mentions for units were possible. (b) Percent seropositivity of nurses (blue bars), physicians (orange bars) and others with patient contact (red bars) by units on which they reported patient contacts. Multiple mentions for units were possible. Lines indicate 95% CIs. The dashed line indicates the overall seropositivity in the study population (2.2%)

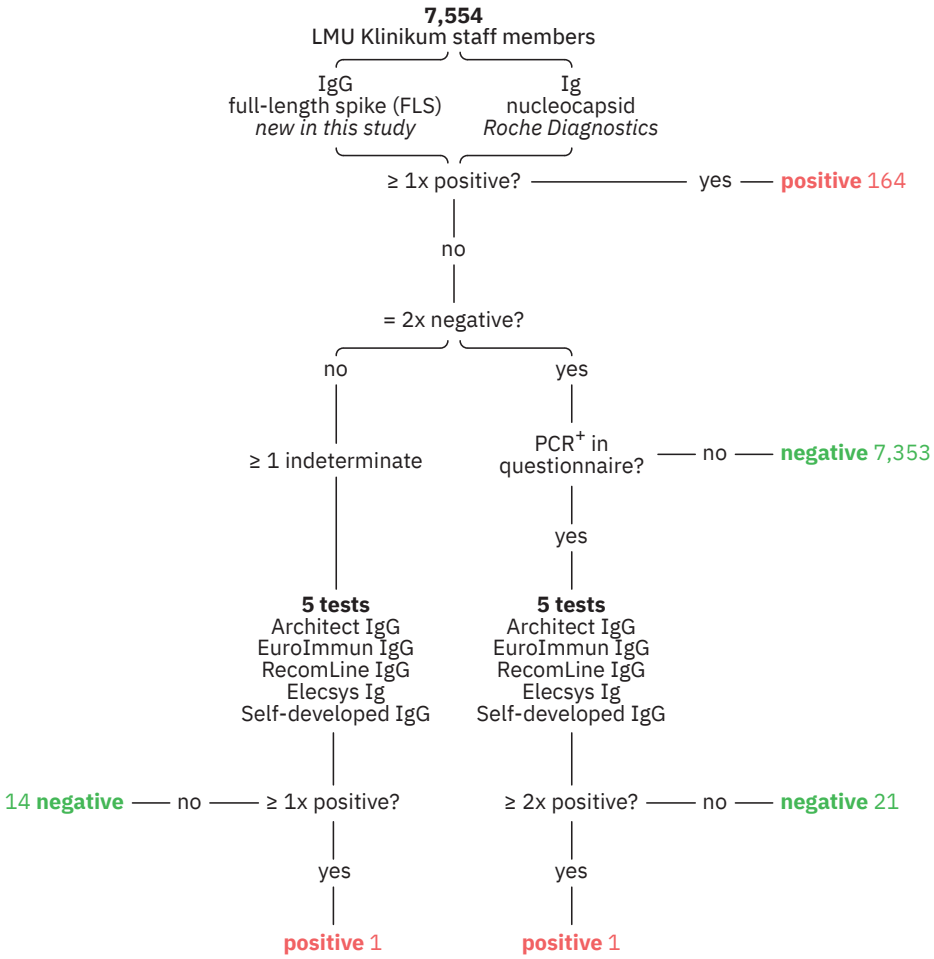
Supplementary Fig. 5. High-risk exposures in the hospital are more frequent on COVID-19 units, seropositivity rates among participants from different study centres and among those with medical preconditions. (a) Relative proportion of high-risk exposures in indicated settings among study participants by their anti-SARS-CoV-2 antibody status and whether they reported to have worked on COVID-19 units. HCWs reporting high-risk exposures in multiple settings are shown as "mixed". (b) Percent anti-SARS-CoV-2 Ab^+ HCWs reporting working primarily at one of the two study centres relative to all HCWs primarily working at that centre divided by being deployed to COVID-19 units or not. (c) Percent anti-SARS-CoV-2 Ab^+ HCWs reporting any of the indicated medical conditions relative to all HCWs who reported the given condition. Study participants reporting multiple conditions are included under each condition. Lines in (b) and (c) indicate 95% CIs.

Supplementary Fig. 6. Symptom combinations among study participants. (a) Absolute frequency of reported symptom combinations among study participants by anti-SARS-CoV-2 antibody status. Colors indicate how many symptoms were in a given combination. The most specific symptom combination was taste disorder, fever, headache and fatigue.

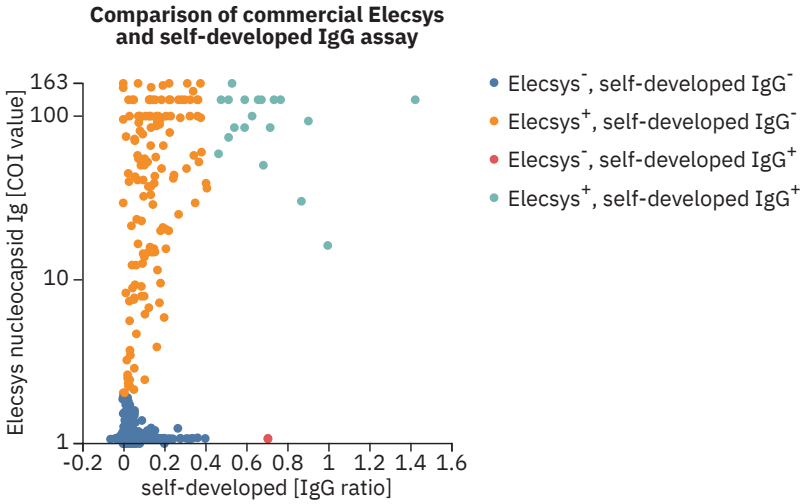
Supplementary Fig. 7. Evaluation of working from home as a precaution. (a) Percentage of staff members reporting high-risk exposures in their community by whether they were working from home as a precaution. (b) Percent staff members from administrative and research occupations by whether they worked from home as a precaution and whether they self-reported high-risk exposures of the indicated type. Focussing on this subgroup, for which working from home was generally available, allowed us to directly compare HCWs in the same occupation who worked from home as a precaution with those who did not. *P*-values were calculated using Fisher's exact test. (c) Percentage of high-risk exposures in the hospital reported to the occupational health office by occupation of the reporting HCW.

Supplementary Fig. 8. Symptoms and high-risk exposures as indications for PCR testing in health care workers. (a) Likelihood of study participants reporting no high-risk exposure (2,482) to get PCR-tested based on which symptoms they indicated. Numbers in braces represent staff members from this group who did get PCR-tested and reported the respective symptom.

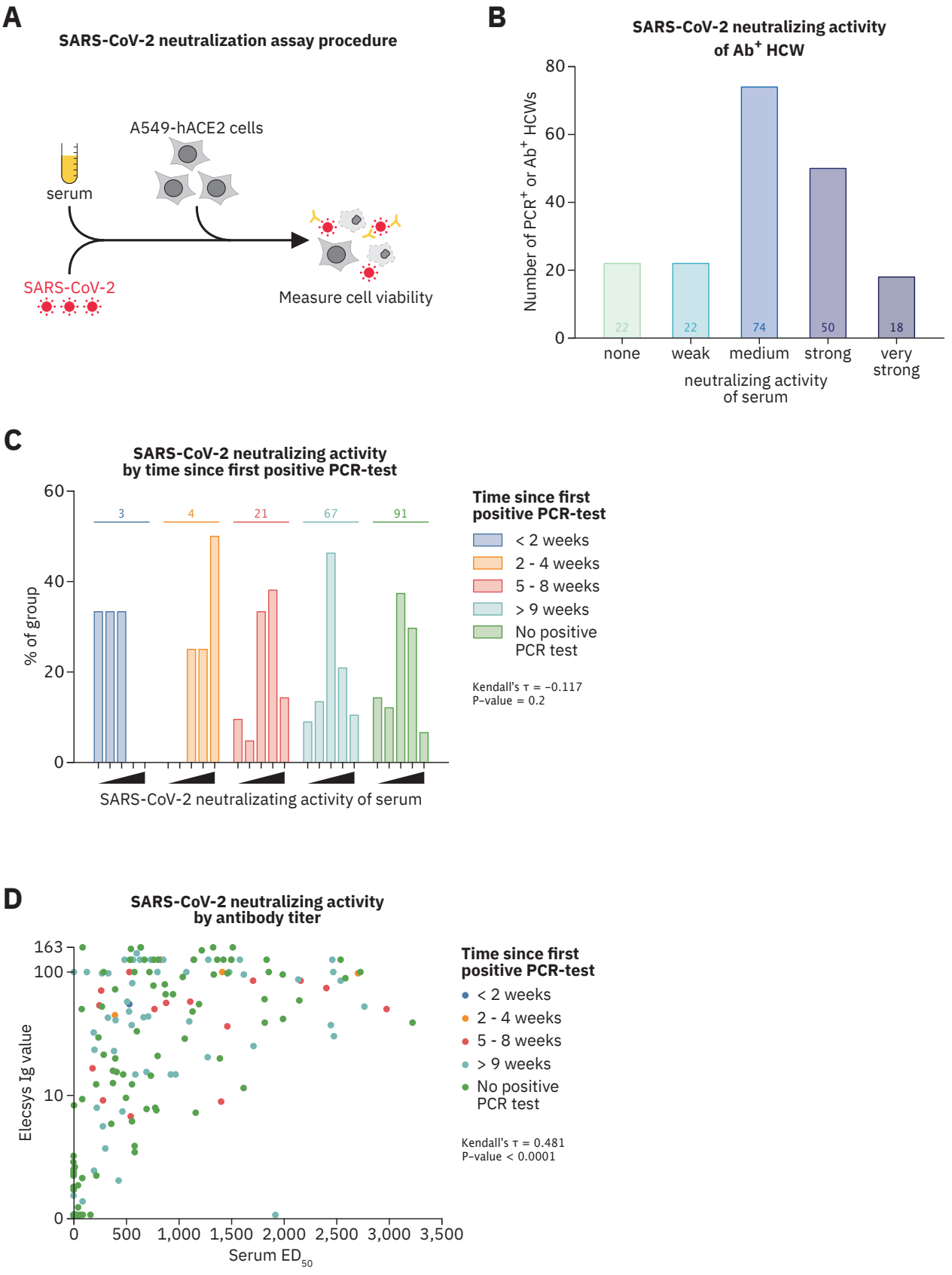
A



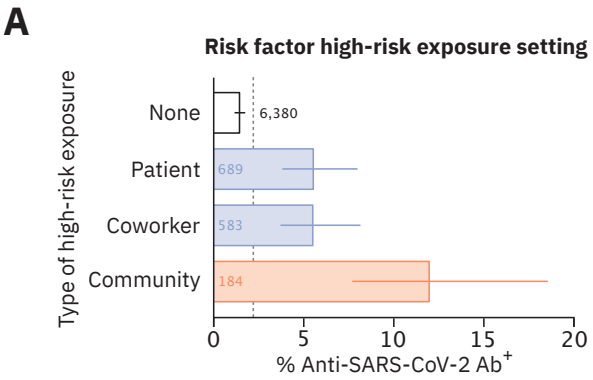
B



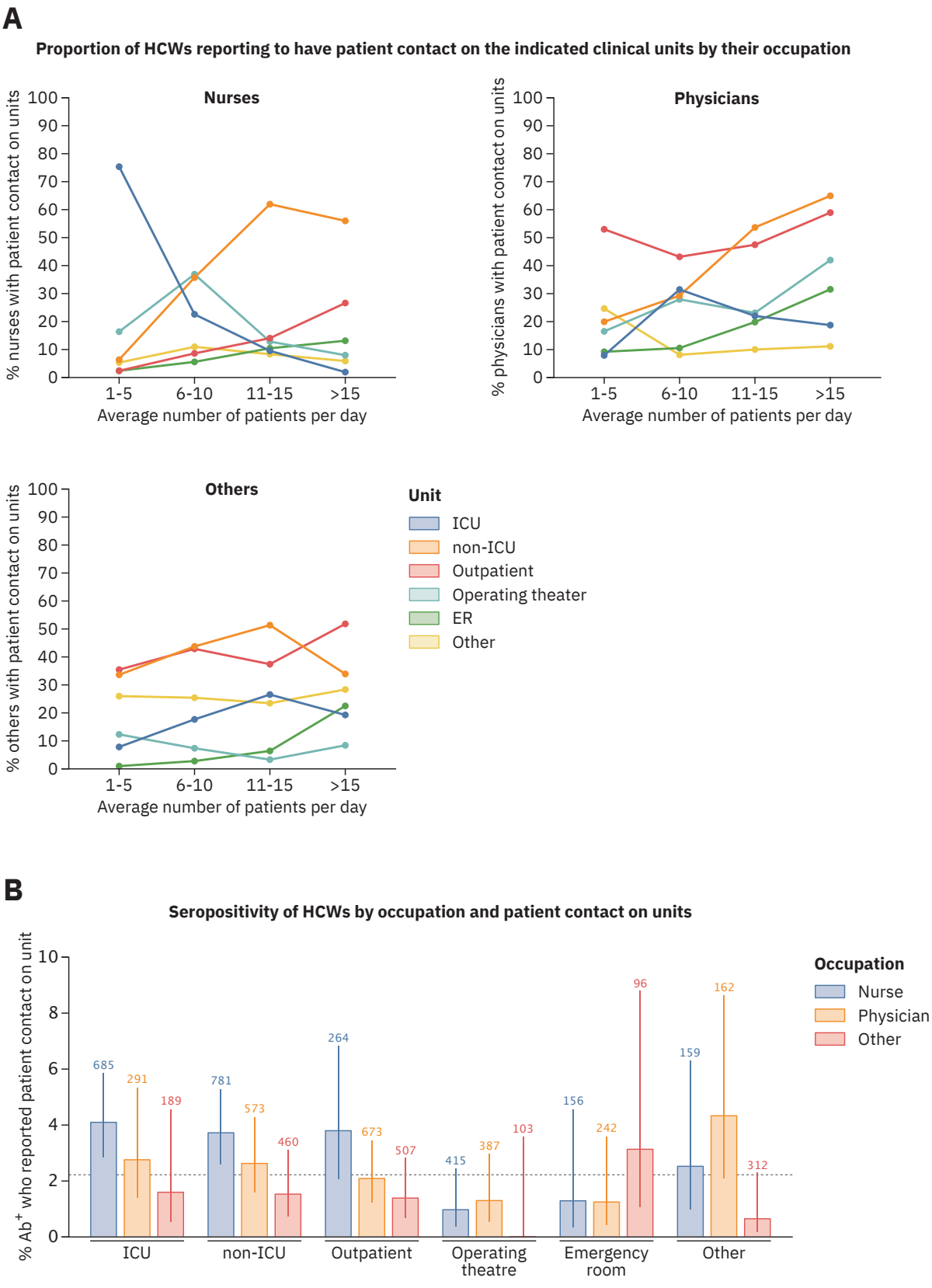
Supplementary Figure 1



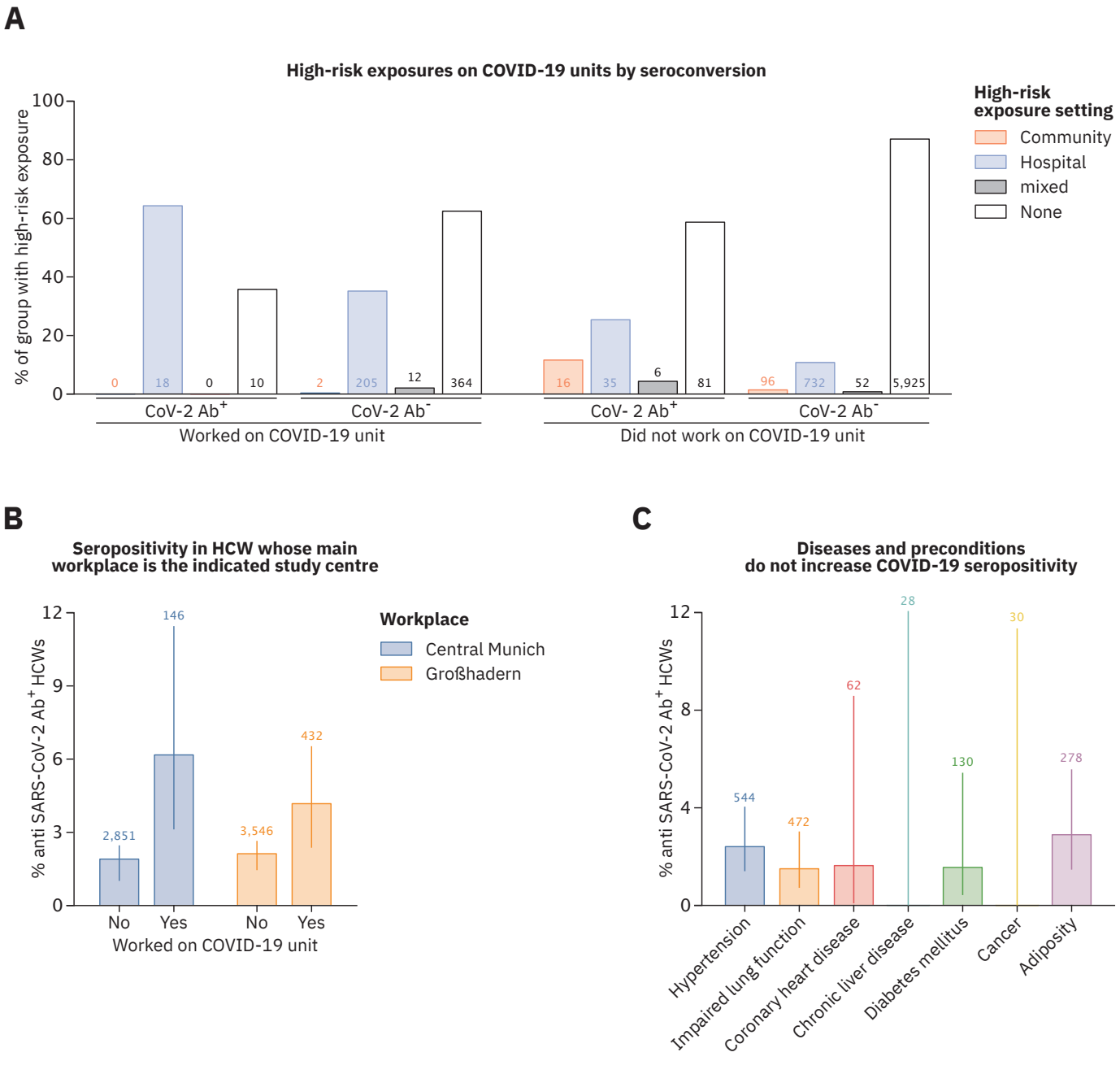
Supplementary Figure 2



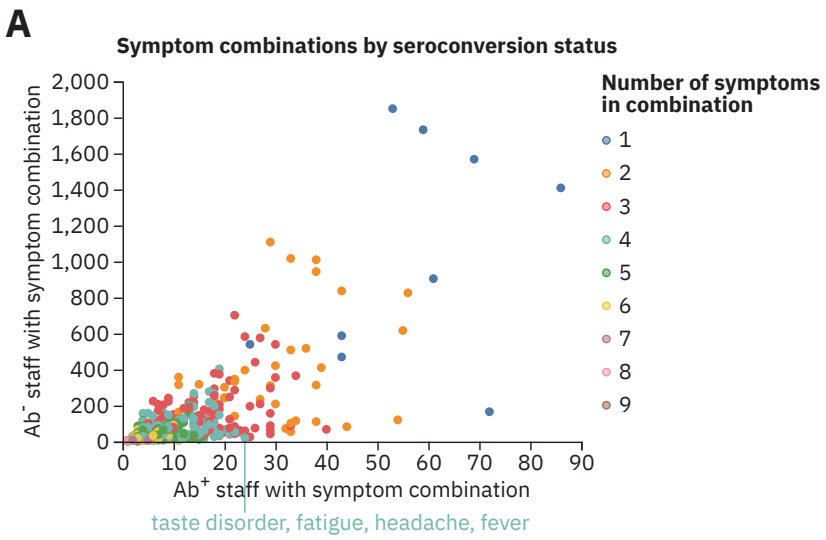
Supplementary Figure 3



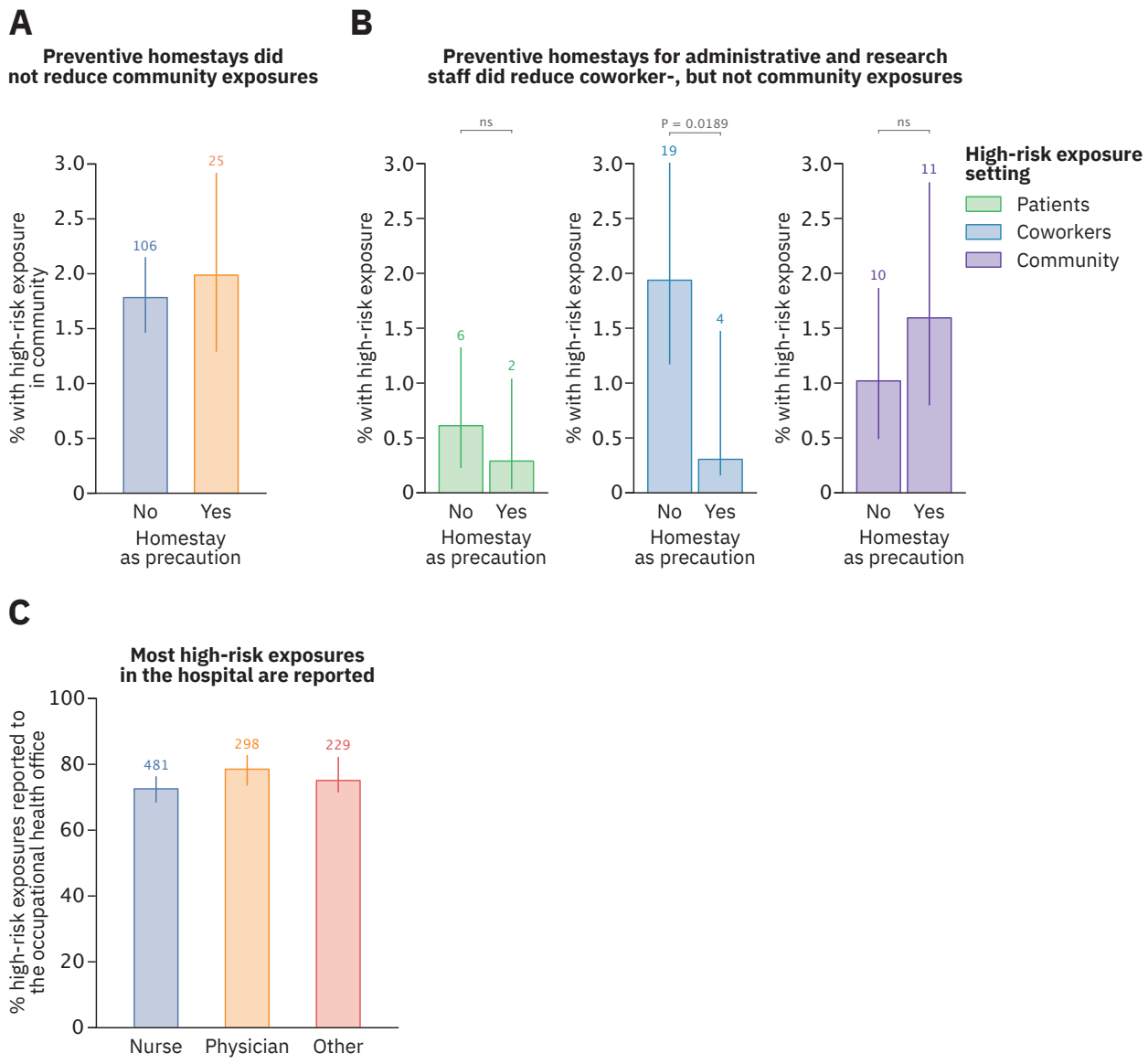
Supplementary Figure 4



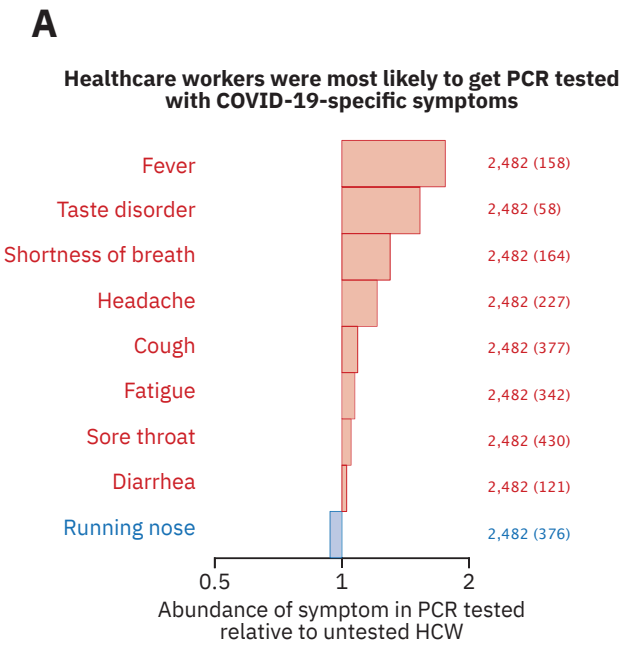
Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7



Supplementary Figure 8

Supplementary Table 1 (part 1). Study questionnaire compared to anti-SARS-CoV-2 antibody status of 7,554 health care workers participating in the study.

Questions	Answers	Anti-SARS-CoV-2 Ab	
		Pos. / total	%
Please state your age.	≤ 30 Y	64 / 2,170	2.95
	31 - 40 Y	39 / 1,951	2.00
	41 - 50 Y	29 / 1,430	2.03
	51 - 60 Y	23 / 1,467	1.57
	> 60 Y	11 / 536	2.05
Please state your gender.	male	51 / 2,118	2.41
	female	115 / 5,431	2.12
	third gender	0 / 5	0.00
Do you take immunosuppressants?	yes	4 / 177	2.26
	no	162 / 7,377	2.20
Were you vaccinated against Influenza in flu season 2019/20?	yes	67 / 2,630	2.55
	no	99 / 4,924	2.01
Are you planning to get vaccinated against Influenza in flu season 2020/21?	yes	85 / 3,390	2.51
	no	49 / 2,719	1.80
	undecided	32 / 1,445	2.21
Would you get vaccinated against SARS-CoV-2 if there was an efficient vaccination available with few side effects?	yes	85 / 4,397	1.93
	no	26 / 913	2.85
	undecided	55 / 2,240	2.46
Do you have any relevant pre-existing medical conditions?	yes	20 / 1,117	1.79
	no	146 / 6,437	2.27
For participants with relevant pre-existing medical conditions: I have the following pre-existing medical conditions:	hypertension	13 / 544	2.39
	lung disease (e.g. asthma, COPD, chronic bronchitis)	7 / 472	1.48
	coronary heart disease	1 / 62	1.61
	chronic liver disease	0 / 28	0.00
	diabetes mellitus	2 /130	1.54
	active cancer	0 / 30	0.00
	obesity	8 / 278	2.88
Do you smoke?	yes	16 / 1,423	1.12
	no	132 / 5,482	2.41
	I stopped smoking within the last 10 Y	18 / 649	2.77
Including yourself, how many adults live in your household?	1	56 / 2,355	2.38
	2	75 / 3,699	2.03
	> 2	35 / 1,500	2.33
How many children under the age of 18 Y live your household?	1	16 / 1,007	1.59
	2	14 / 828	1.69
	3	5 / 176	2.84
	4	1 / 38	2.63
	none	130 / 5,505	2.36
Have you been tested against SARS-CoV-2 using virus specific PCR?	positive (at least once, if multiple tests)	79 / 101	78.22
	negative (all tests, if multiple tests)	16 / 1,423	1.12
	not tested	55 / 5,610	0.98
For positive tested participants: When have you been tested positive for the first time?	< 2 weeks ago	1 / 3	33.33
	2 - 4 weeks ago	4 / 4	100.00
	5 - 8 weeks ago	19 / 22	86.36
	> 9 weeks ago	55 / 72	76.39
For positive tested participants: Did you show any symptoms during that infection?	yes	71 / 84	84.52
	no	8 / 18	44.44

Supplementary Table 1 (part 2). Study questionnaire compared to anti-SARS-CoV-2 antibody status of 7,554 health care workers participating in the study.

Questions	Answers	Anti-SARS-CoV-2 Ab	
		Pos. / total	%
In which department/institute-type do you work?	conservative	78 / 2,164	3.60
	surgical	31 / 1,975	1.57
	pediatrics	16 / 908	1.76
	other clinical	45 / 1,488	3.02
	non-clinical	6 / 1019	0.59
In the last weeks, in which center did you work primarily?	Großhadern	93 / 3,978	2.34
	downtown	63 / 2,997	2.10
	both	3 / 251	1.20
	none of the above	7 / 328	2.13
Are you directly involved in patient care?	yes	123 / 4,729	2.60
	no	34 / 2,825	1.20
For participants working in patient care: I have the following profession:	nurse	68 / 2,185	3.11
	medical technician	3 / 303	0.99
	physical therapist/psychotherapist/occu-pational therapist/speech therapist	5 / 272	1.83
	physician	38 / 1,345	2.83
	other profession	9 / 624	1.44
For participants working in patient care: How many patients do you see per day on average?	< 5 patients	33 / 1,293	2.55
	5 - 10 patients	27 / 1,397	1.93
	11 - 15 patients	21 / 736	2.85
	> 15 patients	42 / 1,303	3.22
For participants working in patient care: Where do you primarily have direct contact with patients?	outpatient unit	31 / 1,444	2.15
	emergency unit	8 / 494	1.62
	normal care unit	51 / 1,814	2.81
	ICU/monitoring unit	39 / 1,165	3.35
	operation theater	9 / 905	0.99
	other	13 / 633	2.05
For participants working in patient care: Did you work on a COVID-19 ward?	yes	28 / 611	4.58
	no	95 / 4,118	2.31
For participants not working in patient care: What is your working area:	transportation	1 / 28	3.57
	cleaning personnel	4 / 119	3.36
	office work/ IT	15 / 822	1.82
	research	12 / 977	1.23
	medical institute without direct patient contact	2 / 210	0.95
	other	9 / 669	1.35
For participants not working in patient care: I had the following contacts with patients/patient material:	short patient contacts (e.g. transportation, cleaning, in the office)	8 / 634	1.26
	contact with patient material (e.g. in the laboratory)	1 / 360	0.28
	no relevant contact to patients/ patient material	34 / 1,831	1.86
In the last three months, have you worked from home for at least 1 week?	yes, as a prophylactic measure	22 / 1,377	1.60
	yes, because I was in quarantine (infected with SARS-CoV-2 or contact to COVID-19 patient)	37 / 164	22.56
	no	107 / 6,013	1.78

Supplementary Table 1 (part 3). Study questionnaire compared to anti-SARS-CoV-2 antibody status of 7,554 health care workers participating in the study.

Questions	Answers	Anti-SARS-CoV-2 Ab	
		Pos. / total	%
How long did you work from home, or how long were you quarantined?	< 1 week	5 / 362	1.38
	1 week	1 / 212	0.47
	2 weeks	31 / 364	8.51
	3 weeks	12 / 158	7.59
	4 weeks	8 / 176	4.55
	5 weeks	5 / 77	6.49
	> 5 weeks	16 / 509	3.14
	no answer	88 / 5,696	1.54
Do you suspect that you have been exposed to SARS-CoV-2 in any of the following scenarios?	patient contact	97 / 3,798	2.55
	contact to infected colleagues	87 / 4,141	2.10
	private contact (not at work)	83 / 3,966	2.09
	no increased exposure	8 / 1,382	0.58
Did you have contact to SARS-CoV-2 infected patients and at least one of the following criteria was met? - at least 15 min face-to-face contact without protective gear (at least protective mask worn by patient and study participant) - direkt contact to body fluids, especially fluids originating from the respiratory tract - performing aerosol forming measures (e.g. tracheal aspiration) - medical examination or nursing without protective gear and < 2 m distance to patient	yes	38 / 689	5.52
	no	128 / 6,865	1.88
If a contact to a SARS-CoV-2 infected patient following the criteria above occured: Was this contact reported to the occupational health office?	yes	33 / 461	7.16
	no	5 / 228	2.19
Did you have contact to SARS-CoV-2 infected colleagues and ≥ 1 of the following criteria was met? - at least 15 min face-to-face contact without protective gear (at least protective mask worn by patient and study participant) - direkt contact to body fluids, especially fluids originating from the respiratory tract	yes	32 / 583	5.49
	no	134 / 6,971	1.92
If a contact to a SARS-CoV-2 infected colleague following the criteria above occured: Was this contact reported to the occupational health office?	yes	31 / 503	6.16
	no	1 / 80	1.25
Did you have contact to SARS-CoV-2 infected individuals outside of work and ≥ 1 of the following criteria was met? - at least 15 min face-to-face contact without protective gear (at least protective mask worn by patient and study participant) - direkt contact to body fluids, especially fluids originating from the respiratory tract	yes	22 / 184	11.96
	no	144 / 7,370	1.95

Supplementary Table 1 (part 4). Study questionnaire compared to anti-SARS-CoV-2 antibody status of 7,554 health care workers participating in the study.

Questions	Answers	Anti-SARS-CoV-2 Ab	
		Pos. / total	%
If a contact to a SARS-CoV-2 infected individual outside of work following the criteria above occured: Were you contacted by the public health authority?	yes	18 / 80	22.50
	no	4 / 104	3.85
In the last three months, did you experience any cold-like symptoms?	yes	120 / 2,986	4.02
	no	46 / 4,568	1.01
If cold-like symptoms were experienced: Which of the following symptoms did you experience?	fever > 38 °C	43 / 517	8.32
	cough	69 / 1,641	4.20
	shortness of breath	43 / 635	6.77
	fatigue	86 / 1499	5.74
	running nose	59 / 1,795	3.29
	sore throat	53 / 1,906	2.78
	unusual headache	61 / 970	6.29
	diarrhea	25 / 569	4.39
	taste disorder	72 / 242	29.75

Supplementary Table 2 (part 1). Study questionnaire compared to anti-SARS-CoV-2 antibody status of 7,554 health care workers participating in the study (German).

Questions	Answers	Anti-SARS-CoV-2 Ab	
		Pos. / total	%
Bitte geben Sie hier Ihr Alter an.	≤ 30 Jahre	64 / 2,170	2.95
	31 - 40 Jahre	39 / 1,951	2.00
	41 - 50 Jahre	29 / 1,430	2.03
	51 - 60 Jahre	23 / 1,467	1.57
	> 60 Jahre	11 / 536	2.05
Bitte geben Sie hier Ihr Geschlecht an.	männlich	51 / 2,118	2.41
	weiblich	115 / 5,431	2.12
	divers	0 / 5	0.00
Nehmen Sie immunsuppressive oder immunmodulierende Medikamente?	ja	4 / 177	2.26
	nein	162 / 7,377	2.20
Haben Sie sich in der Saison 2019/2020 gegen Influenza impfen lassen?	ja	67 / 2,630	2.55
	nein	99 / 4,924	2.01
Planen Sie, sich in der nächsten Saison 2020/2021 gegen Influenza impfen zu lassen?	ja	85 / 3,390	2.51
	nein	49 / 2,719	1.80
	vielleicht	32 / 1,445	2.21
Bei Verfügbarkeit eines nebenwirkungsarmen und effizienten Impfstoffes gegen SARS-CoV-2 würde ich mich impfen lassen?	ja	85 / 4,397	1.93
	nein	26 / 913	2.85
	vielleicht	55 / 2,240	2.46
Liegen bei Ihnen relevante Vorerkrankungen vor?	ja	20 / 1,117	1.79
	nein	146 / 6,437	2.27
Bei Probanden mit relevanten Vorerkrankungen: Bei mir liegen folgende Vorerkrankungen vor:	Bluthochdruck	13 / 544	2.39
	Lungenerkrankungen (z.B. Asthma, COPD, chronische Bronchitis)	7 / 472	1.48
	Koronare Herzerkrankung	1 / 62	1.61
	Chronische Lebererkrankung	0 / 28	0.00
	Diabetes mellitus	2 /130	1.54
	Aktive Krebserkrankung	0 / 30	0.00
	Adipositas	8 / 278	2.88
Planen Sie, sich in der nächsten Saison 2020/2021 gegen Influenza impfen zu lassen?	ja	16 / 1,423	1.12
	nein	132 / 5,482	2.41
	Ex-Raucher (in den letzten 10 Jahren)	18 / 649	2.77
Wie viele erwachsene Personen leben insgesamt in Ihrem Haushalt (mit Ihnen eingeschlossen)?	1	56 / 2,355	2.38
	2	75 / 3,699	2.03
	> 2	35 / 1,500	2.33
Wie viele Kinder unter 18 Jahre leben in Ihrem Haushalt?	1	16 / 1,007	1.59
	2	14 / 828	1.69
	3	5 / 176	2.84
	4	1 / 38	2.63
	keine	130 / 5,505	2.36
Wurden Sie bereits per PCR (Abstrich) auf SARS-CoV-2 getestet?	positiv (mindestens einmal, falls Mehrfachtestung)	79 / 101	78.22
	negativ (immer negativ, falls Mehrfachtestung)	16 / 1,423	1.12
	nicht getestet	55 / 5,610	0.98
Für positiv getestete Teilnehmer: Wann wurden Sie zum 1. Mal positiv getestet?	vor < 2 Wochen	1 / 3	33.33
	vor 2 - 4 Wochen	4 / 4	100.00
	vor 5 - 8 Wochen	19 / 22	86.36
	vor > 9 Wochen	55 / 72	76.39

Supplementary Table 2 (part 2). Study questionnaire compared to anti-SARS-CoV-2 antibody status of 7,554 health care workers participating in the study (German).

Questions	Answers	Anti-SARS-CoV-2 Ab	
		Pos. / total	%
Für positiv getestete Teilnehmer: Hatten sie bei dieser Infektion Symptome?	ja	71 / 84	84.52
	nein	8 / 18	44.44
In welcher Klinik/Organisationseinheit sind Sie am Klinikum beschäftigt?	konservativ	78 / 2,164	3.60
	chirurgisch	31 / 1,975	1.57
	Pädiatrie	16 / 908	1.76
	andere klinische Bereiche	45 / 1,488	3.02
	nicht-klinische Bereiche	6 / 1019	0.59
Waren Sie in den letzten Wochen primär in der Innenstadt oder in Großhadern tätig?	Großhadern	93 / 3,978	2.34
	Innenstadt	63 / 2,997	2.10
	sowohl als auch	3 / 251	1.20
	weder noch	7 / 328	2.13
Arbeiten Sie in der unmittelbaren Patientenversorgung?	ja	123 / 4,729	2.60
	nein	34 / 2,825	1.20
Für Teilnehmer aus der direkten Patientversogung: In welcher?	Pflege	68 / 2,185	3.11
	Technischer Assistenzberuf (MTA/MTRA/…)	3 / 303	0.99
	Physiotherapie/Psychotherapie/Ergotherapie/Logopädie	5 / 272	1.83
	Arzt/Ärztin	38 / 1,345	2.83
	andere	9 / 624	1.44
Für Teilnehmer aus der direkten Patientversogung: Wieviele Patienten behandeln Sie durchschnittlich pro Tag?	< 5 Patienten	33 / 1,293	2.55
	5 - 10 Patienten	27 / 1,397	1.93
	11 - 15 Patienten	21 / 736	2.85
	> 15 Patienten	42 / 1,303	3.22
Für Teilnehmer aus der direkten Patientversogung: Wo sehen Sie überwiegend Patienten?	Ambulanz	31 / 1,444	2.15
	Notaufnahme	8 / 494	1.62
	Normalstation	51 / 1,814	2.81
	Intensivstation/IMC	39 / 1,165	3.35
	OP	9 / 905	0.99
	andere	13 / 633	2.05
Für Teilnehmer aus der direkten Patientversogung: Waren Sie auf einer COVID-Schwerpunktstation eingesetzt?	ja	28 / 611	4.58
	nein	95 / 4,118	2.31
Für Teilnehmer außerhalb der direktion Patientenversorgung: In welchem Bereich sind Sie tätig?	Transport	1 / 28	3.57
	Reinigung	4 / 119	3.36
	Büro/EDV	15 / 822	1.82
	Forschung	12 / 977	1.23
	Medizinisches Institut ohne direkten Patientenkontakt	2 / 210	0.95
	anderer	9 / 669	1.35
Für Teilnehmer außerhalb der direktion Patientenversorgung: Ich bin wie folgt mit Patienten/Material in Kontakt gekommen	Kurze Kontakte mit Patienten (z.B. Transport, Reinigung, Sekretariat)	8 / 634	1.26
	Kontakt mit Patientenmaterial (z.B. im Labor)	1 / 360	0.28
	Kein relevanter Kontakt zu Patienten/Patientenmaterial	34 / 1,831	1.86
Waren Sie in den letzten 3 Monaten mindestens 1 Woche durchgehend im Home Office tätig?	ja, prophylaktisch	22 / 1,377	1.60
	ja, da in Quarantäne (Kontakt zu COVID Patient oder selbst infiziert)	37 / 164	22.56
	nein	107 / 6,013	1.78

Supplementary Table 2 (part 3). Study questionnaire compared to anti-SARS-CoV-2 antibody status of 7,554 health care workers participating in the study (German).

Questions	Answers	Anti-SARS-CoV-2 Ab	
		Pos. / total	%
Wie lange war die Dauer des Home Office oder der Quarantäne?	< 1 Woche	5 / 362	1.38
	1 Woche	1 / 212	0.47
	2 Wochen	31 / 364	8.51
	3 Wochen	12 / 158	7.59
	4 Wochen	8 / 176	4.55
	5 Wochen	5 / 77	6.49
	> 5 Wochen	16 / 509	3.14
	keine Antwort	88 / 5,696	1.54
In welcher Situationen können Sie sich vorstellen, Kontakt mit dem Virus gehabt zu haben?	Patientenkontakt	97 / 3,798	2.55
	Kontakt mit Mitarbeitern	87 / 4,141	2.10
	Kontakt außerhalb der Arbeit	83 / 3,966	2.09
	keine erhöhte Exposition	8 / 1,382	0.58
Hatten Sie wissentlich Kontakt zu Patienten, die positiv für SARS-CoV-2 getestet wurden? Mindestens eines der folgenden Kriterien muss erfüllt sein - mindestens 15-minütiger Gesichts- ("face-to-face") Kontakt ohne Schutzausrüstung (mindestens MNS bei Patient und Mitarbeiter), z.B. im Rahmen eines Gesprächs - direkter Kontakt zu Sekreten oder Körperflüssigkeiten, insbesondere zu respiratorischen Sekreten, wie z. B. Kontakt zu Erbrochenem, Mund-zu-Mund Beatmung, Anhusten, Anniesen etc. - Durchführung aerosolbildender Maßnahmen (z.B. Absaugen) - Kontakt zum bestätigten COVID-19-Fall im Rahmen von Pflege oder medizinischer Untersuchung (< 2m), ohne verwendete Schutzausrüstung.	ja	38 / 689	5.52
	nein	128 / 6,865	1.88
Falls ein Kontakt zu einem mit SARS-CoV-2 infizierten Patienten auftrat, der die in der Vorfrage genannten Kriterien erfüllt: Wurde der Kontakt dem betriebsärztlichen Dienst gemeldet?	ja	33 / 461	7.16
	nein	5 / 228	2.19
Hatten Sie wissentlich Kontakt zu auf SARS-CoV-2 positiv getesteten Mitarbeitern? Mindestens eines der folgenden Kriterien muss erfüllt sein: - mindestens 15-minütiger Gesichts- ("face-to-face") Kontakt ohne Schutzausrüstung (mindestens MNS bei Patient und Mitarbeiter), z.B. im Rahmen eines Gesprächs - direktem Kontakt zu Sekreten oder Körperflüssigkeiten, insbesondere zu respiratorischen Sekreten, wie z. B. Küssen, Anhusten, Anniesen, etc.	ja	32 / 583	5.49
	nein	134 / 6,971	1.92

Supplementary Table 2 (part 4). Study questionnaire compared to anti-SARS-CoV-2 antibody status of 7,554 health care workers participating in the study (German).

Questions	Answers	Anti-SARS-CoV-2 Ab	
		Pos. / total	%
Falls ein Kontakt zu einem mit SARS-CoV-2 infizierten Kollegen auftrat, der die in der Vorfrage genannten Kriterien erfüllt: Wurde der Kontakt dem betriebsärztlichen Dienst gemeldet?	ja	31 / 503	6.16
	nein	1 / 80	1.25
Hatten Sie wissentlich Kontakt zu auf SARS-CoV-2 positiv getesteten Personen im privaten Umfeld? Mindestens eines der folgenden Kriterien muss erfüllt sein:			
- mindestens 15-minütigem Gesichts- ("face-to-face") Kontakt ohne Schutzausrüstung (mindestens MNS bei beiden Personen), z.B. im Rahmen eines Gesprächs	ja	22 / 184	11.96
	nein	144 / 7,370	1.95
- direktem Kontakt zu Sekreten oder Körperflüssigkeiten, insbesondere zu respiratorischen Sekreten, wie z. B. Küssen, Kontakt zu Erbrochenem, Mund-zu-Mund Beatmung, Anhusten, Anniesen, etc.			
Falls ein Kontakt zu einer mit SARS-CoV-2 infizierten Person im privaten Umfeld auftrat, der die in der Vorfrage genannten Kriterien erfüllt: Wurden Sie in der Folge vom Gesundheitsamt kontaktiert?	ja	18 / 80	22.50
	nein	4 / 104	3.85
Hatten Sie in den letzten 3 Monaten erkältungsähnliche Symptome?	ja	120 / 2,986	4.02
	nein	46 / 4,568	1.01
Falls erkältungsähnliche Symptome auftraten: Welche der folgenden Symptome sind aufgetreten?	Fieber > 38 °C	43 / 517	8.32
	Husten	69 / 1,641	4.20
	Kurzatmigkeit	43 / 635	6.77
	verstärkte Müdigkeit	86 / 1499	5.74
	Schnupfen	59 / 1,795	3.29
	Halsschmerzen	53 / 1,906	2.78
	Kopfschmerzen (die so nicht für sie üblich sind)	61 / 970	6.29
	Durchfall	25 / 569	4.39
	Geschmacksstörungen	72 / 242	29.75

Supplementary Table 3. Assay specificity determination of different anti-SARS-CoV-2 antibody detection assays in serum samples from healthy adult blood donors and children/adolescents (< 18 years) collected prior to december 2019.

Assay	Sample description	False positve / Total	Specificity (%)	95% CI (%)
Architect Assay	Adults	4 / 888	99.55	98.85 - 99.82
	Children	0 / 264	100.00	98.57 - 100.00
	Total	**4 / 1,152**	**99.65**	**99.11 - 99.86**
EuroImmun Assay	Adults	15 / 888	98.31	97.23 - 98.97
	Children	7 / 264	97.35	94.63 - 98.71
	Total	**22 / 1,152**	**98.09**	**97.13 - 98.74**
Self-Developed Assay	Adults	1 / 888	99.89	99.36 - 99.99
	Children	0 / 264	100.00	98.57 - 100.00
	Total	**1 / 1,152**	**99.91**	**99.51 - 100.00**
recomLine Assay	Adults	3 / 184	98.37	95.32 - 99.44
	Children	2 / 153	98.69	95.36 - 99.64
	Total	**5 / 337**	**98.52**	**96.57 - 99.36**
Elecsys Assay	Adults	0 / 888	100.00	99.57 - 100.00
	Children	0 / 264	100.00	98.57 - 100.00
	Total	0 / **1,152**	**100.00**	**99.67 - 100.00**

Binominal confidence intervals were computed using the Wilson score interval.

Supplementary Table 4. Assay sensitivity of anti-SARS-CoV-2 antibody detection assays in 332 serum samples from 99 COVID-19 patients collected between 0 and 16 weeks after the onset of symptoms.

Assay	Positve / Total	Sensitivity (%)	95% CI (%)
Architect Assay	86 / 98	87.76	79.81 - 92.85
EuroImmun Assay	85 / 99	85.86	77.65 - 91.39
Self-Developed Assay	74 / 97	76.29	66.93 - 83.65
recomLine Assay	88 / 97	90.72	83.30 - 95.04
Elecsys Assay	88 / 99	88.89	81.19 - 93.68

Binominal confidence intervals were computed using the Wilson score interval. The mean semi-quantitative results of all samples from the same patient was used to calculate the sensitivity, if more than one sample from the same patient was available.

Supplementary Table 5. Time resolved information on measures taken to prevent SARS-CoV-2 spread at the multicenter hospital until August 12, 2020.

Measures	Start date	End date
Prophylactic quarantine for travelers returning from risk areas	Jan 13 2020	-
rRT-PCR testing for all HCWs reporting high-risk exposures to SARS-CoV-2 infected individuals	Feb 28 2020	-
Voluntary rRT-PCR testing for HCWs reporting symptoms	Feb 28 2020	-
Prophylactic quarantine for non-essential HCWs reporting high-risk exposures to SARS-CoV-2 infected individuals	Feb 28 2020	-
Isolation of COVID-19 patients on specialised units	Mar 1 2020	-
Prohibition of business trips to risk areas for HCWs	Mar 6 2020	-
Cancellation and ban of meetings including larger groups of individuals	Mar 6 2020	Jun 6 2020
General visitation ban	Mar 17 2020	May 8 2020
No admission of patients for elective treatment	Mar 19 2020	Jun 7 2020
Face masks compulsory for all staff members	Mar 23 2020	-
Close-down of cafeterias and staff restaurants	Mar 28 2020	-
Face masks compulsory for patients during moving in the hospitals	Apr 6 2020	-
rRT-PCR testing for patients administerd to sugery upon admission	Apr 14 2020	-
Face mask compulsory for patients	Apr 15 2020	-
Allowance of one registered visitor for 1 h/day per patient	May 8 2020	May 29 2020
Allowance of several visitors per patient and day	May 29 2020	Jul 17 2020
rRT-PCR testing for all patients upon admission	Jun 4 2020	-
Allowance of one visitor for 1 h/day per patient	Jul 17 2020	-

Supplementary Table 6 (part 1). Anti-SARS-CoV-2 positivity rates of 7,554 health care workers from different departments/institutes and COVID-19 cases among staff members reported to the occupational health office.

Department/institute	Anti-SARS-CoV-2 Ab		Reported COVID-19
	Pos. / total	%	cases
conservative, internal medicine	**53 / 1,157**	**4.58**	**82**
Department for Palliative Medicine†	1 / 67	1.49	0
Department of Infectious Diseases and Tropical Medicine*	0 / 51	0.00	0
Medical Clinic and Outpatient Clinic I*	11 / 230	4.78	19
Medical Clinic and Outpatient Clinic II*	4 / 123	3.25	12
Medical Clinic and Outpatient Clinic III*	18 / 307	5.86	18
Medical Clinic and Outpatient Clinic IV*	15 / 329	4.56	29
Medical Clinic and Outpatient Clinic V*	4 / 50	8.00	4
conservative, non-internal medicine	**45 / 1,911**	**2.35**	**22**
Central Emergency Department, Campus Großhadern*	1 / 54	1.85	4
Department for Aneasthesiology*	13 / 562	2.31	8
Department for Neurology and Friedrich Baur Institute*	7 / 231	3.03	4
Department for Nuclear Medicine†	3 / 81	3.70	2
Department for Psychiatry and Psychotherapy*	5 / 327	1.53	0
Department for Radiation Therapy and Radiation Oncology*	1 / 132	0.76	0
Department for Radiology*	5 / 217	2.30	0
Departmet for Dermatology and Allergology*	7 / 155	4.52	3
Institute for Clinical Neuroimmunology*	1 / 67	1.49	0
Institute for Diagnostical and Interventional Neuroradiology*	0 / 21	0.00	0
Institute for General Practice*	0 / 14	0.00	1
Institute of Occupational, Social and Environmental Medicine*	2 / 50	4.00	0
surgical	**30 / 1,952**	**1.54**	**20**
Department for General, Visceral, and Transplant Surgery*	1 / 230	0.43	1
Department for Gynecology and Obstetrics*	8 / 375	2.13	5
Department for Hand, Plastic, and Aesthetic Surgery*	0 / 30	0.00	0
Department for Heart Surgery*	5 / 115	4.35	1
Department for Neurosurgery*	1 / 137	0.73	2
Department for Ophthalmology*	2 / 154	1.30	1
Department for Oral and Maxillofacial Surgery*	1 / 58	1.72	3
Department for Orthopedics, Physical Medicine and Rehabilitation*	3 / 216	1.39	0
Department for Otorhinolaryngology*	3 / 121	2.48	1
Department for Thoracic Surgery*	1 / 24	4.17	0
Department for Trauma, and Reconstructive Surgery*	1 / 185	0.54	4
Department for Urology*	1 / 110	0.91	1
Department for Vascular Surgery*	0 / 24	0.00	0
Outpatient Clinic for Dental Prosthetics*	2 / 66	3.03	1
Outpatient Clinic for Orthodontics*	0 / 32	0.00	0
Outpatient Clinic for Tooth Preservation and Parodontology*	0 / 59	0.00	0
Outpatient Surgery Center*	1 / 16	6.25	0

†clinical departments/institutes that did not deploy personnel to COVID-19 units (non-COVID-19 response),
*clinical departments/institutes that deployed personnel to COVID-19 units (COVID-19 response)

Supplementary Table 6 (part 2). Anti-SARS-CoV-2 positivity rates of 7,554 health care workers from different departments/institutes and COVID-19 cases among staff members reported to the occupational health office.

Department/institute	Anti-SARS-CoV-2 Ab		Reported COVID-19
	Pos. / total	%	cases
pediatric	**16 / 908**	**1.76**	**8**
Children's Palliative Center Munich†	1 / 39	2.56	0
Department for Child and Adolescent Psychiatry, Psychosomatics, and Psychotherapy*	4 / 121	3.31	1
Department for Pediatric Cardiology and Intensive Care*	1 / 61	1.64	0
Department for Pediatric Surgery, Dr. von Haunersches Kinderspital*	1 / 133	0.75	0
Department for Pediatrics, Dr. von Haunersches Kinderspital*	9 / 554	1.62	7
Other departments with patient contact*	**6 / 207**	**2.90**	**0**
non-clinical	**15 / 1,419**	**1.06**	**14**
Accouting	0 / 36	0.00	0
Administrative Departments of the Board	1 / 60	1.67	0
Administrative Departments of the Commercial Management	0 / 42	0.00	0
Catering	0 / 40	0.00	1
Central Sterile Services	0 / 31	0.00	0
Department for Clinical Pharmacology	0 / 25	0.00	0
Department for Construction and Technical Facilities	1 / 108	0.93	0
Department for Medical Technology and IT	0 / 102	0.00	0
Department for Patient Logistics	0 / 22	0.00	0
Department for Patient Management	2 / 128	1.56	1
Department for Procurement and Economy	1 / 85	1.18	3
Department for Prophylaxis and Epidemiology of Cardiovascular Diseases	0 / 54	0.00	0
Department for Transfusion Medication, Cell Therapeutics and Hemostaseology	0 / 63	0.00	0
Dispensary	0 / 90	0.00	0
HR Department	1 / 54	1.85	1
Institute for Didactics and Medical Education Research	0 / 17	0.00	0
Institute for Human Genetics	0 / 11	0.00	0
Institute for Molecular Musculoskeletal Research	0 / 2	0.00	0
Institute for Psychiatric Phenomics and Genetics	0 / 7	0.00	0
Institute for Stroke and Dementia Research	2 / 116	1.72	1
Institute for Emergency Medicine and Medicine Management	0 / 33	0.00	0
Institute for Surgical Research	1 / 23	4.35	0
Institute for Laboratory Medicine	0 / 7	0.00	5
Occupational Health Office	0 / 2	0.00	0
other departments without patient contact	6 / 261	2.30	2

†clinical departments/institutes that did not deploy personnel to COVID-19 units (non-COVID-19 response),
*clinical departments/institutes that deployed personnel to COVID-19 units (COVID-19 response)

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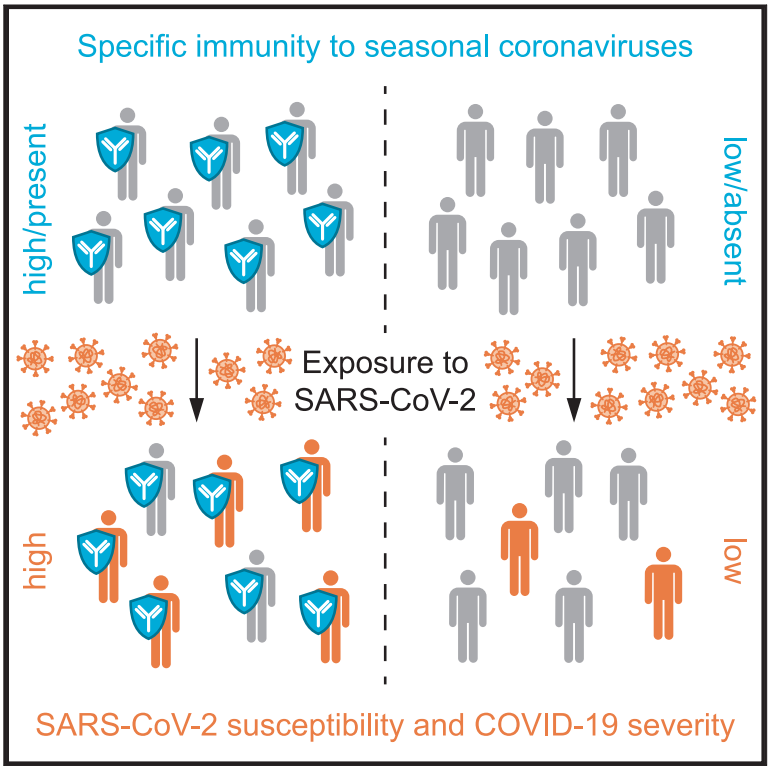
Evidence for increased SARS-CoV-2 susceptibility and COVID-19 severity related to pre-existing immunity to seasonal coronaviruses

Graphical abstract



Highlights

- Antibody levels against SARS-CoV-2 and human seasonal coronaviruses are assessed
- Specific anti-seasonal coronavirus antibodies are elevated in patients with COVID-19
- Anti-seasonal coronavirus antibodies are largely independent from COVID-19 course
- Pre-existing seasonal coronavirus immunity may increase susceptibility to SARS-CoV-2

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In brief

Wratil et al. find specific antibody responses against seasonal human coronaviruses, which cause the common cold, to be elevated in patients with COVID-19 compared to pre-pandemic blood donors. This specific immunity is likely pre-existing in patients and increases their susceptibility to SARS-CoV-2 and severity of COVID-19.

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Evidence for increased SARS-CoV-2 susceptibility and COVID-19 severity related to pre-existing immunity to seasonal coronaviruses

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SUMMARY

The importance of pre-existing immune responses to seasonal endemic coronaviruses (HCoVs) for the susceptibility to SARS-CoV-2 infection and the course of COVID-19 is the subject of an ongoing scientific debate. Recent studies postulate that immune responses to previous HCoV infections can either have a slightly protective or no effect on SARS-CoV-2 pathogenesis and, consequently, be neglected for COVID-19 risk stratification. Challenging this notion, we provide evidence that pre-existing, anti-nucleocapsid antibodies against endemic α-coronaviruses and S2 domain-specific anti-spike antibodies against β-coronavirus HCoV-OC43 are elevated in patients with COVID-19 compared to pre-pandemic donors. This finding is particularly pronounced in males and in critically ill patients. Longitudinal evaluation reveals that antibody cross-reactivity or polyclonal stimulation by SARS-CoV-2 infection are unlikely to be confounders. Thus, specific pre-existing immunity to seasonal coronaviruses may increase susceptibility to SARS-CoV-2 and predispose individuals to an adverse COVID-19 outcome, guiding risk management and supporting the development of universal coronavirus vaccines.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) caused by the novel human viral pathogen severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) developed into a pandemic with more than 242.4 million confirmed cases and 4.93 million deaths thus far (Center for Systems and Science Engineering at John Hopkins University, 2021). Defining parameters that can influence susceptibility to SARS-CoV-2 or that contribute to the high clinical variability of COVID-19 are critical to aid risk stratification, guided application of preventive measures, and COVID-19 management.

There are four species of endemic, seasonal coronaviruses (HCoVs) that typically cause mildly symptomatic respiratory tract infections in humans but are genetically dissimilar and display varying host cell tropism (Pyrc et al., 2006). Two of them,

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HCoV-229E and HCoV-NL63, belong to the taxonomic genus of α-coronaviruses, while the other two, HCoV-HKU1 and HCoV-OC43, belong to the genus of β-coronaviruses that includes SARS-CoV-2. HCoV infections are frequent (Killerby et al., 2018; Masse et al., 2020; Severance et al., 2008), and a longitudinal survey indicated that protective HCoV immunity may be short-lived (Edridge et al., 2020).

It has been hypothesized that previous encounters with HCoVs provide cross-protective immunity to SARS-CoV-2 (Braun et al., 2020). Corroborating this hypothesis, Sagar et al. (2021) suggested that recent HCoV infections can be associated with reduced COVID-19 severity. Moreover, a protective role of pre-existing T cells reactive to HCoVs in SARS-CoV-2 infection was suggested (Bacher et al., 2020; Loyal et al., 2021).

Anderson et al. (2021) recently reported on the potential influence of humoral HCoV immunity on the susceptibility to SARS-CoV-2 and the course of COVID-19: in pre-pandemic sera collected from individuals who became subsequently infected by SARS-CoV-2, no differences in IgG-type antibody responses to the spike protein of β-coronavirus HCoV-OC43 were observed compared to sera from individuals not infected by SARS-CoV-2. Furthermore, there was no relationship between pre-pandemic anti-HCoV-OC43 spike antibody levels and COVID-19 severity. In patients with COVID-19, IgG antibodies reactive to the spike protein of HCoV-OC43, primarily targeting the S2 domain, were boosted in the first 7 days of hospitalization, but the magnitude of this increase was not correlated to disease severity. The authors concluded that humoral immune responses to HCoVs are not associated with protection against SARS-CoV-2 infection and do not impact the severity of COVID-19. Contradicting this notion, our findings indicate that a genus- and antigen-specific, pre-existing immunity to HCoVs can, in fact, increase SARS-CoV-2 susceptibility and COVID-19 severity.

RESULTS

Levels of specific antibodies reactive to the nucleocapsid or spike antigens of seasonal coronaviruses are elevated in patients with COVID-19 compared to pre-pandemic donors

In a broader methodological approach, we monitored IgG-type antibody levels against the nucleocapsid and the spike S1 domain proteins of SARS-CoV-2 and all four seasonal coronaviruses as well as against full-length spike protein of SARS-CoV-2, HCoV-NL63, and HCoV-OC43 in pre-pandemic sera from 888 healthy adults as well as in 314 sera longitudinally collected from 96 patients with COVID-19 (see STAR Methods and Figure S1). We utilized a newly launched commercial line immunoassay (recomLine) and a recently developed bead-based multiplex immunoassay (MultiCoV-Ab) (STAR Methods and Becker et al., 2021). Specificities and sensitivities of these assays for anti-SARS-CoV-2 antibodies and correlative analyses for anti-HCoV antibodies in pre-pandemic and sera of patients with COVID-19 are provided in STAR Methods, Table S1, and Figure S2.

Analyzing the mean of all sampling time points for each donor, we observed drastically increased levels of disease-specific antibodies against the nucleocapsid, full-length spike protein, and spike S1 domain antigen of SARS-CoV-2 in patients with COVID-19 compared to pre-pandemic donors, as expected (Figure 1, green; Figure S3, green). Surprisingly, in both assays, mean antibody levels against the nucleocapsid of α-coronaviruses, HCoV-229E and HCoV-NL63, were significantly elevated in the COVID-19 cohort compared to the group of pre-pandemic donors (Figure 1, brown and yellow). Anti-nucleocapsid responses to β-coronavirus HCoV-HKU1 were also elevated in patients with COVID-19 compared to pre-pandemic donors, albeit less pronounced and only in the Multi-CoV-Ab assay (Figure 1, blue). Anti-nucleocapsid responses to the β-coronavirus HCoV-OC43 were similar between the study groups (Figure 1, purple). Furthermore, critically ill patients compared to less severely affected cases i.e., "non-critical" (defined according to WHO guidelines 2020), had increased antibody titers against the nucleocapsid of the two α-coronaviruses and HCoV-HKU1 (Figure 1, brown, yellow, blue), but not of SARS-CoV-2 or HCoV-OC43 (Figure 1, green and purple). In contrast, full-length spike-specific antibodies targeting HCoV-OC43, but not those targeting HCoV-NL63, were significantly increased in patients with COVID-19 compared to pre-pandemic donors (Figure 1, third row, yellow and purple). Of note, antibody responses to the spike S1 domain of β-coronavirus HCoV-OC43 were, in turn, reduced in critically ill patients compared to pre-pandemic donors (Figure 1, lower row, purple) and indifferent between the study groups for the other HCoVs tested (Figure 1, lower row, brown, orange, and blue). Qualitative evaluation of the recomLine assay showed both significantly increased numbers of patients with COVID-19 positive for anti-nucleocapsid antibodies recognizing α-coronaviruses compared to pre-pandemic donors, and more critically ill patients being positive for antibodies targeting HCoV-229E than non-critically ill (Table S2).

Anti-HCoV antibody concentrations remain largely unaltered in patients with COVID-19 during the disease course, indicating that high antibody responses against seasonal coronaviruses were pre-existing in these patients

Two confounders could potentially contribute to the increased concentrations of antibodies against seasonal coronaviruses observed in patients with COVID-19: (1) cross-reactivity of anti-SARS-CoV-2 antibodies with the assays' HCoV antigen components and (2) polyclonal stimulation of pre-existing, HCoV-specific plasma cells by SARS-CoV-2 infection. To address both scenarios, we first explored longitudinal changes in antibody levels of those 28 patients with COVID-19 in our cohort who had donated sera both in the first 2 weeks after symptom onset and at later time points in the disease course. Expectedly, specific antibody responses to the nucleocapsid, full-length spike protein, and spike S1 domain of SARS-CoV-2 drastically increased during the disease course in this cohort (Figure 2, green). In contrast, titers of antibodies against all four HCoVs remained largely unaltered (Figure 2, brown, yellow, blue, and purple). Only, anti-full-length spike antibody levels against HCoV-NL63 increased in the first 2 weeks after the onset of symptoms and decreased thereafter (Figure 2, lower left, yellow).

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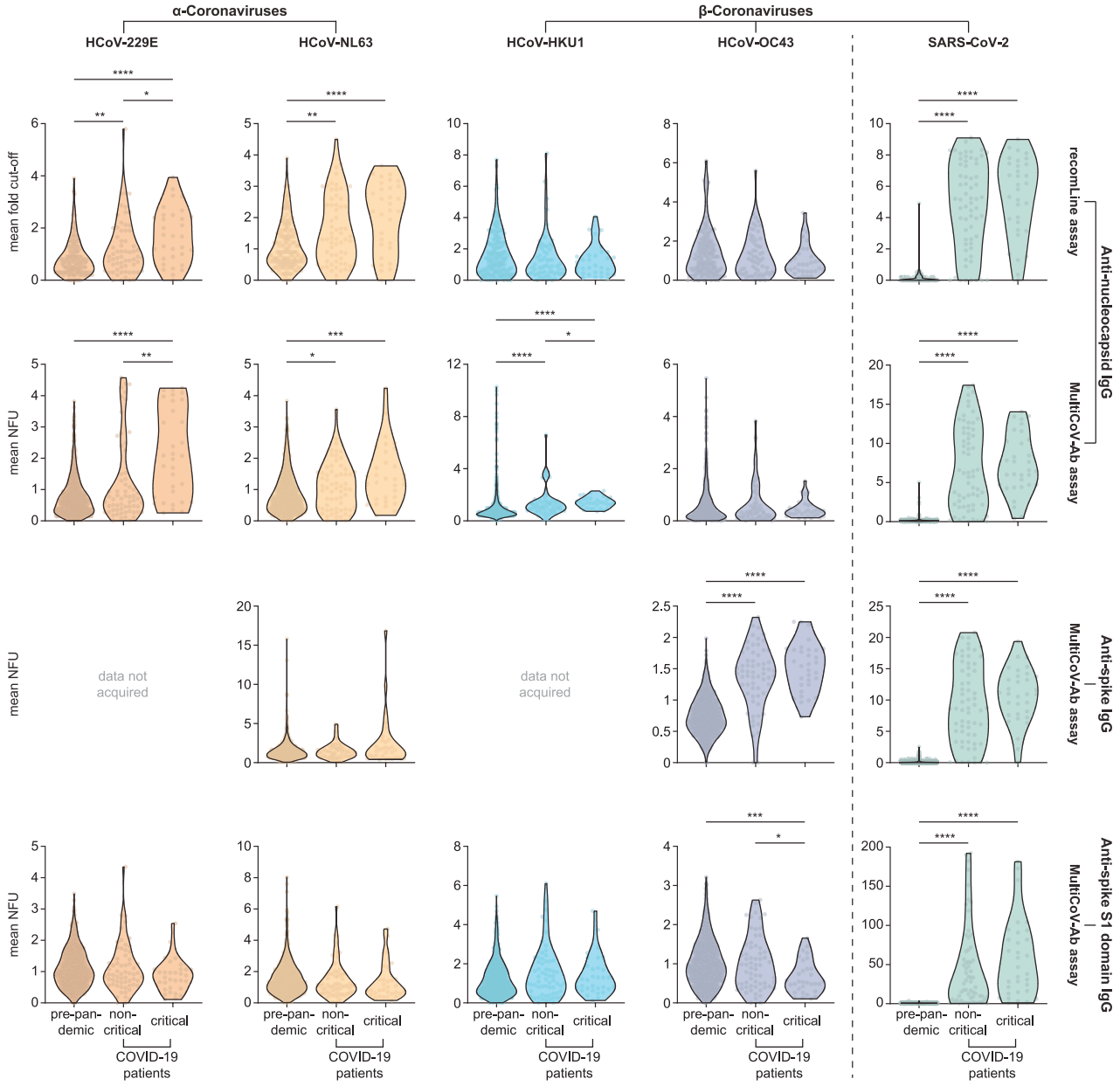

Figure 1. Anti-nucleocapsid and anti-spike S1 domain antibody levels in sera from pre-pandemic donors and patients with COVID-19

Eight hundred and eighty-eight pre-pandemic sera from healthy adult blood donors (184 in case of the line immunoassay), 153 samples from 32 critically ill patients with COVID-19 (161 in case of the recomLine assay, critical), and 142 samples from 64 less severely affected patients with COVID-19 (143 in case of the recomLine assay, non-critical) were analyzed for their antibody levels against HCoV-229E, -NL63, -HKU1, and -OC43, as well as SARS-CoV-2. Mean antibody levels per donor/patient (dots) are depicted as violin plots for every group (pre-pandemic, as well as critical and non-critical COVID-19). Differences in the assays' antibody responses comparing the groups were tested for their statistical significance via Kruskal-Wallis test and pairwise comparisons using Wilcoxon rank-sum test with continuity correction. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001. NFU, normalized fluorescence units.

Second, we compared mean antibody levels in these 28 patients with COVID-19 in sera collected from the third week after the onset of symptoms onward relative to those obtained in the first 2 weeks. Inherent to the newly mounting immune response, we observed markedly increased antibody titers against the pandemic SARS-CoV-2 in this longitudinal comparison (Figure 3A, green). In the same comparison of specimens, however, most anti-HCoV antibody responses showed only insignificant changes (Figure 3A, brown, yellow, blue, and purple, respectively). Anti-nucleocapsid antibody levels against HCoV-229E were slightly, but significantly elevated in the recomLine assay, possibly hinting at weak cross-reactivity (Figure 3A, top left, brown). Conversely, anti-nucleocapsid antibody responses to HCoV-229E and HCoV-NL63 (Figure 3B, upper row, brown

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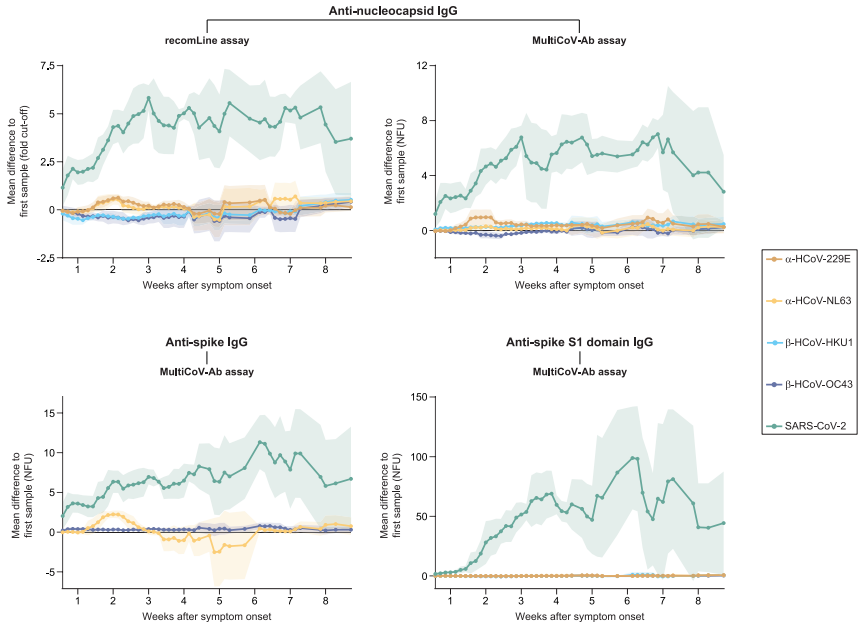

Figure 2. Longitudinal antibody level changes in 28 patients with COVID-19

One-hundred and seventy-four sera from 28 patients with COVID-19 who donated specimens both in the first 2 weeks after symptom onset and at later time points were analyzed. The five time point rolling averages for differences in antibody levels compared to the first sample donated by each individual patient are shown. Shaded areas depict standard deviations. NFU, normalized fluorescence units.

and yellow) as well as anti-full-length spike antibody levels against HCoV-OC43 (Figure 3B, lower left, purple) were markedly elevated in these 28 individuals with COVID-19 compared to pre-pandemic donors. Anti-spike S1 domain antibody levels against HCoV-OC43 in critically ill patients, on the other hand, were significantly decreased in this comparison (Figure 3B, lower right, purple). Collectively, these results largely exclude a relevant cross-reactivity of anti-SARS-CoV-2 antibodies in HCoV serology or a polyclonal stimulation of HCoV-specific plasma cells after SARS-CoV-2 infection. We conclude that high antibody titers to the nucleocapsid of HCoV-229E and HCoV-NL63, as well as full-length spike antigen of HCoV-OC43, were most likely pre-existing in these patients with COVID-19.

In patients with COVID-19, antibody responses to SARS-CoV-2 and seasonal coronaviruses often show sex-specific differences, and interleukin-6 levels at admission correlate significantly with disease severity in multivariate analyses

We compared the mean antibody levels for each patient with COVID-19 enrolled in this study to additional health record data. Our analysis revealed that patients' age, their interleukin-6 (IL-6) levels both upon admission and at their peak, and the duration of their hospitalization or the time they spent on intensive care units (ICUs) showed only weak correlations with their anti-SARS-CoV-2 and anti-HCoV antibody responses, respectively (Figure 4A).

Male patients compared to females had significantly higher antibody levels against SARS-CoV-2 (Figure 4B, green) and against the nucleocapsid of both α-coronaviruses as well as HCoV-OC43, in both assays utilized (Figure 4B, brown, yellow, purple). Anti-nucleocapsid responses to HCoV-HKU1 were significantly elevated in males in the MultiCoV-Ab assay (Figure 4B, blue). Anti-OC43 antibodies against full-length spike protein were increased in males compared to females (Figure 4B, third row, purple). Conversely, anti-spike S1 domain antibodies were significantly decreased in the same comparison (Figure 4B, bottom row, purple). Patients' comorbidities had, in most cases, no effect on anti-SARS-CoV-2 anti-HCoV antibody titers (Figure 4C).

In multivariate analyses, we investigated whether the disease severity of individuals with COVID-19 correlated with antibody levels against SARS-CoV-2 and seasonal coronaviruses while also considering the aforementioned data from patients' health records. Data on hospitalization and admission to ICU were excluded from this multivariate analysis because they were, among others, used to define the patients' disease severity (WHO guidelines, 2020) and were thus considered dependent variables. In all assays, COVID-19 severity correlated significantly with patients' IL-6 levels at admission ($p \leq 0.0140$; supplemental information). Peak IL-6 responses or patients' mean antibody levels correlated with disease severity only in specific assays (supplemental information). However, patients' age, sex, or presence of comorbidities did not correlate with disease severity in this multivariate analysis (supplemental information).

DISCUSSION

In summary, our study provides evidence that specific pre-existing adaptive immunity to seasonal coronaviruses is associated with increased susceptibility to SARS-CoV-2 infection and adverse disease outcome. The mode of action underlying these findings is unclear. We hypothesize a direct or indirect enhancement of early stages of SARS-CoV-2 infection on the nasal or oral mucosa or in the respiratory tract, or an antibody-dependent cellular cytotoxicity influencing immunopathology in lung tissue mediated by specific pre-existing antibodies against seasonal coronaviruses. Regarding anti-nucleocapsid antibody responses, a recent study suggested that lectin pathway recognition molecules of the complement system, including the effector enzyme MASP-2, can directly bind to SARS-CoV-2 nucleocapsid protein, with subsequent activation of lectin pathway-mediated C3b and C4b deposition (Ali et al., 2021). Conceivably, pre-existing anti-nucleocapsid antibodies against

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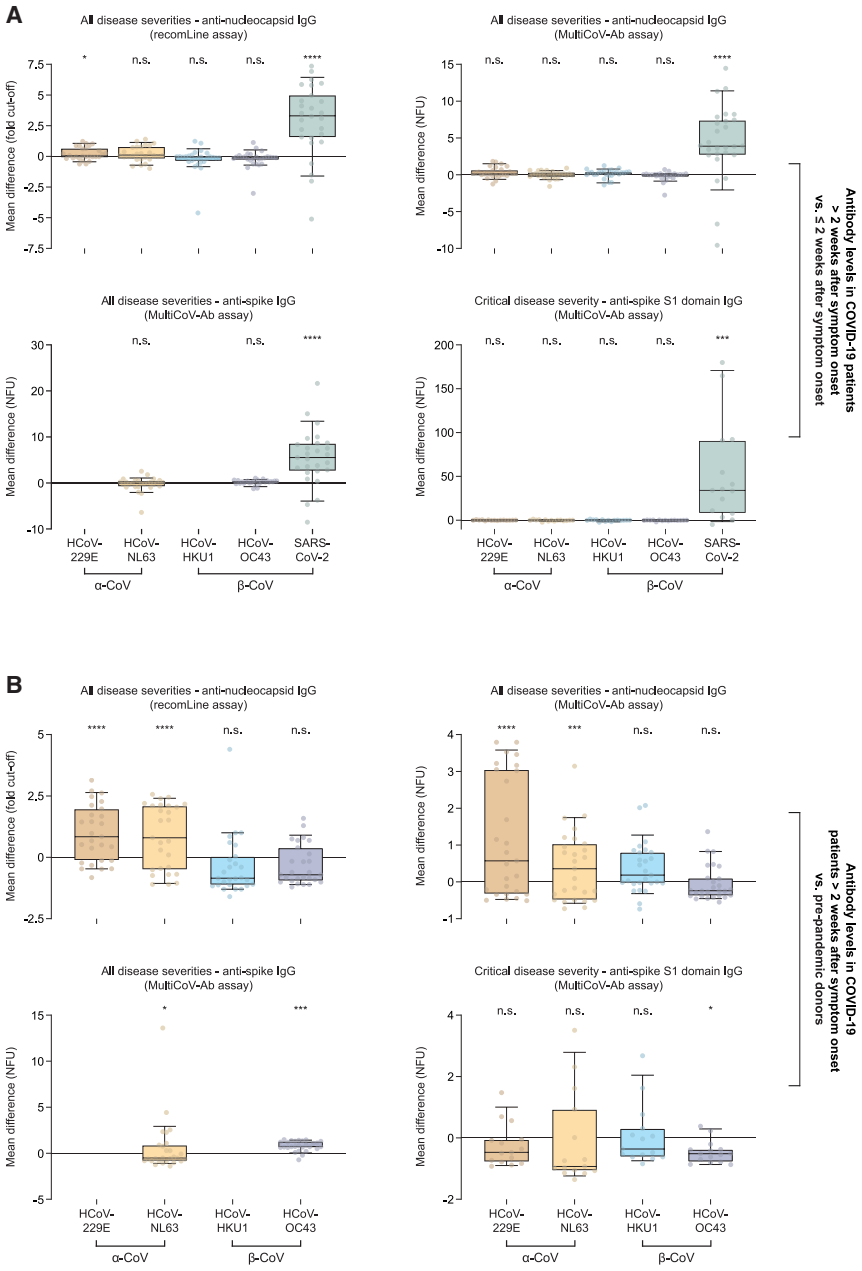

Figure 3. Comparison of antibody levels in 28 patients with COVID-19 at later time points with earlier time points and pre-pandemic specimens

Sixty-nine sera collected in the first 2 weeks after symptom onset from 28 patients with COVID-19, 105 sera collected after the first 2 weeks after symptom onset from the same patients, and 888 pre-pandemic sera from healthy adult blood donors (184 in case of the recomLine assay) were analyzed. Differences in mean antibody levels for each patient comparing samples obtained more than 2 weeks after symptom onset with those from the first 2 weeks after symptom onset and mean antibody levels from pre-pandemic adults (dots) are depicted as boxplots with whiskers between the 10th and 90th percentiles for the following groups: patients with COVID-19 more than 2 weeks after symptom onset versus less than 2 weeks after symptom onset (A), patients with COVID-19 more than 2 weeks after symptom onset versus pre-pandemic donors (B). Differences in antibody levels in each group were analyzed for their statistical significance using two-tailed, paired t tests in (A) and two-tailed, unpaired t tests in (B). ***p ≤ 0.001, ****p ≤ 0.0001; n.s., not significant; NFU, normalized fluorescence units; α-CoV, α-coronaviruses; β-CoV, β-coronaviruses.

seasonal coronaviruses may cross-react with SARS-CoV-2 nucleocapsid released from infected, dying cells in the respiratory tract negatively modulating the development of thromboembolism and aggravating disease outcome.

During the validation of the MultiCoV-Ab assay (Becker et al., 2021), similar, albeit less pronounced, trends for elevated anti-nucleocapsid IgG titers against HCoV-229E and HCoV-NL63 were observed in relation to individuals' SARS-CoV-2 serostatus. Another study conducted in healthcare workers found decreased levels of nucleocapsid-specific antibodies against seasonal coronaviruses in symptomatic individuals with COVID-19 compared to those with asymptomatic disease (Ortega et al., 2021). Of particular note, the COVID-19 cohorts in the former studies consisted mainly of non-hospitalized patients with asymptomatic or mild disease severity (79.1%; Becker et al., 2021) (99.2%; Ortega et al., 2021), whereas our current study had a substantially lower proportion of mildly affected patients with COVID-19 (26.0%, STAR Methods).

Our data support the notion of a SARS-CoV-2 susceptibility- and COVID-19 severity-enhancing effect related to high abundance of nucleocapsid-specific antibodies against α-coronaviruses and possibly β-coronavirus HCoV-HKU1. Two other studies monitored anti-nucleocapsid responses to seasonal coronaviruses in COVID-19 cases via the recomLine assay and observed decreased anti-HCoV-OC43 antibody titers in critically ill patients compared to less severely affected (Dugas et al., 2020, 2021). Utilizing the same assay, we observed a similar albeit statistically insignificant trend toward low anti-HCoV-OC43 nucleocapsid antibody levels in critically ill patients. This result, however, could not be confirmed in the MultiCoV-Ab assay.

Furthermore, our findings indicate that SARS-CoV-2 susceptibility is enhanced by pre-existing antibodies targeting the spike antigen of HCoV-OC43. Regarding humoral responses to seasonal coronavirus spike protein, several studies observed

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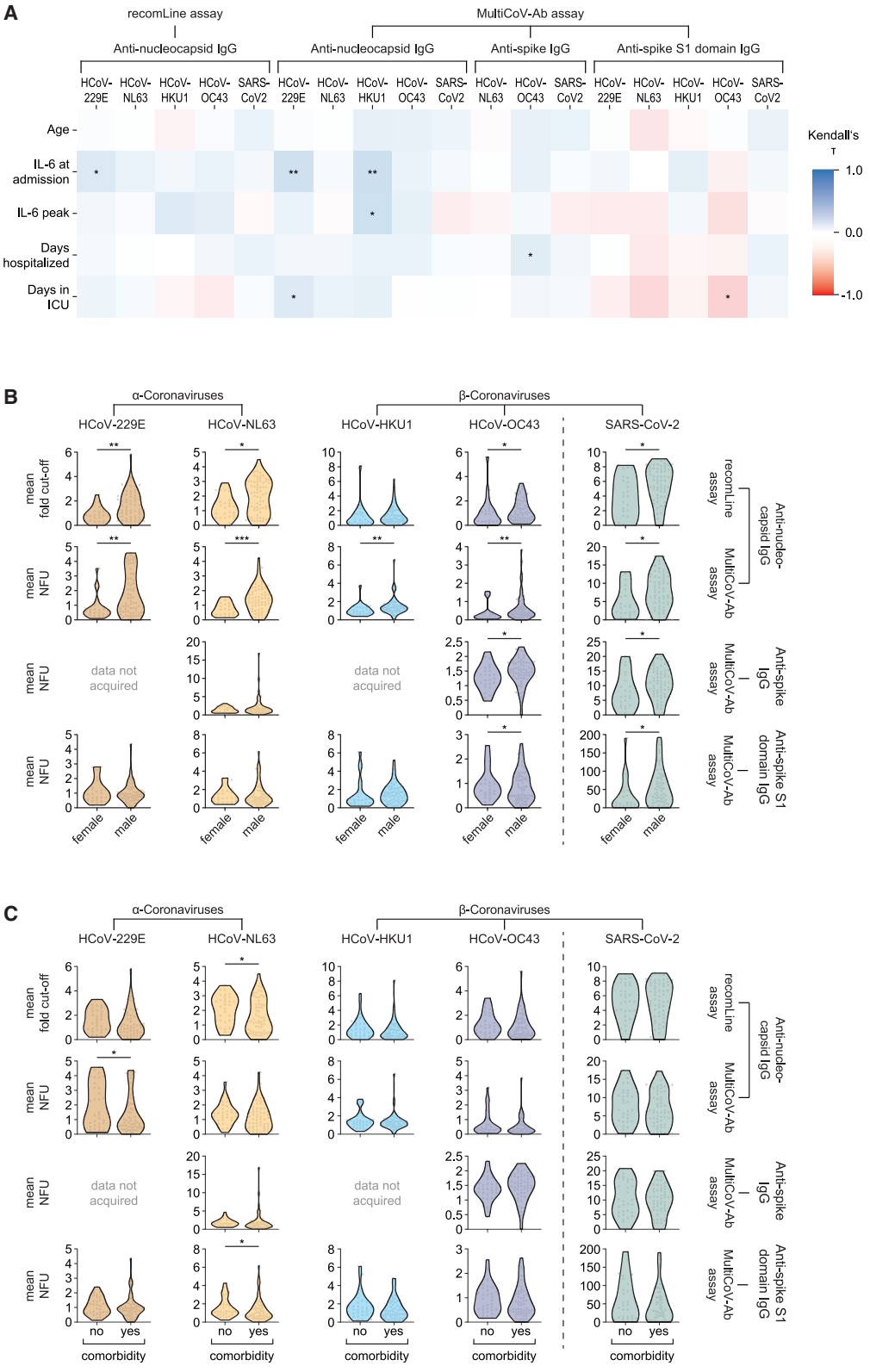

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elevated antibody levels against HCoV-OC43 in patients with COVID-19 (Prévost et al., 2020; Anderson et al., 2021) and vaccinees (Tauzin et al., 2021) compared to uninfected, non-vaccinated individuals, corroborating our results. However, longitudinal and cross-sectional analyses suggested that these increased anti-HCoV-OC43 spike antibody titers were likely not pre-existing, but dependent on either the COVID-19 disease course (Prévost et al., 2020; Anderson et al., 2021) or vaccination (Tauzin et al., 2021), and mainly mediated by antibodies targeting the S2 domain of the viral spike (Anderson et al., 2021). In line with these findings, our data suggest that high anti-HCoV-OC43 spike antibody levels in COVID-19 are likely due to increased concentrations of antibodies targeting the S2 domain. Furthermore, decreased anti-spike S1 domain responses were observed in critically ill patients compared to pre-pandemic donors. Our longitudinal assessment, on the other hand, revealed high, yet stable and COVID-19 disease course-independent antibody levels against the full-length spike antigen of HCoV-OC43 and against the nucleocapsid of seasonal α-coronaviruses, indicating that these elevated antibody concentrations were, indeed, pre-existing. These discrepant results could be due to differences in the COVID-19 patient cohorts: the former studies included lower rates of severely and critically ill patients with COVID-19 (8.9%; Prévost et al., 2020) (14.0%; Anderson et al., 2021), whereas the percentage of such cases was more than 3-fold higher in our patient cohort (44.8%). Thus, our findings could potentially be more applicable to severe COVID-19. Of note, Prévost et al. (2020) did not perform longitudinal antibody analyses in the same patients, but cross-sectional analyses in dissimilar patient groups. The alterations in anti-spike antibodies against HCoV-OC43 in individuals with COVID-19 observed by Prévost et al. (2020), therefore, could underlie inter-individual rather than longitudinal changes in serological responses. Moreover, differences in the type and specificities of the assays utilized to detect anti-full-length spike antibodies against HCoV-OC43 and their cross-reactivity to anti-SARS-CoV-2 antibodies could contribute to the different results obtained by Prévost et al. (2020) and Anderson et al. (2021) compared to ours. Unfortunately, a well-validated, broadly available anti-HCoV spike antibody assay is lacking. All studies on serological responses against these antigens currently relied on self-developed methods and the analytical performances of these are difficult to compare.

Adding to the discussion, Sokal et al. (2021) found fractions of SARS-CoV-2 spike protein-specific memory B cells that were cross-reactive for HCoV-HKU1 and HCoV-OC43 as well as B cells specific for HCoV-HKU1 or HCoV-OC3 spike protein among PBMCs from four patients with COVID-19 3 months after infection with SARS-CoV-2. The abundance of these HCoV antigen-specific cells declined over time. However, the authors were unable to investigate the influence of SARS-CoV-2-specific, cross-reactive memory B cells on the overall serological responses against the novel coronavirus, in particular at earlier time points after infection. Furthermore, it was not addressed in this study whether the declining numbers of HCoV-specific memory B cells were associated with SARS-CoV-2 infection itself or due to COVID-19-independent, rapid fluctuations of HCoV antibody responses as observed by Edridge et al. (2020).

Comparing antibody responses against seasonal coronavirus in patients with COVID-19 with additional health record data we found that these responses are largely independent from age, having comorbidities, the time patients spent hospitalized or on ICU, and IL-6 levels. Interestingly, the group of male patients showed, in most instances, significantly increased anti-nucleocapsid antibody titers against seasonal coronaviruses. In multivariate analyses, we found IL-6 levels, especially those measured at admission, to correlate with disease severity, in line with recent studies (Leisman et al., 2020).

A study conducted by Sagar et al. (2021) proposed that acute HCoV infections can be associated with reduced COVID-19 severity. Data from medical records on PCR testing for acute HCoV infections were analyzed retrospectively in this investigation and not adaptive immune responses to individual HCoVs that we unveil as relevant in our study.

Anderson et al. (2021) suggested that pre-existing IgG-type antibody responses to the spike antigen of β-coronaviruses HCoV-OC43 in patient sera collected up to 7 years before SARS-CoV-2 infection do not influence susceptibility to the novel coronavirus and COVID-19 severity. However, Anderson et al. (2021) did not investigate the role of nucleocapsid-specific antibody responses to α-coronaviruses as a critical and predisposing factor for COVID-19. Moreover, HCoV antibody titers have been reported to decay or fluctuate considerably within months after infection or re-infection (Edridge et al., 2020), questioning the validity of the interpretation of pre-existing HCoV immunity at the time of SARS-CoV-2 exposure in patient-matched reference sera, which sometimes date back many years (Anderson et al., 2021). Based on the limited dataset that only assessed anti-HCoV-antibodies targeting the spike protein, Anderson et al. (2021) concluded that humoral adaptive immunity to seasonal coronaviruses is not associated with protection from infection or an altered disease course. Contradicting this notion, we provide evidence that pre-existing, humoral immunity reflected by specific antibodies recognizing either the nucleocapsid of seasonal α-coronaviruses or the spike antigen of HCoV-OC43 increases SARS-CoV-2 susceptibility. We propose that seasonal coronavirus serology can serve as a marker to guide clinical risk stratification and that individuals with recently resolved seasonal coronavirus infections may benefit from advanced preventive measures against COVID-19. Our findings fuel efforts to develop a universal vaccine that mitigates the immunological crosstalk between coronaviruses of different species and its

Figure 4. Comparison of mean antibody levels in patients with COVID-19 with additional health record data

Mean antibody levels in 96 patients with COVID-19 were compared to additional health record data. Kendall's correlation coefficients (τ) between quantitative assay results and age, interleukin-6 levels at admission as well as at their individual peak, days patients spend hospitalized or admitted to intensive care units (ICUs) are depicted in (A). (B and C) Mean antibody levels in the same patients (dots) compared to sex (B) and presence of comorbidities (C) as violin plots. In (B) and (C), differences between the groups were analyzed for their statistical significance using the Wilcoxon rank-sum test with continuity correction. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. NFU, normalized fluorescence units.

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potentially negative effects on the outcome of subsequent, possibly lethal coronavirus infections.

Limitations of the study

The results of our longitudinal data analysis cannot completely exclude the possibility of cross-reactive anti-SARS-CoV-2 antibodies that bind seasonal coronavirus antigens, thus contributing to the elevated anti-HCoV antibody titers observed in our assays. Furthermore, we cannot fully rule out polyclonal stimulation of HCoV-specific plasma cells after SARS-CoV-2 infection. However, for these scenarios to potentially contribute to our findings they would have to be (1) specific for certain antigens of individual HCoV species, (2) increase quickly already in the earliest days after SARS-CoV-2 infection, and (3) be stable over several weeks and independent from the COVID-19 disease course. Taken together, this seems unlikely. To corroborate our results, studies on matched sera from individuals with COVID-19 collected shortly before infection and during the disease course should be conducted. Also, the role of low antibody responses against the spike S1 domain found in critically ill patients should be investigated further. Furthermore, future work should seek to identify factors that drive humoral immunity toward strong, specific anti-HCoV responses.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.celrep.2021.110169.

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AUTHOR CONTRIBUTIONS

P.R.W. and O.T.K. conceived the study. P.R.W. and O.T.K. drafted the first version of the manuscript. N.A.S., A.D., N.S.-M., L.K., and V.H. contributed to drafting sections of the manuscript. P.R.W., N.A.S., B.K., A.D., and L.K. performed data analysis. P.R.W., N.A.S., B.K., A.O., P.M.S., A.R., M.G., and S.S. conducted laboratory experiments. A.D., D.J., M.B., U.R., and N.S.-M. performed the MultiCoV-Ab experiments and data normalization. M.M., J.C.H., C.S., J.M., M.R., J.B., S.K., B.Z., M.v.B.-B., J.E., and N.S.-M. participated in the study design. All authors contributed to the interpretation of data and approved the final manuscript.

DECLARATION OF INTERESTS

N.S.-M. was a speaker at Luminex user meetings in the past. The Natural and Medical Sciences Institute at the University of Tübingen is involved in applied research projects as a fee for services with Luminex. The remaining authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
R-phycoerythrin labelled goat-anti-human IgG	Dianova	Cat#JIM-109-116-098; RRID: AB_2337678
Biological samples		
Pre-pandemic serum samples from healthy adult blood donors (anonymized)	Blutspendedienst des Bayerischen Roten Kreuzes	N/A
Serum specimens from patients with COVID-19 (pseudonymized)	This study	N/A
Chemicals, peptides, and recombinant proteins		
HCoV-OC43 spike protein	Sino Biological	Cat#40607-V08B
Critical commercial assays		
recomLine SARS-CoV-2 IgG line immunoassay	Mikrogen	Cat#7374
Deposited data		
Pseudonymized patient record data and raw data from serum antibody measurements in patients and pre-pandemic donors	This study	Mendeley: https://doi.org/10.17632/stfw4w4vjh.1
Oligonucleotides		
Primer: CAG promoter forward CTT CTG GCG TGT GAC CGG	This study	N/A
Primer: CAG promoter reverse CAT GGT GGC CTT TGC CAA	This study	N/A
Primer: T4 foldon forward AAG TGG CCT AGC GGG CGC TTG GTC CCA CGT G	This study	N/A
Primer: T4 foldon reverse AAG ATC TGC TAG CTC GAG TCG C	This study	N/A
Primer: NL63-S1 forward CAT TTT GGC AAA GGC CAC CAT GAA GCT GTT CCT GAT CCT GC	This study	N/A
Primer: NL63-S1 reverse GGA GGA ATT TGC AGG AAT CAG GGA ACC GTC AG	This study	N/A
Primer: NL63-S2 forward CCC TGA TTC CTG CAA ATT CCT CCG ACA ACG GTA TCT	This study	N/A
Primer: NL63-S2 reverse CCA AGC GCC CGC TAG GCC ACT TGA TGT AGT TCT CGA A	This study	N/A
Recombinant DNA		
Plasmid: pCAGGS	NovoPro	Cat#V008798
Plasmid: pCAGGS encoding SARS-CoV-2 trimeric spike	Amanat et al. (2020)	N/A
Plasmids: pCAGGS encoding spike S1 domains of SARS-CoV-2, HCoV-229E, HCoV-NL63, HCoV-HKU1 or HCoV-OC43	Becker et al. (2021)	N/A
Plasmids: pRSET2b encoding nucleocapsid proteins of SARS-CoV-2, HCoV-229E, HCoV-NL63, HCoV-HKU1 or HCoV-OC43	Becker et al. (2021)	N/A
Plasmid: pCMV3-C-FLAG encoding HCoV-NL63 spike gene ORF cDNA	Sino Biological	Cat#VG40604-CF
Software and algorithms		
recomScan 3.4	Mikrogen	Cat#31006
xPOTENT 4.3	Luminex	Cat# XPON-UPGRD-FM3D

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Prism 9.3.0	GraphPad	www.graphpad.com
R version 4.1.1	R Foundation	www.r-project.org
R package tidyverse 1.3.1	Wickham et al. (2019)	cran.r-project.org/package=tidyverse
R package caret 6.0-90	RStudio	cran.r-project.org/package=caret
R package MASS 7.3-54	Venables and Ripley (2002)	cran.r-project.org/package=MASS
Other		
Dynablot Plus strip processor	Dynex Technologies	Cat#D7144-P6-E
Flexmap 3D	Luminex	Cat#FLEXMAP-3D
Biomek i7	Beckman	Cat#B87587

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Oliver T. Keppler (keppler@mvp.lmu.de).

Materials availability

All unique/stable reagents generated in this study are available from the Lead Contact with a completed Materials Transfer Agreement.

Data and code availability

All raw data corresponding to pseudonymized patient record data and serum antibody measurements in patients with COVID-19 and pre-pandemic donors have been deposited to Mendeley Data (https://doi.org/10.17632/stfw4w4vjh.1).

This paper does not report original code.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Patients and specimens

We established a collection of pre-pandemic serum samples from 888 healthy adult blood donors (collected prior to December 2019 in Germany) whose health record data were anonymized. Furthermore, we included a set of 314 serum specimens collected between March 8, 2020, and July 7, 2020, from 96 patients infected with SARS-CoV-2 at the LMU Klinikum, Munich, Germany. Patients are part of the COVID-19 Registry of the LMU Klinikum (CORKUM, WHO trial id DRKS00021225) and the study was approved by the local ethics committee (No: 20-245). All patients were tested positive for SARS-CoV-2 by rRT-PCR in nasopharyngeal or oropharyngeal swabs. The median age of the 96 patients with COVID-19 examined in this study was 61 years (interquartile range 50 to 71 years), and 26.0% (25/96) of these individuals were female. Clinical data, including symptoms and symptom onsets, were obtained from health records. Immunocompromised individuals were excluded from this study. If the time of symptom onset was not stated e.g., in asymptomatic patients, we substituted this information with the time of the first SARS-CoV-2-PCR-positive result. This was the case for 26.0% (25/96) of patients. We categorized the disease severity of patients with COVID-19 following the WHO guidelines "Clinical Management of COVID-19": asymptomatic (no clinical signs of infection), mild (symptomatic patients without evidence of viral pneumonia or hypoxia), moderate (clinical signs of pneumonia, including fever, cough, dyspnoea), severe (clinical signs of pneumonia, plus one of the following: respiratory rate > 30 /min, severe respiratory distress, $SpO_2 < 90\%$ on room air), critical (one of the following: acute respiratory distress syndrome, sepsis, septic shock). Five patients contributing a total of 15 samples were categorized as asymptomatic, 19 patients contributing 35 samples as mild, 29 patients contributing 57 samples as moderate, 11 patients contributing 41 samples as severe, and 32 patients contributing 166 samples as critical (Figure S1). Due to the anonymization of pre-pandemic blood donors, these individuals could not be age-matched to the patients with COVID-19 examined in this study.

METHOD DETAILS

Coronavirus antigens

For the expression and purification of SARS-CoV-2 full-length spike protein as well as the nucleocapsid and spike S1 domain antigens of SARS-CoV-2 and HCoVs used in the MultiCoV-Ab assay, well-described plasmids were utilized (Becker et al., 2021; Amanat

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et al., 2020). To generate the expression construct of HCoV-NL63 full-length spike protein, four DNA fragments were amplified by PCR. Fragment 1 comprising a part of the CAG promotor sequence was amplified from pCAGGS expression plasmid encoding the SARS-CoV-2 trimeric spike (Amanat et al., 2020) using CAG promoter forward and reverse primers. Fragment 2 comprises the DNA encoding the T4 foldon and was amplified from the same plasmid using T4-foldon forward and reverse primers. Fragment 3 corresponds to the S1 fragment of HCoV-NL63 and was amplified from pCMV3-C-FLAG encoding HCoV-NL63 spike gene ORF cDNA using NL63-S1 forward and reverse primers. Fragment 4 corresponding to S2 fragment of HCoV-NL63 was amplified from the same cDNA template using NL63-S2 forward and reverse primers. Individual amplified DNA fragments were fused by overlap extension and cloned into XbaI and NotI sites of a pCAGGS expression vector. The newly generated expression construct encoding the full-length spike protein of HCoV-NL63 was verified by DNA sequencing. Protein expression and purification of antigens used in the MultiCoV-Ab assay was performed as previously described (Becker et al., 2021). HCoV-OC3 spike protein was purchased.

Antibody detection assays

The commercial recomLine SARS-CoV-2 IgG line immunoassay was used to determine the presence of IgG-type anti-nucleocapsid-specific antibodies against SARS-CoV-2 and HCoVs in serum specimens. The assay was performed in accordance with the manufacturers' instructions. Briefly, test strips were incubated with 20μL serum diluted in 2 mL wash buffer on a Dynablot Plus strip processor. Following washing with wash buffer, conjugation solution and, after additional washing, substrate solution were added. After incubating the test strips for 8 min in substrate solution, strips were rinsed with deionized water and subsequently dried between 2 layers of absorbent paper. Quantitative results for the recomLine assay were obtained by analyzing test strips with the recomScan software. According to the manufacturer's guidelines, the "fold cut-off" value was determined by subtracting the signal of interest with that of the internal cut-off band.

Further, the previously described MultiCoV-Ab multiplex immunoassay was employed that detects the presence of IgG antibodies against several SARS-CoV-2 and HCoVs antigens, including the S1 domain of viral spike protein and the nucleocapsid antigen (SARS-CoV-2 and all HCoVs), as well as the full-length spike antigen (SARS-CoV-2, HCoV-NL63 and HCoV-OC43) (Becker et al., 2021). Briefly, serum samples diluted 1:400 were incubated with antigens immobilized on magnetic beads in 384-well plates. Following washing with phosphate buffered saline containing 0.05% (v/v) Tween-20 and incubation with R-phycoerythrin labelled goat-anti-human IgG, antibody binding was measured on a FLEXMAP 3D running the xPONENT software v4.3. Normalization values were calculated by dividing the mean fluorescence intensity for each sample by those of plate-by-plate quality controls. Liquid handling was, in part, carried out on a Biomek i7.

Determination of assay specificities

The specificity of the two assays used in this study for SARS-CoV-2-specific IgG-type antibodies was measured in 888 (184 in case of the recomLine assay) pre-pandemic sera from healthy adult blood donors (see STAR Methods section 'patients and specimens'). With 1/184 false positive results, the recomLine assay had a specificity for anti-nucleocapsid antibodies against SARS-CoV-1 of 99.5% (95% CI – 97.0% to 99.9%, Table S1). While MultiCoV-Ab normally uses a dual full-length spike and RBD cut-off system to determine positivity (Becker et al., 2021), specificities and sensitivities for the detection of antibodies against nucleocapsid, full-length spike and spike S1 domain antigens were analyzed separately for the purposes of this study. The MultiCoV-Ab assay had false positive rates of 4/888 for nucleocapsid-specific, 18/888 for full-length spike-specific and 21/888 spike S1 domain-specific anti-SARS-CoV-2 antibodies translating into specificities of 99.6% (95% CI – 98.9% to 99.8%), 98.0% (95% CI – 96.8 to 98.7) and 97.6% (95% CI – 96.4 to 98.5%), respectively (Table S1). Out of the four false positive samples in the anti-SARS-CoV-2 nucleocapsid antibody component of the Multi-CoV-Ab assay, none was positive in the anti-SARS-CoV-2 full-length spike antibody component, and one was positive in the anti-SARS-CoV-2 spike S1 domain antibody component of the same assay.

Infections with seasonal coronaviruses are frequent (Killerby et al., 2018), especially in children (Masse et al., 2020). We were unable to establish a cohort of individuals that were verifiably never infected by one or more HCoVs and, thus, can be assumed to be negative for long-lasting IgG-type antibodies against these viruses. As a consequence, the specificity for the two assays used in this study to detect anti-HCoV antibodies could not be analyzed.

In sera from pre-pandemic adults analyzed for nucleocapsid-specific antibodies by the recomLine assay, 28.8% (53/184) were positive for antibodies targeting HCoV-229E, 45.1% (83/184) for antibodies targeting HCoV-NL63, 57.6% (106/184) for antibodies targeting HCoV-HKU1, and 53.8% (99/184) for antibodies against HCoV-OC43, respectively (Table S2). 59.2% (109/184) of tested pre-pandemic samples were positive for more than one anti-HCoV-antibody analyzed via the recomLine assay, and 15.8% (29/184) were positive for antibodies against all HCoVs. However, since the specificity of the two assays for detecting HCoV-specific antibodies could not be measured, we mainly focused on comparing rather quantitative antibody levels than qualitative assay results in this study.

Determination of assay sensitivities

Sensitivities of the recomLine and the MultiCoV-Ab assays in detecting anti-SARS-CoV-2 antibodies were calculated from mean antibody levels of every patient in the study cohort (see STAR Methods section 'patients and specimens' and Figure S1). With mean IgG-type anti-SARS-CoV-2 nucleocapsid antibody levels of 84/95 patients with COVID-19 being positive, the overall sensitivity of the recomLine assay was 88.4% (95% CI – 80.5% to 93.4%, Table S1). Similarly, the MultiCoV-Ab assay was positive in 83/95

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patients for nucleocapsid-specific and in 86/95 patients for full-length spike as well as spike S1 domain-specific anti-SARS-CoV-2 antibodies translating into sensitivities of 87.4% (95% CI – 79.2% to 92.6%) and 90.5% (95% CI – 83.0% to 94.9%), respectively (Table S1).

We were unable to establish a cohort of patients with acute, primary HCoV infection since infections with HCoVs are frequent (Killerby et al., 2018; Masse et al., 2020), the prevalence of long-lasting IgG-type antibodies is high (Severance et al., 2008), and re-infections are likely to occur (Edridge et al., 2020). Accordingly, we were unable to formally determine the sensitivity of the recomLine and MultiCoV-Ab assays for IgG-type anti-HCoV antibodies.

Assay correlation

We correlated quantitative results of all samples from pre-pandemic donors and patients with COVID-19 in both assays for HCoV-specific antibodies. Pearson correlations for different assays and assay components were similar comparing data from pre-pandemic donors and patients with COVID-19 (Figure S2). Results for anti-HCoV antibodies targeting the same antigen from HCoVs of the same taxonomic genus (α- or β-coronaviruses) correlated stronger than those targeting different antigens or HCoVs from different genera (Figure S2). This indicates that the specificity of the assays for similar antigens from HCoVs of the same genus is possibly decreased or that cross-reacting antibodies within the same genus are frequent, in line with data from other studies (Becker et al., 2021; Edridge et al., 2020).

QUANTIFICATION AND STATISTICAL ANALYSIS

Multivariate analysis was performed using logistic regression, with disease severity (critical or non-critical) as dependent and quantitative antibody levels, age, sex, comorbidities and IL-6 levels as independent variables (Data S1). As no additional health record data was available for pre-pandemic donors, pre-pandemic samples had to be excluded from the multivariate analysis.

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Supplemental information

Evidence for increased SARS-CoV-2 susceptibility and COVID-19 severity related to pre-existing immunity to seasonal coronaviruses

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Supplemental Information

Data S1. Multivariate analysis of patient data, Related to STAR Methods.

Multivariate analysis was performed using logistic regression, with disease severity (critical or non-critical) as dependent and quantitative antibody levels, age, sex, comorbidities and IL-6 levels as independent variables.

recomLine anti-nucleocapsid IgG HCoV 229E:

```
Call:
glm(formula = d$COVID.19_Severity_Binned ~ Assay_result + d$Age + d$Sex +
d$Comorbidities + d$IL6_Admission + d$IL6_Peak, family = binomial(link = "logit"))

Deviance Residuals:
Min             1Q              Median          3Q              Max
-2.4588         -0.2616         0.1724          0.4161          1.5913

Coefficients:
                    Estimate    Std. Error  z value     Pr(>|z|)
(Intercept)         7.992915    3.219701    2.483       0.01305
Assay_result        -0.249074   0.326058    -0.764      0.44493
d$Age               -0.044493   0.036190    -1.229      0.21891
d$MaleSex           -1.147000   1.182823    -0.970      0.33219
d$Comorbidities_yes -0.357018   0.987477    -0.362      0.71769
d$IL6_Admission     -0.026530   0.009783    -2.712      0.00669
d$IL6_Peak          -0.001389   0.000841    -1.652      0.09859

(Dispersion parameter for binomial family taken to be 1)
Null deviance: 88.239 on 64 degrees of freedom
Residual deviance: 39.517 on 58 degrees of freedom
(31 missing observations were deleted)
AIC: 53.517
Number of Fisher Scoring iterations: 8
```

recomLine anti-nucleocapsid IgG HCoV-NL63:

```
Call:
glm(formula = d$COVID.19_Severity_Binned ~ Assay_result + d$Age + d$Sex +
d$Comorbidities + d$IL6_Admission + d$IL6_Peak, family = binomial(link = "logit"))

Deviance Residuals:
Min             1Q              Median          3Q              Max
-2.6388         -0.2661         0.1447          0.4225          1.5927
```

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	8.6536867	3.4216635	2.529	0.01144
Assay_result	-0.4447192	0.3938486	-1.129	0.25883
d$Age	-0.0451992	0.0370132	-1.221	0.22202
d$Male_Sex	-1.0952441	1.1675533	-0.938	0.34821
d$Comorbidities_yes	-0.5435209	1.0172223	-0.534	0.59312
d$IL6_Admission	-0.0263973	0.0098655	-2.676	0.00746
d$IL6_Peak	-0.0014357	0.0008462	-1.697	0.08978

(Dispersion parameter for binomial family taken to be 1)
Null deviance: 88.239 on 64 degrees of freedom
Residual deviance: 38.750 on 58 degrees of freedom
(31 missing observations were deleted)
AIC: 52.75
Number of Fisher Scoring iterations: 8

recomLine anti-nucleocapsid IgG HCoV-HKU1:

Call:
glm(formula = d$COVID.19_Severity_Binned ~ Assay_result + d$Age + d$Sex +
d$Comorbidities + d$IL6_Admission + d$IL6_Peak, family = binomial(link = "logit"))

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.60482	-0.09684	0.20811	0.42963	1.59237

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	7.172919	3.187291	2.250	0.02442
Assay_result	0.641608	0.343148	1.870	0.06152
d$Age	-0.056921	0.038907	-1.463	0.14347
d$Male_Sex	-1.035782	1.101640	-0.940	0.34711
d$Comorbidities_yes	0.885900	1.206261	0.734	0.46269
d$IL6_Admission	-0.033363	0.012419	-2.686	0.00722
d$IL6_Peak	-0.002153	0.001079	-1.995	0.04606

(Dispersion parameter for binomial family taken to be 1)
Null deviance: 88.239 on 64 degrees of freedom
Residual deviance: 34.937 on 58 degrees of freedom
(31 missing observations were deleted)
AIC: 48.937
Number of Fisher Scoring iterations: 8

recomLine anti-nucleocapsid HCoV-OC43:

Call:
glm(formula = d$COVID.19_Severity_Binned ~ Assay_result + d$Age + d$Sex +
d$Comorbidities + d$IL6_Admission + d$IL6_Peak, family = binomial(link = "logit"))

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.61379	-0.04863	0.11609	0.31741	1.55619

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	7.436234	3.609519	2.060	0.03938
Assay_result	1.533964	0.699228	2.194	0.02825
d$Age	-0.058602	0.042265	-1.387	0.16559
d$Male_Sex	-1.320542	1.271993	-1.038	0.29919
d$Comorbidities_yes	0.395148	1.159875	0.341	0.73334
d$IL6_Admission	-0.035169	0.013320	-2.640	0.00828
d$IL6_Peak	-0.003033	0.001469	-2.065	0.03895

(Dispersion parameter for binomial family taken to be 1)
Null deviance: 88.239 on 64 degrees of freedom
Residual deviance: 30.877 on 58 degrees of freedom
(31 missing observations were deleted)
AIC: 44.877
Number of Fisher Scoring iterations: 9

recomLine anti-nucleocapsid IgG SARS-CoV-2:

Call:
glm(formula = d$COVID.19_Severity_Binned ~ Assay_result + d$Age + d$Sex +
d$Comorbidities + d$IL6_Admission + d$IL6_Peak, family = binomial(link = "logit"))

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.45567	-0.14122	0.08876	0.42943	1.69320

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	10.343689	4.076942	2.537	0.01118
Assay_result	-0.301504	0.204489	-1.474	0.14037
d$Age	-0.053748	0.039257	-1.369	0.17095
d$Male_Sex	-0.889153	1.161933	-0.765	0.44413
d$Comorbidities_yes	-0.420277	1.013340	-0.415	0.67833
d$IL6_Admission	-0.030195	0.010826	-2.78	0.00529
d$IL6_Peak	-0.002047	0.001024	-2.000	0.04551

(Dispersion parameter for binomial family taken to be 1)
Null deviance: 88.239 on 64 degrees of freedom
Residual deviance: 37.448 on 58 degrees of freedom
(31 missing observations were deleted)
AIC: 51.448
Number of Fisher Scoring iterations: 8

MultiCoV-Ab anti-nucleocapsid IgG HCoV-229E:

Call:
glm(formula = d$COVID.19_Severity_Binned ~ Assay_result + d$Age + d$Sex +
d$Comorbidities + d$IL6_Admission + d$IL6_Peak, family = binomial(link = "logit")):

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.6287	-0.2872	0.1164	0.3524	1.5888

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	8.926854	3.511526	2.542	0.0110
Assay_result	-0.575487	0.330974	-1.739	0.0821
d$Age	-0.041085	0.036927	-1.113	0.2659
d$Male_Sex	-1.014368	1.237669	-0.820	0.4125
d$Comorbidities_yes	-1.093028	1.136586	-0.962	0.3362
d$IL6_Admission	-0.024057	0.009793	-2.456	0.0140
d$IL6_Peak	-0.001713	0.000898	-1.908	0.0564

(Dispersion parameter for binomial family taken to be 1)
Null deviance: 88.239 on 64 degrees of freedom
Residual deviance: 36.953 on 58 degrees of freedom
(31 missing observations were deleted)
AIC: 50.953
Number of Fisher Scoring iterations: 8

MultiCoV-Ab anti-nucleocapsid IgG HCoV-NL63:

Call:
glm(formula = d$COVID.19_Severity_Binned ~ Assay_result + d$Age + d$Sex +
d$Comorbidities + d$IL6_Admission + d$IL6_Peak, family = binomial(link = "logit")):

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.7492	-0.2313	0.1186	0.4785	1.5253

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	9.6996495	3.7242773	2.604	0.00920
Assay_result	-0.9351666	0.5942018	-1.574	0.11553
d$Age	-0.0580449	0.0395097	-1.469	0.14180
d$Male_Sex	-0.6046866	1.2328601	-0.490	0.62380
d$Comorbidities_yes	-0.3038257	0.9829524	-0.309	0.75725
d$IL6_Admission	-0.0306021	0.0106505	-2.873	0.00406
d$IL6_Peak	-0.0014241	0.0008495	-1.676	0.09365

(Dispersion parameter for binomial family taken to be 1)
Null deviance: 88.239 on 64 degrees of freedom
Residual deviance: 37.127 on 58 degrees of freedom
(31 missing observations were deleted)
AIC: 51.127
Number of Fisher Scoring iterations: 8

MultiCoV-Ab anti-nucleocapsid IgG HCoV-HKU1:

Call:
glm(formula = d$COVID.19_Severity_Binned ~ Assay_result + d$Age + d$Sex +
d$Comorbidities + d$IL6_Admission + d$IL6_Peak, family = binomial(link = "logit")):

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.2749	-0.1596	0.2394	0.5255	1.7290

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	6.9370456	3.0883987	2.246	0.0247
Assay_result	0.7622557	0.5591984	1.363	0.1728
d$Age	-0.0565598	0.0384412	-1.471	0.1412
d$Male_Sex	-1.2874256	1.1227392	-1.147	0.2515
d$Comorbidities_yes	0.6534988	1.1457791	0.570	0.5684
d$IL6_Admission	-0.0305615	0.0110764	-2.759	0.0058
d$IL6_Peak	-0.0017036	0.0009291	-1.834	0.0667

(Dispersion parameter for binomial family taken to be 1)
Null deviance: 88.239 on 64 degrees of freedom
Residual deviance: 38.189 on 58 degrees of freedom
(31 missing observations were deleted)
AIC: 52.189
Number of Fisher Scoring iterations: 8

MultiCoV-Ab anti-nucleocapsid IgG HCoV-OC43:

Call:
glm(formula = d$COVID.19_Severity_Binned ~ Assay_result + d$Age + d$Sex +
d$Comorbidities + d$IL6_Admission + d$IL6_Peak, family = binomial(link = "logit")):

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.56707	-0.06025	0.10037	0.33643	1.32197

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	6.592636	3.390925	1.944	0.05187
Assay_result	3.130125	1.672732	1.871	0.06131
d$Age	-0.046352	0.039852	-1.163	0.24479
d$Male_Sex	-1.221146	1.166980	-1.046	0.29537
d$Comorbidities_yes	0.337086	1.220931	0.276	0.78248
d$IL6_Admission	-0.037365	0.013929	-2.683	0.00731
d$IL6_Peak	-0.002063	0.001205	-1.713	0.08680

(Dispersion parameter for binomial family taken to be 1)
Null deviance: 88.239 on 64 degrees of freedom
Residual deviance: 32.213 on 58 degrees of freedom
(31 missing observations were deleted)
AIC: 46.213
Number of Fisher Scoring iterations: 8

MultiCoV-Ab anti-nucleocapsid IgG SARS-CoV-2:

Call:
glm(formula = d$COVID.19_Severity_Binned ~ Assay_result + d$Age + d$Sex +
d$Comorbidities + d$IL6_Admission + d$IL6_Peak, family = binomial(link = "logit")):

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.2274	-0.2391	0.1377	0.4847	1.6397

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	8.3349848	3.3673715	2.475	0.01332
Assay_result	-0.0778296	0.1004437	-0.775	0.43842
d$Age	-0.0437103	0.0370584	-1.179	0.23820
d$Male_Sex	-1.1227285	1.1708042	-0.959	0.33759
d$Comorbidities_yes	-0.3647847	0.9942303	-0.367	0.71369
d$IL6_Admission	-0.0274779	0.0096858	-2.837	0.00456
d$IL6_Peak	-0.0016608	0.0009564	-1.736	0.08249

(Dispersion parameter for binomial family taken to be 1)
Null deviance: 88.239 on 64 degrees of freedom
Residual deviance: 39.597 on 58 degrees of freedom
(31 missing observations were deleted)
AIC: 53.597
Number of Fisher Scoring iterations: 8

MultiCoV-Ab anti-full-length spike IgG HCoV-NL63:

Call:
glm(formula = d$COVID.19_Severity_Binned ~ Assay_result + d$Age + d$Sex +
d$Comorbidities + d$IL6_Admission + d$IL6_Peak, family = binomial(link = "logit")):

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.2825	-0.2311	0.1572	0.4103	1.7489

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	9.0514863	3.6267732	2.496	0.01257
Assay_result	-0.4196069	0.3634773	-1.154	0.24833
d$Age	-0.0644189	0.0427527	-1.507	0.13187
d$Male_Sex	-0.7586666	1.1858050	-0.640	0.52231
d$Comorbidities_yes	0.3479846	1.0299132	0.338	0.73546
d$IL6_Admission	-0.0279588	0.0101403	-2.757	0.00583
d$IL6_Peak	-0.0013589	0.0008441	-1.610	0.10742

(Dispersion parameter for binomial family taken to be 1)
Null deviance: 88.239 on 64 degrees of freedom
Residual deviance: 37.896 on 58 degrees of freedom
(31 missing observations were deleted)
AIC: 51.896
Number of Fisher Scoring iterations: 8

MultiCoV-Ab anti-full-length spike HCoV-OC43:

Call:
glm(formula = d$COVID.19_Severity_Binned ~ Assay_result + d$Age + d$Sex +
d$Comorbidities + d$IL6_Admission + d$IL6_Peak, family = binomial(link = "logit")):

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.6636	-0.2901	0.1254	0.4366	1.5938

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	9.702358	3.863512	2.511	0.01203
Assay_result	-1.192470	1.123376	-1.062	0.28846
d$Age	-0.047806	0.036747	-1.301	0.19327
d$Male_Sex	-1.097940	1.155607	-0.950	0.34206
d$Comorbidities_yes	-0.128770	0.983232	-0.131	0.89580
d$IL6_Admission	-0.027887	0.009759	-2.858	0.00427
d$IL6_Peak	-0.001606	0.000889	-1.807	0.07082

(Dispersion parameter for binomial family taken to be 1)
Null deviance: 88.239 on 64 degrees of freedom
Residual deviance: 38.936 on 58 degrees of freedom
(31 missing observations were deleted)
AIC: 52.936

MultiCoV-Ab anti-full-length spike IgG SARS-CoV-2:

Call:
glm(formula = d$COVID.19_Severity_Binned ~ Assay_result + d$Age + d$Sex +
d$Comorbidities + d$IL6_Admission + d$IL6_Peak, family = binomial(link = "logit"))

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.42977	-0.10008	0.04344	0.40484	1.65760

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	12.884307	4.589526	2.807	0.00500
Assay_result	-0.256129	0.119014	-2.152	0.03139
d$Age	-0.046642	0.037084	-1.258	0.20849
d$Male_Sex	-1.773021	1.341044	-1.322	0.18613
d$Comorbidities_yes	-1.027937	1.115105	-0.922	0.35662
d$IL6_Admission	-0.033467	0.011241	-2.977	0.00291
d$IL6_Peak	-0.002719	0.001233	-2.205	0.02746

(Dispersion parameter for binomial family taken to be 1)
Null deviance: 88.239 on 64 degrees of freedom
Residual deviance: 33.620 on 58 degrees of freedom
(31 missing observations were deleted)
AIC: 47.62
Number of Fisher Scoring iterations: 8

MultiCoV-Ab anti-spike S1 domain IgG HCoV-229E

Call:
glm(formula = d$COVID.19_Severity_Binned ~ Assay_result + d$Age + d$Sex +
d$Comorbidities + d$IL6_Admission + d$IL6_Peak, family = binomial(link = "logit")):

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.0202	-0.1432	0.1426	0.3906	1.8640

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	7.6531043	3.5030183	2.185	0.02891
Assay_result	1.0707801	0.7878301	1.359	0.17410
d$Age	-0.0492439	0.0405033	-1.216	0.22406
d$Male_Sex	-1.4908385	1.2260208	-1.216	0.22399
d$Comorbidities_yes	-0.6268878	1.0855593	-0.577	0.56362
d$IL6_Admission	-0.0314618	0.0110862	-2.838	0.00454
d$IL6_Peak	-0.0013326	0.0008702	-1.531	0.12566

(Dispersion parameter for binomial family taken to be 1)
Null deviance: 88.239 on 64 degrees of freedom
Residual deviance: 37.660 on 58 degrees of freedom
(31 missing observations were deleted)
AIC: 51.66
Number of Fisher Scoring iterations: 8

MultiCoV-Ab anti-spike S1 domain IgG HCoV-NL63:

Call:
glm(formula = d$COVID.19_Severity_Binned ~ Assay_result + d$Age + d$Sex +
d$Comorbidities + d$IL6_Admission + d$IL6_Peak, family = binomial(link = "logit")):

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.1831	-0.1274	0.1628	0.4847	1.4713

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	6.7411356	3.1298060	2.154	0.03125
Assay_result	0.4320627	0.3516539	1.229	0.21920
d$Age	-0.0278579	0.0371272	-0.750	0.45305
d$Male_Sex	-1.6189670	1.2503970	-1.295	0.19540
d$Comorbidities_yes	-0.5539335	1.0679679	-0.519	0.60398
d$IL6_Admission	-0.0334206	0.0118973	-2.809	0.00497
d$IL6_Peak	-0.0012707	0.0008609	-1.476	0.13995

(Dispersion parameter for binomial family taken to be 1)
Null deviance: 88.239 on 64 degrees of freedom
Residual deviance: 38.582 on 58 degrees of freedom
(31 missing observations were deleted)
AIC: 52.582
Number of Fisher Scoring iterations: 8

MultiCoV-Ab anti-spike-S1 domain IgG HCoV-HKU1:

Call:
glm(formula = d$COVID.19_Severity_Binned ~ Assay_result + d$Age + d$Sex +
d$Comorbidities + d$IL6_Admission + d$IL6_Peak, family = binomial(link = "logit")):

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.30641	-0.05738	0.09455	0.42991	1.70781

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	8.501005	3.833462	2.218	0.02658
Assay_result	1.045286	0.486096	2.150	0.03153
d$Age	-0.057205	0.041239	-1.387	0.16539
d$Male_Sex	-1.212629	1.258575	-0.963	0.33530
d$Comorbidities_yes	-0.459557	1.076778	-0.427	0.66953
d$IL6_Admission	-0.041278	0.014487	-2.849	0.00438
d$IL6_Peak	-0.002291	0.001127	-2.034	0.04199

(Dispersion parameter for binomial family taken to be 1)
Null deviance: 88.239 on 64 degrees of freedom
Residual deviance: 33.908 on 58 degrees of freedom
(31 missing observations were deleted)
AIC: 47.908
Number of Fisher Scoring iterations: 9

MultiCoV-Ab anit-spike S1 domain IgG HCoV-OC43:

Call:
glm(formula = d$COVID.19_Severity_Binned ~ Assay_result + d$Age + d$Sex +
d$Comorbidities + d$IL6_Admission + d$IL6_Peak, family = binomial(link = "logit")):

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.2230	-0.1026	0.1608	0.4476	1.5951

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	5.8717060	3.2669150	1.797	0.07228
Assay_result	1.5307731	0.8992969	1.702	0.08872
d$Age	-0.0367974	0.0367676	-1.001	0.31692
d$Male_Sex	-1.0907544	1.1947670	-0.913	0.36127
d$Comorbidities_yes	-0.0447493	1.0295468	-0.043	0.96533
d$IL6_Admission	-0.0355695	0.0125756	-2.828	0.00468
d$IL6_Peak	-0.0010922	0.0008903	-1.227	0.21990

Dispersion parameter for binomial family taken to be 1)
Null deviance: 88.239 on 64 degrees of freedom
Residual deviance: 36.536 on 58 degrees of freedom
(31 missing observations were deleted)
AIC: 50.536
Number of Fisher Scoring iterations: 8

MultiCoV-Ab anti-spike S1 domain IgG SARS-CoV-2:

Call:
glm(formula = d$COVID.19_Severity_Binned ~ Assay_result + d$Age + d$Sex +
d$Comorbidities + d$IL6_Admission + d$IL6_Peak, family = binomial(link = "logit")):

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.91907	-0.22784	0.09311	0.38766	1.83059

Coefficients:

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	9.2486737	3.6603942	2.527	0.01151
Assay_result	-0.0149871	0.0079460	-1.886	0.05928
d$Age	-0.0371202	0.0404572	-0.918	0.35887
d$Male_Sex	-1.3322131	1.1847316	-1.124	0.26081
d$Comorbidities_yes	-0.9800549	1.1007894	-0.890	0.37329
d$IL6_Admission	-0.0302960	0.0105671	-2.867	0.00414
d$IL6_Peak	-0.0018264	0.0009189	-1.988	0.04685

(Dispersion parameter for binomial family taken to be 1)
Null deviance: 88.239 on 64 degrees of freedom
Residual deviance: 36.297 on 58 degrees of freedom
(31 missing observations were deleted)
AIC: 50.297
Number of Fisher Scoring iterations: 8

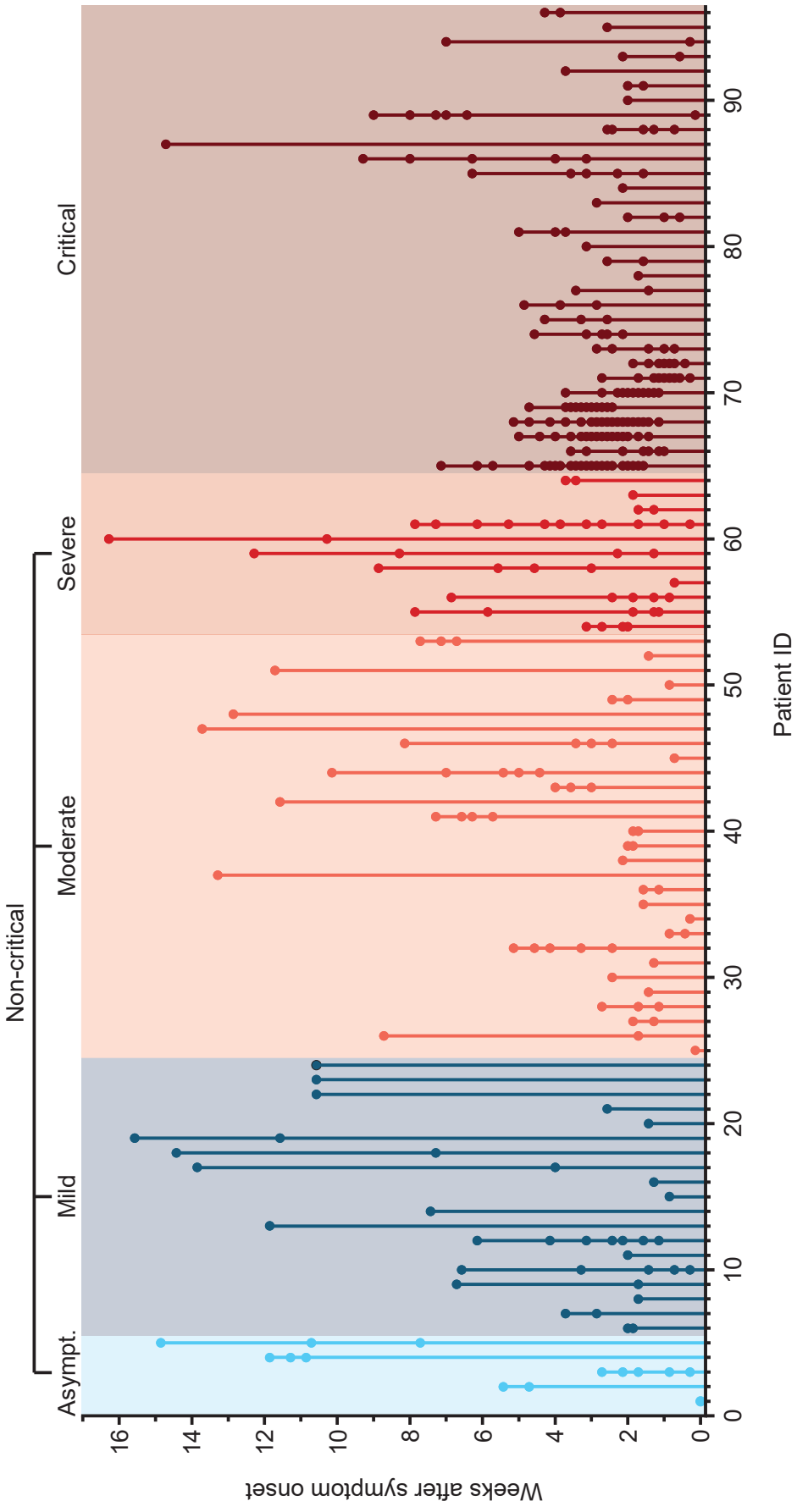
Non-critical
Asympt.
Mild
Moderate
Severe
Critical
Weeks after symptom onset
0
2
4
6
8
10
12
14
16
Patient ID
0
10
20
30
40
50
60
70
80
90

Figure S1

Figure S1. Sampling timetable for serum specimens from COVID-19 patients included in the study, STAR Methods.

314 serum samples from 96 COVID-19 patients were analyzed. Each colored line represents a patient (patient ID, x-axis) and colored dots along those lines represent serum samples obtained from each patient arranged by the time from symptom-onset to sampling (weeks after symptom onset, y-axis). Patients' disease severity was classified and is color-coded: asymptomatic – light blue, mild – dark blue, moderate – light red, severe – red, critical – dark red. If the time of symptom onset was not available, this information was substituted with the time of the first SARS-CoV-2-PCR-positive result. This was the case for 26.0 % (25/96) of patients.

Figure S2

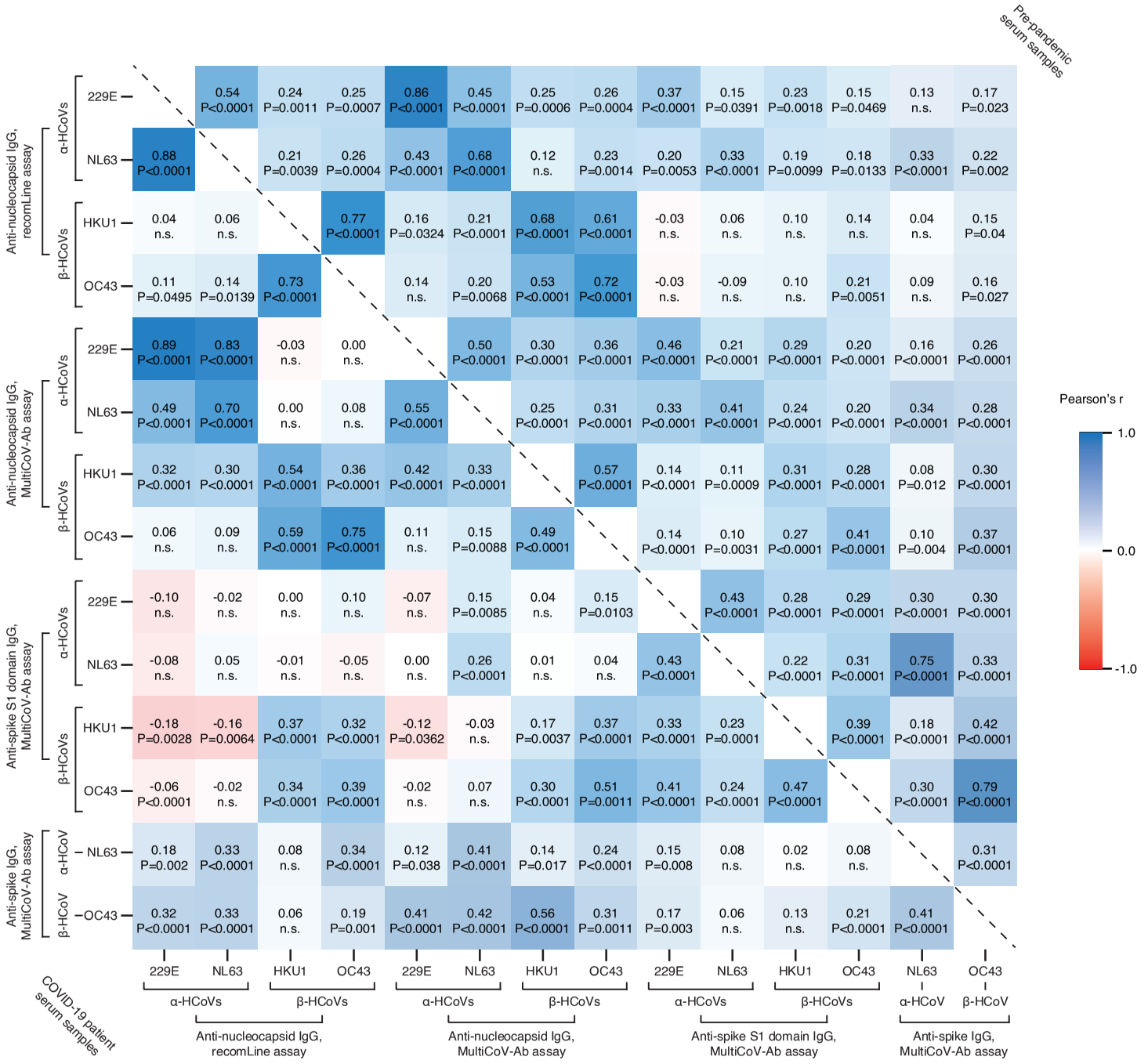
Pre-pandemic serum samples
COVID-19 patient serum samples
Anti-nucleocapsid IgG, recomLine assay
Anti-nucleocapsid IgG, MultiCoV-Ab assay
Anti-spike S1 domain IgG, MultiCoV-Ab assay
Anti-spike IgG, MultiCoV-Ab assay
α-HCoVs
β-HCoVs
229E
NL63
HKU1
OC43
Pearson's r
1.0
0.0
-1.0

Figure S2. Assay correlations for anti-HCoV antibody levels, Related to STAR Methods.

Pearson correlation coefficients (r) between quantitative assay results obtained from 888 pre-pandemic samples (184 in case of the recomLine assay) are depicted in the top right half of the correlation matrix as well as from 314 serum samples from 96 COVID-19 patients in the bottom left half and indicated by color from -1.0 (red) to 1.0 (blue). P-Values as indicators for statistical significance of the calculated Pearson correlations are shown. n.s. – not significant.

Figure S3

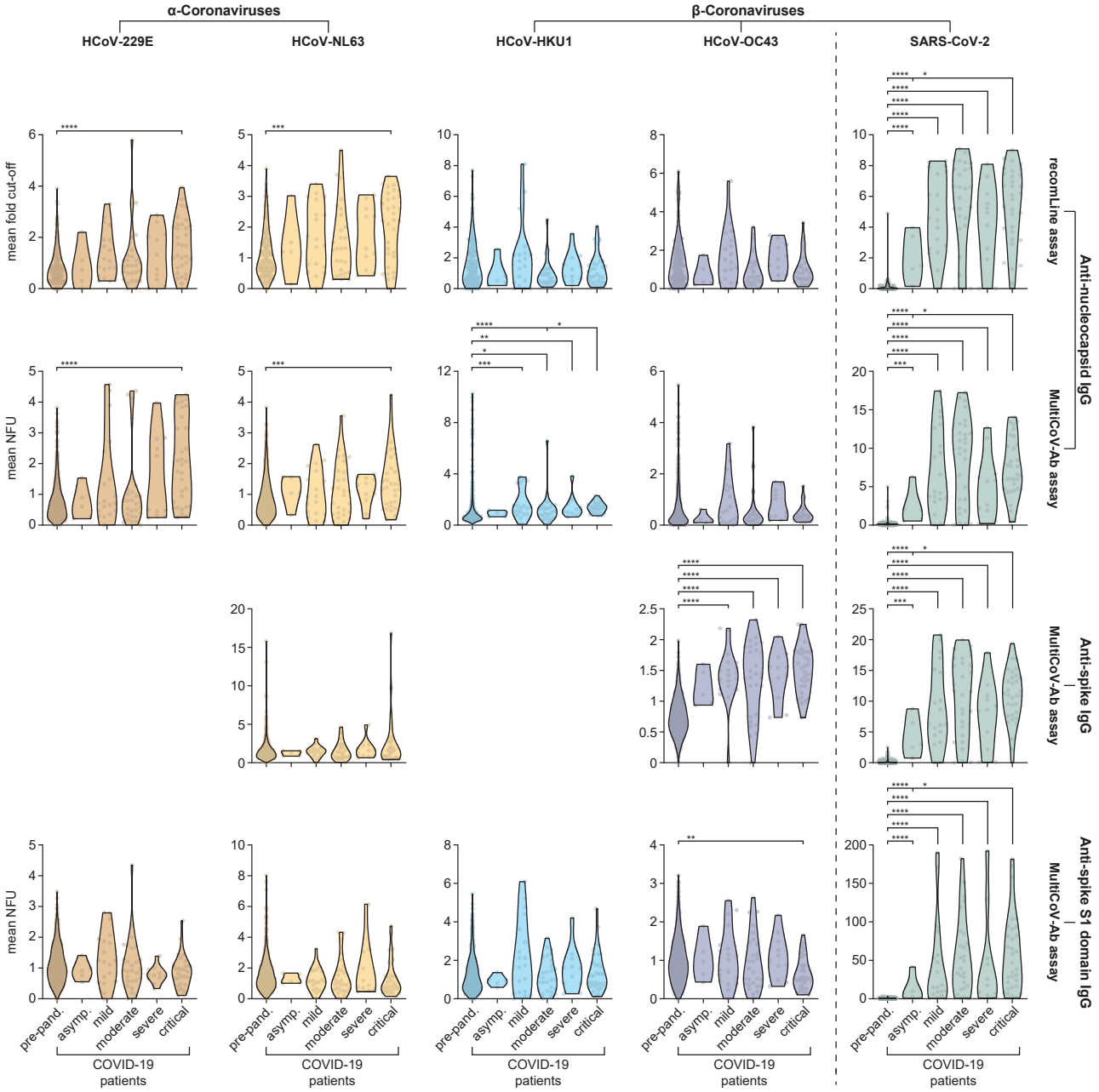
α-Coronaviruses
β-Coronaviruses
HCoV-229E
HCoV-NL63
HCoV-HKU1
HCoV-OC43
SARS-CoV-2
mean fold cut-off
mean NFU
recomLine assay
MultiCoV-Ab assay
Anti-nucleocapsid IgG
Anti-spike IgG
Anti-spike S1 domain IgG
pre-pand.
asymp.
mild
moderate
severe
critical
COVID-19 patients

Figure S3. Anti-nucleocapsid and anti-spike S1 domain antibody levels in sera from pre-pandemic donors and COVID-19 patients, Related to Figure 1.

888 pre-pandemic sera from healthy adult blood donors (184 in case of the recomLine assay), 153 samples from 32 critically ill COVID-19 patients (161 in case of the recomLine assay, critical), 40 samples from 11 severely ill COVID-19 patients (39 in case of the recomLine assay), 56 samples from 28 moderately ill COVID-19 patients (55 in case of the recomLine assay), 33 samples from 19 mildly affected COVID-19 patients (35 in case of the recom-Line assay), and 13 samples from 5 asymptomatic (14 in case of the recomLine assay) COVID-19 patients were analyzed for their antibody levels against HCoV-229E, -NL63, -HKU1, and -OC43, as well as SARS-CoV-2. Mean antibody levels per donor/patient (dots) are depicted as violin plots for every group. Differences in the assays' antibody responses comparing the groups were tested for their statistical significance via Kruskal-Wallis test and pairwise comparisons using Wilcoxon rank sum test with continuity correction. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, $^{****}P \leq 0.0001$. NFU – normalized fluorescence units.

Table S1. Assay specificity and sensitivity of anti-SARS-CoV-2 antibody detection assays in serum samples from healthy adult blood donors collected prior to December 2019 and COVID-19 patients. Related to STAR Methods.

Assay	Specificity in % (95% CI)	False Positive/ Total	Sensitivity in % (95% CI)	Positive/ Total
Anti-SARS-CoV-2 nucleocapsid, recomLine assay	99.46 (96.99 to 99.90)	1/184	88.42 (80.45 to 93.41)	84/95
Anti-SARS-CoV-2 nucleocapsid, MultiCoV-Ab assay	99.55 (98.85 to 99.82)	4/888	87.37 (79.21 to 92.62)	83/95
Anti-SARS-CoV-2 full-length spike, MultiCoV-Ab assay	97.97 (96.82 to 98.71)	18/888	90.53 (82.97 to 94.94)	86/95
Anti-SARS-CoV-2 spike S1 domain, MultiCoV-Ab assay	97.64 (96.41 to 98.45)	21/888	90.53 (82.97 to 94.94)	86/95

Binominal 95% confidence intervals (95% CI) were computed using the Wilson score interval. The mean of all samples from the same patient was used to calculate the sensitivity, if more than one sample from the same patient was available. When applying the dual spike and RBD cut-off system, the specificity and sensitivity of the MulitCoV-Ab assay were 100% (0 false positive /888 total) and 94.74% (90 positive /95 total), respectively.

Table S2. Positivity rates for anti-HCoV nucleocapsid antibodies in pre-pandemic serum samples and COVID-19 Patients. Related to Figure 1.

	HCoV-229E		HCoV-NL63		HCoV-OC43		HCoV-HKU1	
	% Pos	**95 % CI**	**% Pos**	**95 % CI**	**% Pos**	**95 % CI**	**% Pos**	**95 % CI**
Pre-pandemic								
Total	28.8	(22.8 to 35.7)	45.1	(38.1 to 52.3)	53.8	(46.6 to 60.9)	57.6	(50.4 to 64.5)
COVID-19								
Non-critical	47.6	(35.8 to 59.7)	63.5	(51.1 to 74.3)	50.8	(38.8 to 62.7)	44.4	(32.8 to 56.7)
Critical	71.9	(54.6 to 84.4)	78.1	(61.2 to 89.0)	37.5	(22.9 to 54.7)	46.9	(30.9 to 50.7)
Total	55.8	(45.8 to 65.4)	68.4	(58.5 to 76.9)	46.3	(36.6 to 56.3)	45.3	(35.6 to 55.3)
Pre-pand. vs.								
Non-critical COVID-19	$P = 0.0085$		$P = 0.0133$		n.s.		n.s.	
Critical COVID-19	$P < 0.0001$		$P = 0.0009$		n.s.		n.s.	
Total COVID-19	$P < 0.0001$		$P = 0.0002$		n.s.		n.s.	
Critical COVID-19 vs.								
Non-critical COVID-19	$P = 0.0298$		n.s.		n.s.		n.s.	

If there were several samples from the same donor with disconcordant qualitative results for a specific anti-hCoV nucleocapsid antibody, this donor counted as positive for evaluation if the mean of all semi-quantitative results was above the threshold for positivity. Data for statistical significance was computed using Fisher's Exact Test. Binominal 95% confidence intervals (95 % CI) were calculated via the Wilson score interval. n.s. – not significant; pos. – positive

ARTICLES
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Three exposures to the spike protein of SARS-CoV-2 by either infection or vaccination elicit superior neutralizing immunity to all variants of concern

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Infection-neutralizing antibody responses after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection or coronavirus disease 2019 vaccination are an essential component of antiviral immunity. Antibody-mediated protection is challenged by the emergence of SARS-CoV-2 variants of concern (VoCs) with immune escape properties, such as omicron (B.1.1.529), which is rapidly spreading worldwide. Here we report neutralizing antibody dynamics in a longitudinal cohort of coronavirus disease 2019 convalescent and infection-naive individuals vaccinated with mRNA BNT162b2 by quantifying SARS-CoV-2 spike protein antibodies and determining their avidity and neutralization capacity in serum. Using live-virus neutralization assays, we show that a superior infection-neutralizing capacity against all VoCs, including omicron, developed after either two vaccinations in convalescents or a third vaccination or breakthrough infection of twice-vaccinated, naive individuals. These three consecutive spike antigen exposures resulted in an increasing neutralization capacity per anti-spike antibody unit and were paralleled by stepwise increases in antibody avidity. We conclude that an infection-plus-vaccination-induced hybrid immunity or a triple immunization can induce high-quality antibodies with superior neutralization capacity against VoCs, including omicron.

The World Health Organization classified B.1.1.529 (Omicron) on 26 November 2021 as a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variant of concern (VoC). Omicron has since become the dominant VoC in most countries[1]. Earlier VoCs showed either an enhanced ability for transmission (VoCs Alpha (B.1.1.7) and Delta (B.1.617.2)) or a partial immune escape with variable effects on neutralization by polyclonal serum antibodies (VoCs Beta (B.1.351), Gamma (P.1/B.1.1.28)) and Delta)[2–7]. A striking characteristic of the VoC Omicron, which apparently developed independently, is the large number of amino acid substitutions, insertions and deletions in the viral spike protein—32 compared with the original Wuhan-hu-1 virus[8]—that likely contribute to its extraordinarily rapid spread in the population. The number of epitopes in the spike protein, which are relevant for neutralization and are targeted by polyclonal antibody responses in coronavirus disease 2019 (COVID-19) convalescent or vaccinated naive individuals, is an important determinant of the genetic barrier to viral escape from humoral immunity[6,9]. Thus, physician–scientists anticipated early on Omicron's potential for a pronounced immune escape.

Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection[10]. Affinity maturation of neutralizing antibodies can markedly alter their capacity to control SARS-CoV-2 variants[11]. In general, somatic hypermutations in variable regions of antibodies increase their binding affinity depending on type and duration of antigen exposure[6,12]. Affinity maturation can markedly expand the breadth and efficiency of neutralizing antibodies against SARS-CoV-2 infection[13]. This may

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even enable the neutralization of emerging virus variants that have evolved to escape neutralization by ancestral antibodies.

In this Article, we characterized the antibody response in a longitudinal cohort of 98 convalescent individuals, infected with SARS-CoV-2 during the first pandemic wave in spring 2020, and 73 infection-naive individuals matched for sex, age, working conditions and risk factors[14]. We quantified anti-spike IgG titers, IgG antibody avidity and infection-neutralizing capacity in serum samples from these two groups collected after the first, second and third vaccinations with the mRNA BNT162b2 COVID-19 vaccine. The aim of the study was to characterize the dynamics of infection neutralization against SARS-CoV-2 and its VoCs after different timely spaced infection events and vaccinations.

Results

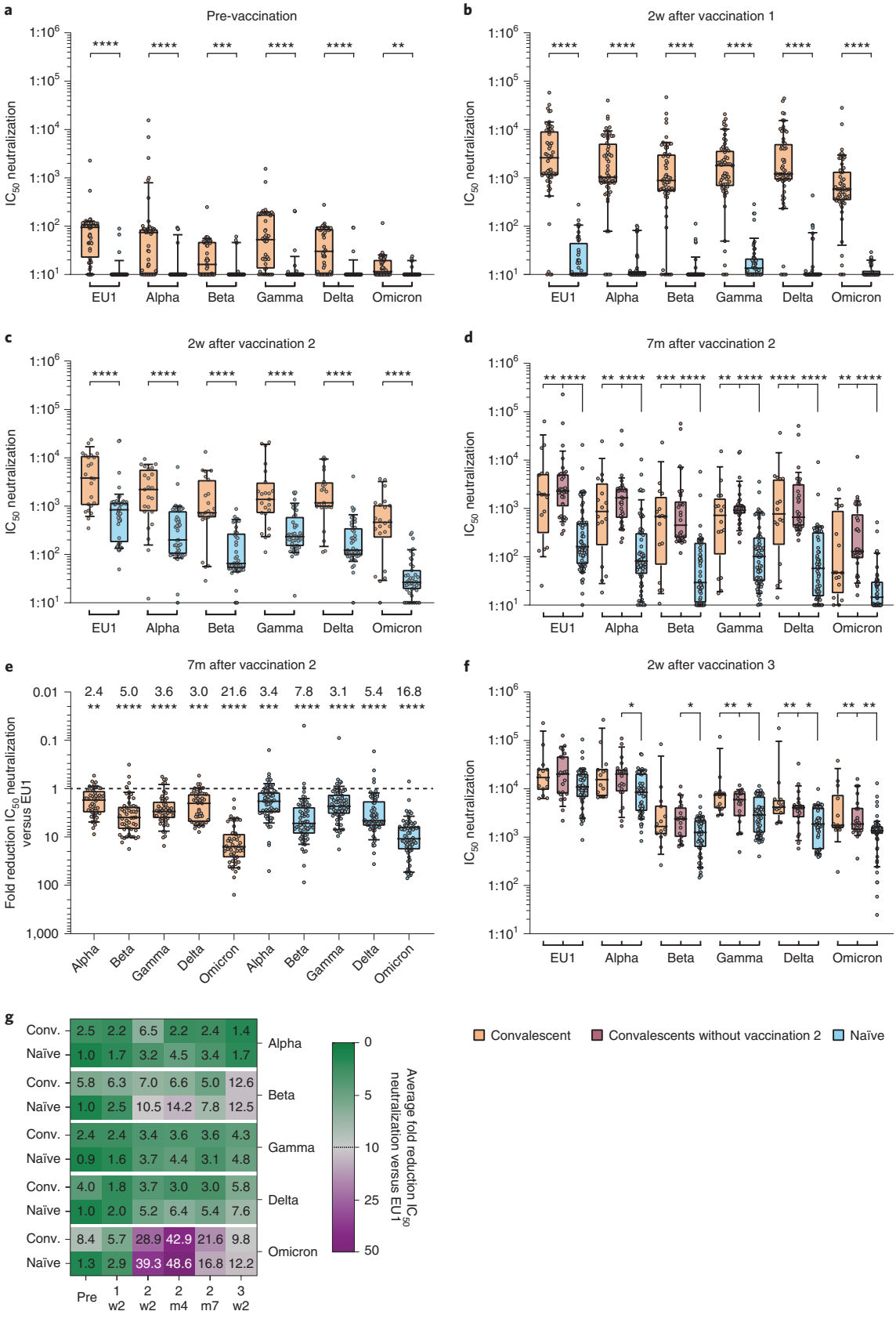
Convalescents develop a higher neutralization capacity against all SARS-CoV-2 variants of concern than naive individuals after vaccination. We established a cohort of 98 convalescents from mild COVID-19 (for details, see Supplementary Table 1 and Extended Data Fig. 1 and work by Koerber et al.[14]), of which 6 were excluded because of suspected SARS-CoV-2 re-exposure and 62 were followed up after vaccination. Then, 73 infection-naive individuals were randomly matched for age, sex and infection exposure risk. These individuals were continuously followed since the first wave of the COVID-19 pandemic in spring 2020, through their initial COVID-19 vaccinations with mRNA BNT162b2 in early 2021 and after a third vaccination during the last quarter of 2021, with a total of 486 serum samples collected. In this cohort, we determined the dynamics of SARS-CoV-2 spike protein antibodies and serum-neutralization capacity against the early clinical SARS-CoV-2 isolate B.1.177 (EU1) and all five VoCs: B.1.1.7 (Alpha), B.1.351 (Beta), P.1/B.1.1.28.1 (Gamma), B.1.617.2 (Delta), as well as B.1.1.529 (Omicron, sublineage BA.1; Extended Data Fig. 1). The first (1) and second (2) COVID-19 vaccination were given 3 weeks apart, and the third vaccination dose (3) was applied 9 months later.

To quantify infection neutralization, we used a novel, high-throughput live-virus neutralization assay comprising all known VoCs that were isolated from individuals with COVID-19. Hereby, immortalized human MDA-MB-231 cells expressing the angiotensin-converting enzyme 2 (hACE2) receptor (MDA-MB-231–hACE2 cells)[15,16], which are highly susceptible to SARS-CoV-2 infection and display a strong cytopathic response to infection, allowed for the rapid quantification of neutralizing activities against SARS-CoV-2. Sera from COVID-19 convalescents collected approximately 9 months after infection showed a low-level infection-neutralization capacity against the early 2020 SARS-CoV-2 variant EU1 and against all VoCs (Fig. 1a). After a first vaccination (1) with mRNA BNT162b2, serum-neutralization titers of convalescents showed a 63-fold increase on average, while titers in infection-naive vaccinees remained close to background (Fig. 1b). Neutralization titers in naive individuals markedly increased after vaccination 2, still remaining significantly lower than those of convalescents (Fig. 1c). Interestingly, even at 4 and 7 months after vaccination 2, no significant difference in neutralization capacity was detected comparing convalescents vaccinated once or twice within a 3-week interval (Fig. 1d and Extended Data Fig. 2). Although in naive individuals the infection-neutralization capacity after vaccination 2 was significantly lower than that of vaccinated convalescents (Fig. 1c,d and Extended Data Fig. 2), the relative ability of individual VoCs to escape neutralization relative to EU1 at 7 months after vaccination 2 was similar for convalescent and naive individuals (Fig. 1e and Extended Data Fig. 3). Overall, the infection-neutralization capacity for Omicron and, albeit less pronounced, for Beta was lower than for the other SARS-CoV-2 variants confirming the immune escape properties of these two VoCs (Fig. 1a–e and Extended Data Figs. 2 and 3). Around 40.6% (95% confidence interval: 29.4–52.9%) of naive individuals, but only 4.0% (95% confidence interval: 1.1–13.5%) of convalescents showed no neutralization activity against Omicron 7 months after the initial vaccinations.

Strikingly, after COVID-19 vaccination 3, administered 9 months after vaccinations 1 and 2, the infection-neutralization capacity against all VoCs, including Omicron, reached high levels in both naive and convalescent individuals (Fig. 1f). Again, infection-neutralization capacity remained higher in vaccinated convalescents, and there was no difference whether convalescents had received one or two vaccine doses (Fig. 1f). Figure 1g summarizes neutralization of VoCs compared to that of EU1, highlighting both the prominent immune escape properties of Omicron and the impact of a third vaccination in naive individuals that was able to partially counteract this pathogen's evolution.

Overall, COVID-19 convalescents showed a higher neutralization capacity against all SARS-CoV-2 VoCs compared to

Fig. 1 | Kinetics and comparison of infection-neutralization activities for SARS-CoV-2 variants of concern in naive individuals and convalescents after BNT162b2 vaccination. a–**g**, COVID-19 convalescents (orange), convalescents who received only vaccinations 1 and 3 (red) and naive individuals (blue) at indicated time points before and after BNT162b2 vaccination. **a**–**d**,**f**, Serum IC_{50} values for infection-neutralization capacity of SARS-CoV-2 strain EU1 and VoCs Alpha, Beta, Gamma, Delta and Omicron normalized to 10^7 viral RNA copies shown as boxplots with median, bounds between upper and lower quartiles and whiskers between the 10th and 90th percentiles. Numbers of serum samples analyzed are indicated below, with those against Omicron in parentheses. **a**, 51 (50) SARS-CoV-2 convalescents at approximately 9 months after infection and 34 (29) SARS-CoV-2 naive individuals before vaccination (pre), naive individuals versus convalescents for Omicron $^{**}P = 0.0033$, Beta $^{***}P = 0.0002$, all other VoCs $^{****}P < 0.0001$, all other VoCs $^{****}P < 0.0001$. **b**, 59 (56) convalescents and 48 (42) naive individuals at 2 weeks after vaccination 1 (w2), $^{****}P < 0.0001$. **c**, 23 (22) convalescents and 47 (42) naive individuals at 2 weeks after vaccination 2, $^{****}P < 0.0001$. **d**, 16 (16) convalescents and 65 (64) naive individuals at 7 months (m7) after vaccination 2 and 34 (34) convalescents having received only vaccination 1, naive individuals versus twice-vaccinated convalescents for all variants $^{****}P < 0.0001$, and versus once-vaccinated convalescents for EU1 $^{**}P = 0.0011$, Alpha $^{**}P = 0.0054$, Beta $^{***}P = 0.0004$, Gamma$^{**}P = 0.0031$, Delta $^{****}P < 0.0001$ and Omicron $^{**}P = 0.0034$. **e**, Fold reduction of IC_{50} values comparing neutralization of EU1 with that of VoCs depicted as boxplots with median, bounds between the upper and lower quartiles, and whiskers between the 10th and 90th percentiles in 50 convalescents and 64 naive individuals (blue) at m7; numbers above boxes indicate average (avg.) fold changes comparing EU1 and VoCs; in convalescents comparing EU1 to Alpha $^{**}P = 0.0017$, Delta $^{***}P = 0.0005$ and all other VoCs $^{****}P < 0.0001$, and in naive individuals comparing EU1 and Alpha$^{***}P = 0.0002$ and all other VoCs $^{****}P < 0.0001$. **f**, 14 convalescents and 59 naive individuals at 2 weeks after vaccination 3, and 22 convalescents who received only vaccination 1 and 3; naive individuals versus twice-vaccinated convalescents for Gamma $^{**}P = 0.0064$, Delta $^{**}P = 0.0025$ and Omicron $^{**}P = 0.0069$, and versus three-times-vaccinated convalescents for Alpha $^{*}P = 0.0307$, Beta $^{*}P = 0.0155$, Gamma $^{*}P = 0.0342$, Delta $^{*}P = 0.0115$ and Omicron $^{**}P = 0.0089$. **g**, Heat map illustrating average fold reduction of IC_{50} values for VoCs compared to IC_{50} values for EU1 in convalescent (conv.) and naive participants. Connecting lines indicate statistically significant differences between groups. Absence of connecting lines or asterisks indicates absence of significance. Statistics were calculated using Mann–Whitney *U* test (**a**–**c**), Kruskal–Wallis test with Dunn's multiple-testing correction (**d**,**f**) and two-sided Friedman test with Dunn's multiple-testing correction (**e**). Pre, before first vaccination; 1, first vaccination; 2, second vaccination; 3, third vaccination; w2, 2 weeks after respective vaccination; m4, 4 months after vaccination 2.


a
Pre-vaccination
b
2w after vaccination 1
c
2w after vaccination 2
d
7m after vaccination 2
e
7m after vaccination 2
f
2w after vaccination 3
IC50 neutralization
Fold reduction IC50 neutralization versus EU1
EU1
Alpha
Beta
Gamma
Delta
Omicron
g
Conv.
Naïve
Pre
1 w2
2 w2
2 m4
2 m7
3 w2
Average fold reduction IC50 neutralization versus EU1
Convalescent
Convalescents without vaccination 2
Naïve

infection-naive individuals, even after three vaccinations in the latter. The Omicron VoC is characterized by an unprecedented escape from antibody neutralization in serum samples from convalescents and naive individuals at all time points of this study.

Increased infection-neutralization capacity is associated with higher antibody avidity. The higher neutralization capacity of convalescents in light of the immune escape properties of the Omicron VoC prompted us to investigate the longitudinal dynamics of infection-neutralization capacity and compare these to binding antibody titers against the S1 domain and polyclonal antibody-binding strength to the S1 and S2 ectodomains of the spike protein of the original Wuhan SARS-CoV-2 strain. Serum anti-spike IgG levels reached their maximum in convalescents after one vaccine dose, and in naive individuals after two vaccinations (Fig. 2a). Subsequently, IgG levels declined in both groups at 4 months and even more so at 7 months after vaccination 2, albeit more rapidly in naive individuals (Fig. 2a). After vaccination 3, serum anti-spike IgG levels increased significantly compared with 7 months after the initial vaccinations, on average by a factor of 2.7 and 9.6 for vaccinated convalescent and naive individuals, respectively (Fig. 2a).

The marked decline in serum anti-spike IgG levels in both study groups following vaccination 2 (Fig. 2a) was contrasted by a substantial infection-neutralization capacity of convalescents against all VoCs (Fig. 1d). This lack of an association between antibody titers and infection-neutralization capacity led us to reanalyze the data from our cohort for the dynamics of neutralization activity against the different VoCs over time (Extended Data Fig. 4). We found that neutralization capacity in infection-naive individuals, which was particularly low against Omicron, significantly increased after vaccination 3 (Fig. 2b,c). In convalescent individuals, vaccination 3 further increased their capacity to neutralize EU1 as well as Alpha, Gamma and Omicron, whereas the increase was less pronounced for Beta or Delta VoCs (Fig. 2b,c and Extended Data Figs. 4 and 5). Specifically, the neutralization capacity against Delta, reflected by the 50% inhibitory concentration (IC_{50}) value, showed an 8.1-fold increase in naive individuals, but only a 4.6-fold increase in convalescents (Fig. 2d). Against Omicron, a >42-fold increase in naive individuals and a >14-fold increase in convalescents, respectively, were observed (Fig. 2e), indicating the particular relevance of a third vaccination to be able to neutralize this VoC.

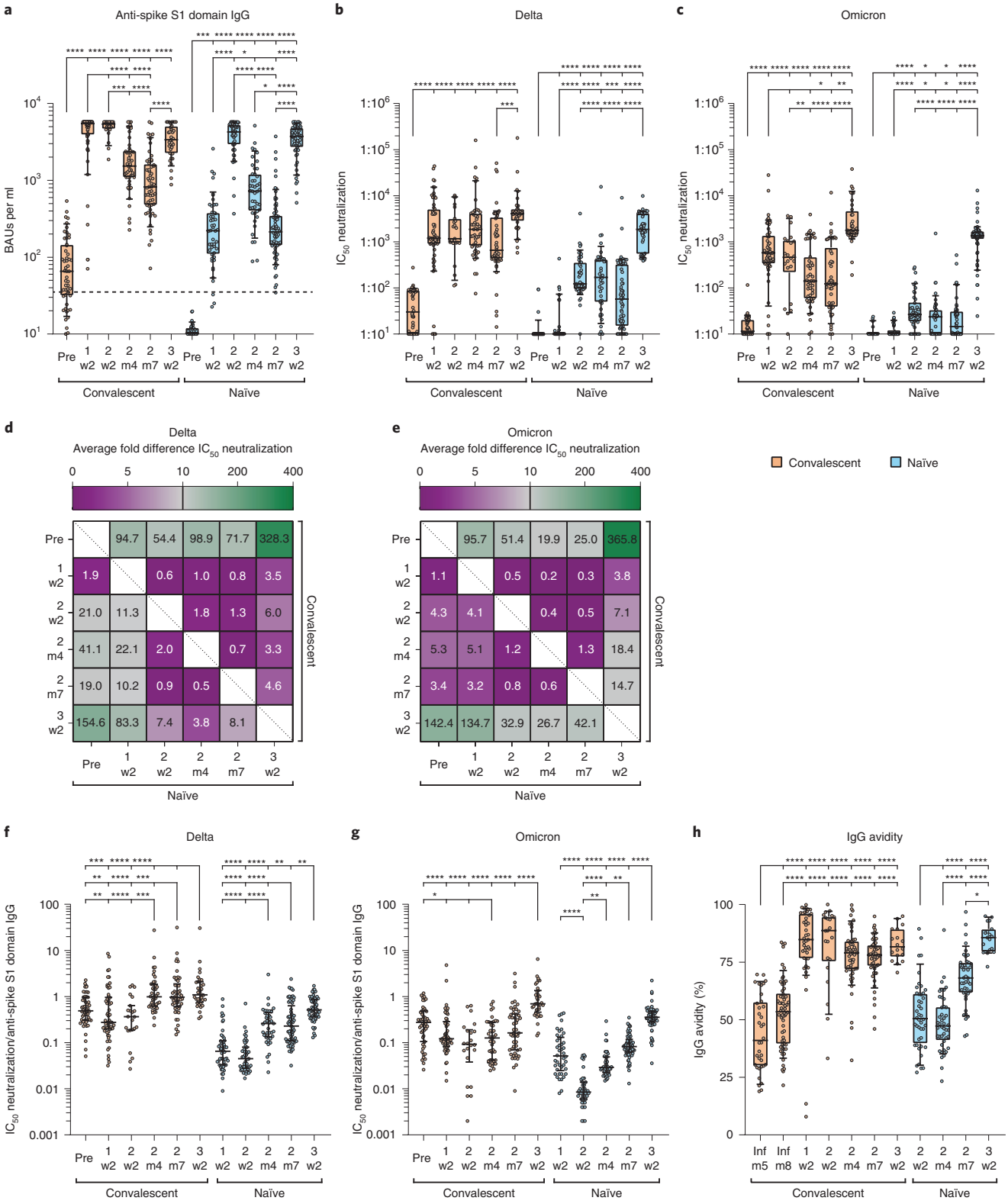
To better assess the relative efficacy of serum antibodies for virus neutralization, we determined the ratio between the IC_{50} neutralization and anti-spike IgG titers. Notably, we observed a high neutralization capacity per antibody unit in sera of convalescents against EU1 and all VoCs, including Omicron, that slightly increased after vaccination 2 and became more pronounced after vaccination 3 (Fig. 2f,g and Extended Data Fig. 6). For naive individuals, in contrast, this ratio was low after vaccinations 1 and 2, increased over time (m4 and m7), and further after vaccination 3, reaching levels comparable to those seen in convalescents (Fig. 2f,g and Extended Data Fig. 6).

Collectively, these results suggest a maturation of antibody responses over time and after each encounter with the SARS-CoV-2 spike protein. Conceptually, this could be due to either an increased breadth of the polyclonal neutralizing antibody repertoire directed against the spike protein or an increase of their strength of binding to the spike protein. To experimentally address the latter, we quantified the avidity of serum IgG binding to the S1/S2 SARS-CoV-2 spike protein ectodomain of the original Wuhan-hu-1 SARS-CoV-2 strain. In convalescent individuals, we detected a step increase in antibody avidity after a single vaccine dose, which remained largely stable over the following 7 months and did not further increase after vaccination 3 (Fig. 2h). This is consistent with a maturation of spike-specific antibodies that have been reported after SARS-CoV-2 infection[17,18] and which required only a single vaccination to reach maximal avidity. Hereby, the long time period of 9 months after infection may have supported a matured antibody response. In naive individuals, however, spike protein-specific antibody avidity only increased 7 months after vaccination 2, and vaccination 3 was required to increase the avidity to levels comparable to those in vaccinated convalescents (Fig. 2f). Taken together, these results suggest that an increase in antibody avidity may be critical for a highly potent infection-neutralization capacity, and provide mechanistic insight into the exceptional benefit of a third vaccination in infection-naive individuals or two timely spaced vaccinations in convalescents to counteract VoCs with immune escape potential such as Omicron.

Delta and Omicron breakthrough infections in twice-vaccinated, naive individuals boost neutralizing responses comparably to a third vaccination. To explore the applicability of the findings in our longitudinal cohort of the high immune-protective benefit of three separate exposures to SARS-CoV-2 spike antigen—either from vaccination alone or from infection and vaccination—in a real-world scenario, we investigated a second cohort of 31 individuals with 16 Delta and 15 Omicron breakthrough infections. Of these, 30 individuals had received two vaccine doses and one person had been

Fig. 2 | Longitudinal analysis of serum antibody titers, infection neutralization of Delta and Omicron variants of concern and antibody avidity following mRNA BNT162b2 vaccination. a, Anti-spike S1 domain IgG titers in 274 sera from 62 convalescents, and 304 sera from 73 naive participants as binding arbitrary units (BAUs) per ml, convalescent $^{***}P=0.0004$, naive pre-vaccination (pre) versus w2 after vaccination (vacc.) 1 $^{***}P=0.0002$, w2 after vacc. 1 versus m4 after vacc. 2 $^{*}P=0.0181$, m4 after vacc. 2 versus w2 after vacc. 3 $^{*}P=0.0123$, convalescent m7 after vacc. 2 versus w2 vacc. 3 $^{***}P=0.0005$, naive w2 after vacc. 1 versus m7 vacc. 2 $^{***}P=0.0003$. **b**,**c**, Serum IC_{50} values for infection-neutralization capacity normalized to 10^7 viral RNA copies of SARS-CoV-2 VoCs Delta in 266 and 296 sera (**b**) and Omicron in 261 and 279 sera (**c**) from 62 convalescents and 73 naive individuals, respectively; convalescent w2 vacc. 1 versus m7 vacc. 2 $^{*}P=0.0357$, and versus w2 vacc. 3 $^{**}P=0.0043$, w2 vacc. 2 versus m4 vacc. 2 $^{**}P=0.0049$, naive pre versus m4 vacc. 2 $^{*}P=0.0197$, and versus m7 vacc. 2 $^{*}P=0.0376$, w2 vacc. 1 versus m4 vacc. 2 $^{*}P=0.0236$, and versus m7 vacc. 2 $^{*}P=0.0043$. **d**,**e**, Heat maps showing average fold changes in IC_{50} values for Delta (**d**) and Omicron (**e**) between the respective time points for convalescent and naive individuals. **f**,**g**, Ratios between infection-neutralization IC_{50} values and anti-spike S1 domain antibody titers for Delta in 263 and 295 sera; convalescent pre versus m4 vacc. 2 $^{**}P=0.0030$, versus m7 vacc. 2 $^{**}P=0.0052$, and versus w2 vacc. 3 $^{***}P=0.0005$, w2 vacc. 2 versus m7 vacc. 2 $^{***}P=0.0003$, and versus m7 vacc. 2 $^{***}P=0.0005$, naive w2 vacc. 1 versus m7 vacc. 2 $^{**}P=0.0027$, and versus w2 vacc. 3 $^{**}P=0.0032$ (**f**); and for Omicron in 258 and 278 sera from 62 convalescents and 73 naive individuals; convalescent pre versus m4 vacc. 2 $^{*}P=0.0340$, naive w2 vacc. 2 versus m4 vacc. 2 $^{**}P=0.0077$, and versus m7 vacc. 2 $^{**}P=0.0011$ (**g**). **h**, IgG-type anti-spike avidity in 288 sera from 90 convalescents, and 150 sera from 47 naive individuals, convalescent pre versus m4 vacc. 2 $^{*}P=0.0340$, naive w2 vacc. 2 versus m4 vacc. 2 $^{**}P=0.0077$, and versus m7 vacc. 2 $^{**}P=0.0011$. **a**–**c**,**h**, Boxplots with median, bounds between upper and lower quartiles, and whiskers between the 10th and 90th percentiles, SARS-CoV-2 convalescents (orange) and naive participants (blue). **a**–**c**,**f**–**h**, Medians are indicated by lines and interquartile ranges (IQRs) by the error bars. Differences between time points analyzed for statistical significance using the Kruskal–Wallis test with Dunn's multiple-testing correction; $^{****}P<0.0001$. Connecting lines indicate statistically significant differences between groups. Absence of connecting lines or asterisks indicates absence of significance. Inf, after infection; m4, 4 months after vaccination; m5, 5 months after infection; m7, 7 months after vaccination; m8, 8 months after infection.

vaccinated with a single dose of Ad26.COV2.S, on average 5 months earlier (Supplementary Table 2). In this second cohort, we determined infection-neutralization titers on average 7 d after PCR-based diagnosis of a breakthrough infection. Remarkably, neutralization titers were significantly higher among these 31 individuals than among twice-vaccinated naive study participants of the first cohort and comparable to those detected in twice-vaccinated convalescent and triple-vaccinated naive individuals of the first cohort 2 weeks after the last vaccination (Fig. 3a). We did not detect significant differences in the infection-neutralization capacity against the



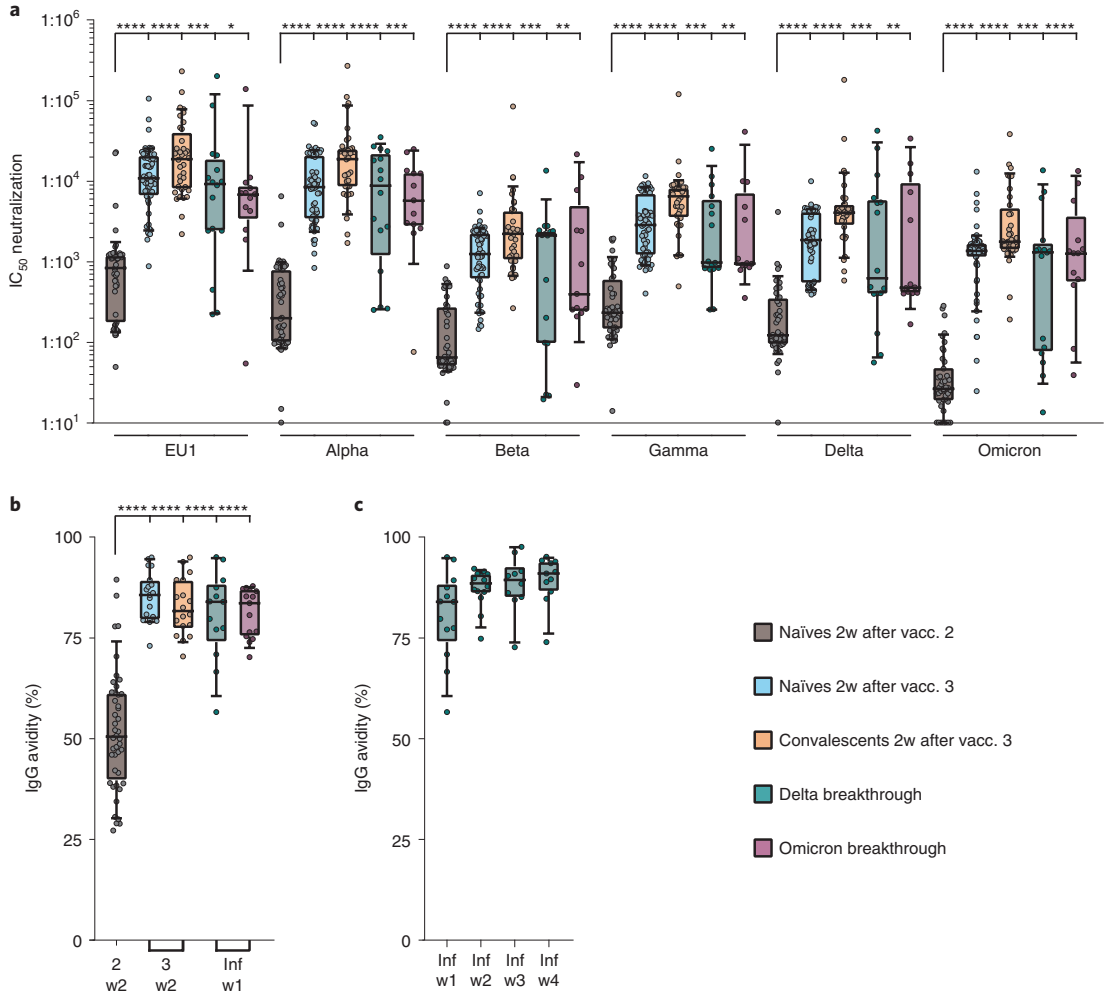


Fig. 3 | Infection-neutralization capacity for SARS-CoV-2 variants of concern after breakthrough infection with Delta and Omicron in vaccinated individuals. **a**, Serum IC_{50} values for infection-neutralization capacity normalized to 10^7 viral RNA copies of SARS-CoV-2 VoCs in 47 naive individuals (42 for Omicron) 2 weeks after vacc. 2 (dark blue), 59 naive individuals (light blue) and 36 convalescents 2 weeks after vacc. 3, as well as 16 and 15 vaccinated individu*als on average 7 d after PCR-confirmed breakthrough infections with Delta (green) and Omicron (purple), respectively; naive individuals 2 weeks after vacc. 2 versus naive individuals and convalescents 2 weeks after vacc. 3 ****$P<0.0001$ for all variants, versus Delta breakthrough infection for EU1 ***$P=0.0007$, Alpha ****$P<0.0001$, Beta ***$P=0.0010$, Gamma ***$P=0.0007$, Delta ***$P=0.0006$ and Omicron ***$P=0.0002$, and versus Omicron breakthrough for EU1 *$P=0.0251$, Alpha ***$P=0.0003$, Beta **$P=0.0024$, Gamma **$P=0.0016$, Delta **$P=0.0022$ and Omicron ****$P<0.0001$. **b**, IgG-type anti-spike antibody avidities in 44 naive participants 2 weeks after vacc. 2 (dark brown), 19 naive (light blue) and 18 convalescent participants 2 weeks after vaccination 3, as well as 13 and 13 vaccinated individuals on average 7 d after PCR-confirmed breakthrough infections with Delta (green) and Omicron (purple), respectively; ****$P<0.0001$. **c**, IgG-type anti-spike antibody avidity in vaccinated individuals on average 7 d ($n=13$), 2 weeks ($n=14$), 3 weeks ($n=10$) and 4 weeks ($n=11$) after PCR-confirmed breakthrough infections with Delta. Data are shown as boxplots with median, bounds between upper and lower quartiles, and whiskers between the 10th and 90th percentiles. Differences between groups were analyzed for their statistical significance using the Kruskal–Wallis test with Dunn's multiple-testing correction. Connecting lines indicate statistically significant differences between groups. Absence of connecting lines or asterisks indicates absence of significance. w1, 7 d after infection; w2, 2 weeks after respective vaccination/infection; w3, 3 weeks after infection; w4, 4 weeks after infection.

different VoCs, including Omicron, between individuals with either Delta or Omicron breakthrough infections (Fig. 3a). Although not statistically significant, individuals 7 d after Delta breakthrough infection seemed to neutralize the Omicron VoC less well. Findings were similar when analyzing only individuals of the second cohort vaccinated twice with mRNA BNT162b2 (Extended Data Fig. 7). This observation corresponded well to the increased antibody avidity to the Wuhan-hu-1 spike protein after a Delta or Omicron breakthrough infection (Fig. 3b). Interestingly, we detected increasing antibody avidity in single individuals over time in a longitudinal analysis following Delta breakthrough infection (Fig. 3c) that did, however, not reach statistical significance.

Together, the results obtained in this independent cohort of vaccinated individuals with newly diagnosed SARS-CoV-2 breakthrough infections corroborated the findings from the longitudinal analysis in the first cohort; both for vaccinated naive individuals and for convalescent individuals, a total of three timely spaced challenges of the immune system with SARS-CoV-2 spike protein, irrespective of the type of exposure, led to superior infection-neutralization capacity.

Discussion

Using a rapid and sensitive high-throughput infection-neutralization assay with replication-competent, clinical isolates of all known SARS-CoV-2 VoCs, we quantified and compared the serum-neutralization

capacity in a longitudinal cohort of COVID-19 convalescents and matched infection-naive individuals before and after vaccination. This allowed us to determine the distinct dynamics of infection-neutralization capacity associated with the type and order of antigen exposure in the form of vaccination or infection. Comparison to a second cohort of vaccinated individuals with recent Delta and Omicron breakthrough infections identified three timely spaced encounters with SARS-CoV-2 spike protein as the common determinant to reach a superior neutralization capacity against all SARS-CoV-2 VoCs, including the emergent Omicron VoC that shows the ability to escape immunity.

We here report four key findings: First, in a direct comparison with all other VoCs, Omicron displays the most pronounced humoral immune escape evading antibody neutralization at early and late time points after vaccination. Second, a 'hybrid immunity' in convalescents after one mRNA vaccination is not further enhanced by a second vaccination after a short time frame of 3 weeks. In contrast, a timely spaced, second vaccination after several months further increases neutralization capacity to combat VoCs such as Omicron with an unprecedented ability of immune escape. Third, in a longitudinal analysis, there is no direct association between anti-spike IgG titers and the infection-neutralization capacity. A stepwise increase in the avidity of SARS-CoV-2 spike-specific antibodies after the first vaccination in convalescents and after the second and third vaccination in naive individuals was noted, consistent with the reported occurrence of affinity-matured memory B cells up to 6 months after infection[19], highlighting that the quality rather than the mere quantity of antibodies is important. Fourth, triple-vaccinated naive individuals reach almost the same level of neutralization capacity against the immune escape VoC Omicron as vaccinated convalescents, as well as individuals who experienced a breakthrough infection with either the Delta or the Omicron VoC. Thus, the more rapid induction of high-avidity antibodies in convalescents after vaccination can be compensated for by three mRNA vaccinations in infection-naive individuals, and also develops after a breakthrough infection in twice-vaccinated individuals.

'Hybrid immunity' was achieved either after two mRNA vaccinations in convalescents (first cohort) or after a SARS-CoV-2 breakthrough infection in naive individuals, who had received a two-dose COVID-19 vaccination regimen (second cohort), both resulting in superior infection-neutralizing immune responses against SARS-CoV-2 VoCs including Omicron. Of note, a robust neutralization response in convalescents was seen already after a single vaccine dose, and a second shot only increased the response if given with a delay. An alternative path toward a comparably high neutralizing immunity is reported here for individuals who were triple vaccinated with BNT162b2, consistent with similar observations by others[20–25].

From our data, we conclude that a superior infection-neutralization capacity against SARS-CoV-2 VoCs, including those with immune escape properties, needs to develop over time following a total of three spike antigen exposures. Our results support the notion that a single infection with SARS-CoV-2 does not provide a similar level of protection as the combination of infection and vaccination. Importantly, the dynamics by which the infection-neutralization capacity increased were paralleled by an enhanced avidity of SARS-CoV-2 spike-binding antibodies providing a critical refinement for predicting the efficacy of protective humoral responses against a range of different VoCs.

Further studies will be required to analyze the breadth of the spike-specific antibody repertoire after repeated vaccinations in naive and convalescent individuals, and to characterize the avidity of spike-specific antibodies generated after infection or vaccination specifically to current and future VoCs. While a superior infection-neutralization capacity against immune escape VoCs is induced by repeated exposure to the original SARS-CoV-2 spike protein as encoded by the BNT162b2 mRNA vaccine, a boosting and refinement of immunity through VoC-specific vaccines may provide higher and long-lasting protection from infection.

It should be noted that this study focused on determining serum infection-neutralization capacity following infection and vaccination as a correlate of protection and identified antibody avidity as an important factor. We, however, lack the information on how the antibody repertoire may evolve over time and did not analyze antibody levels and neutralizing capacity at time points shortly before the third vaccination. The study also provides insights neither into the breadth of antibody responses nor into antibody avidity against the spike of the different VoCs.

Notwithstanding our finding of a superior infection-neutralization capacity after three mRNA vaccinations, protection from severe COVID-19 may already be achieved after two antigen encounters in particular in children and young adults[26]. In this context, cell-mediated immunity elicited by infection or by vaccination likely contributes to protection from severe COVID-19 (ref. [27]). In our study, however, we neither directly assessed the protective efficacy of two versus three antigen doses against severe disease nor addressed the protective effect of T cell responses. Although the development of infection-neutralization capacity mediated by spike-specific antibodies and antiviral T cell immunity have been shown to develop in parallel[14], further studies are required to elucidate whether three timely spaced encounters with spike antigen also accompany a quantitative and qualitative increase in protective T cell immunity.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-022-01715-4.

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Methods

Study participants and sample collection. In a screening effort, 4,554 health care workers were tested for SARS-CoV-2 nucleocapsid-specific antibodies with a commercial chemiluminescence immunoassay (iFlash CLIA, YHLO Biotechnology[28]. Convalescent individuals from SARS-CoV-2 infection in the first pandemic wave in March/April 2020 were identified either by positive PCR or by two to four independent serological assays (specificity of ≥98% for each assay results in a specificity of ≥99.96% for the convalescent cohort)[14]. Naive individuals tested negative in at least two different SARS-CoV-2 nucleocapsid-specific IgG assays. In total, 171 (98 convalescent and 73 naive) individuals were enrolled into a follow-up study that was conducted from April 2020 onwards at the University Hospital rechts der Isar of the Technical University of Munich (Supplementary Table 1). The study scheme is depicted in Extended Data Fig. 8. No statistical methods were used to predetermine sample sizes, but our sample sizes increase those reported in previous publications[20–25]. Studies were approved by the local ethics committee (ethics vote 476/20 and 26/21S-SR) and participants provided written informed consent to study participation and biobanking.

A total of 68 convalescents gave written informed consent for further analyses after their COVID-19 vaccination, and 73 SARS-CoV-2 naive individuals were matched by sex, age, working conditions and risk factors present in the convalescent cohort. Median age was 36 (IQR, 29 to 53) years in naive participants and 40 (IQR, 29 to 54) years in convalescent participants. Approximately 65.8% of naive participants and 57.6% of convalescent participants were female. All naive and 25 of 68 convalescent individuals who were continuously followed received two doses of BNT162b2 mRNA vaccine (Comirnaty, BioNTech/Pfizer) as immunization. The interval between the two vaccinations was, on average, 22 and 21 d for naive and convalescent individuals, respectively. Due to a change in the national guidelines in March 2021, the remaining 43 of 68 convalescents from the first wave were only vaccinated once with BNT162b2 until the middle of 2021, assuming that the prior infection substituted for one vaccination[29]. For all analyses, six convalescent individuals were excluded because they showed ≥fourfold and ≥eightfold increases in a surrogate neutralization and in IC_{50} value for neutralization, respectively, independent of vaccination indicating SARS-CoV-2 re-exposure[14].

Sera from 34 naive and 51 convalescent participants were analyzed before vaccination, from 48 naive and 59 convalescent participants 2 weeks after their initial vaccination and from 47 naive and 23 convalescent participants 2 weeks after the second vaccination. A total of 45 and 72 naive and 51 and 56 convalescent participants were tested 4 and 7 months after their basic immunization, respectively, including 31 and 37 of convalescents who did not receive a second vaccine dose. Finally, sera from 59 naive participants and 36 convalescents were evaluated 2 weeks after receiving an additional BNT162b2 shot as the third immunization after an average of 9 months (Extended Data Fig. 1).

Additionally, a second cohort of 31 individuals with PCR-confirmed breakthrough infections with SARS-CoV-2 Delta or Omicron VoC ≥14 d after vaccination 2 were included (cohort 2; Supplementary Table 2). This study was approved by the local ethics committee (vote 229/21), and all participants provided written informed consent. Median age was 35 (IQR, 31 to 38) years in Delta-infected participants and 41 (IQR, 28 to 49) years in Omicron-infected participants. Specimens were collected on average 7 d (V1), 2 weeks (V2), 3 weeks (V3) and 4 weeks (V4) after the first positive PCR result showing breakthrough infection. VoC-specific PCR and/or whole-genome sequencing identified Delta (B.1.617.2) in respiratory samples of 16/31 and Omicron (B.1.1.529) in respiratory samples of 15/31 individuals. In this cohort, 26 of 31 participants (84%) had received two doses of an mRNA vaccine (22 BNT162b2, 4 mRNA-1273), and 5 of 31 had received a first vaccination with an adenoviral vector vaccine, two of which subsequently received the same vaccine and two were vaccinated with BNT162b2 (Supplementary Table 2). The median time span between the first positive PCR result and a complete vaccination cycle was 141 d (IQR, 99 to 242 d) in Delta-infected participants and 166 d (IQR, 146 to 194 d) in Omicron-infected individuals.

Antibody detection and avidity assays. IgG-type antibody responses to the Wuhan-hu-1 strain S1 domain of SARS-CoV-2 spike antigen were quantified in tenfold diluted serum specimens using the commercial anti-SARS-CoV-2 QuantiVac-ELISA (IgG; EuroImmun). Binding strength of the SARS-Cov-2 IgG antibodies was determined by adaptation of the commercial IgG agile SARS-CoV-2 ELISA (Virion/Serion) using ammonium thiocyanate (Roth, Germany) as the chaotropic agent as described previously[30]. Briefly, serum samples were measured using the IgG agile SARS-CoV-2 ELISA and adjusted to 100 BAUs per ml, according to the standard curve provided by the manufacturer, to exclude an influence of variable antibody concentrations. Then, serum samples were incubated in the plates pre-coated with Wuhan SARS-CoV-2 spike protein ectodomain S1, S2 and receptor binding domain recombinant antigens for 1 h at 37 °C in a humid chamber. After washing, antigen–antibody complexes were incubated in the presence of 1.0 M ammonium thiocyanate or PBS as control for 10 min at room temperature. After washing to remove antibodies bound with low avidity, the ELISA was completed according to the manufacturer's instructions. The relative avidity index was calculated as follows: percentage avidity = IgG concentration ammonium thiocyanate treated)/(IgG concentrations PBS treated) × 100 and is given in percentages[30,31].

SARS-CoV-2 neutralization assay. High-titer virus stocks were generated by infection of Vero-E6 cells (American Type Culture Collection) grown in virus expansion medium (DMEM containing 5% FBS, 100 U ml^{-1} penicillin–streptomycin). Cells were incubated with clinical isolates of different SARS-CoV-2 variants (GISAID EPI ISL: 2450298 (EU1/B.1.177), 2095258 (Alpha/B.1.1.7), 1752394 (Beta/B.1.351), 2095178 (Gamma/P.1/B.1.1.28.1), 2772700 (Delta/B.1.617.2) and 7808190 (Omicron/B.1.1.529, sublineage BA.1)). EU1 and the Omicron VoC were isolated from nasopharyngeal swabs of patients with COVID-19. Virus stocks were expanded by two passages before collection and storage at −80 °C. All virus stocks were only used for infection experiments after sequencing of the complete viral genomes. Virus stocks were characterized by real-time RT–PCR as reported previously[32].

For each individual SARS-CoV-2 VoC, the tissue culture infectious dose resulting in 90% loss of target cell viability ($TCID_{90}$) 48 h after infection was determined using a dilution series of the virus stock on MDA-MB-231 cells (American Type Culture Collection) overexpressing hACE2. For infection neutralization, cells were cultured and infected in 384-well plates (7,500 cells per well). The respective $TCID_{90}$ of each virus stock was incubated for 2 h with different concentrations of each serum to be tested. Subsequently, 10 µl of the virus–serum mixtures were added to 20 µl of medium and added to MDA-MB-231–hACE2 cells. At 48 h after infection, cytopathic effects were recorded by the addition of 10 µl CellTiter-Glo 2.0 reagent (Promega) and subsequent measurement of bioluminescence signals (0.5-s integration time, no filter) to quantify virus-mediated cytotoxicity in target cells.

Statistical analysis. Data and statistical analyses were performed in Prism 9 (GraphPad Software). $TCID_{90}$ values for tissue culture infectious doses and IC_{50} values for neutralization were calculated after normalized, sigmoidal dose–response curve approximation of the respective data.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All primary data that was used to generate the results obtained in this study are available in the source data provided with this paper.

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Author contributions

These authors equally share responsibility for this work: P.A.K., O.T.K. and U.P. Conceptualization: P.A.K., O.T.K. and U.P. Methodology: P.R.W., M.S., A.P., A.W., E.V., N.G., H.M., M.F., C.-C.C., G.L., M.A., E.M.-P., M.M., S.K., H.M., C.C., J.W., D.H., S.K., G.A., T.V., M.P. and O.T.K. Resources: S.B., P.S. and V.F. Formal analysis: C.D., M.M., A.G., S.J., N.G., H.M., M.W., E.V., S.Y., K.T. and C.C. Writing—original draft: P.R.W., A.P., M.S., P.A.K., O.T.K. and U.P. Writing—review and editing: all authors. Visualization: P.R.W., A.P., P.K., O.T.K. and U.P. Supervision: H.B., V.H., B.L., K.U., P.A.K., O.T.K. and U.P. Funding acquisition: B.L., K.U., P.A.K., O.T.K. and U.P.

Competing interests

The authors declare no competing interests concerning the study content. Outside the study, they declare the following competing interests: U.P. is a cofounder, shareholder and board member of SCG Cell Therapy, a member of the scientific advisory board of Leukocare and a member of topic-specific scientific advisory boards of Sanofi-Pasteur, GILEAD and GSK and an ad hoc advisor for BioNTech (without remuneration).

Additional information

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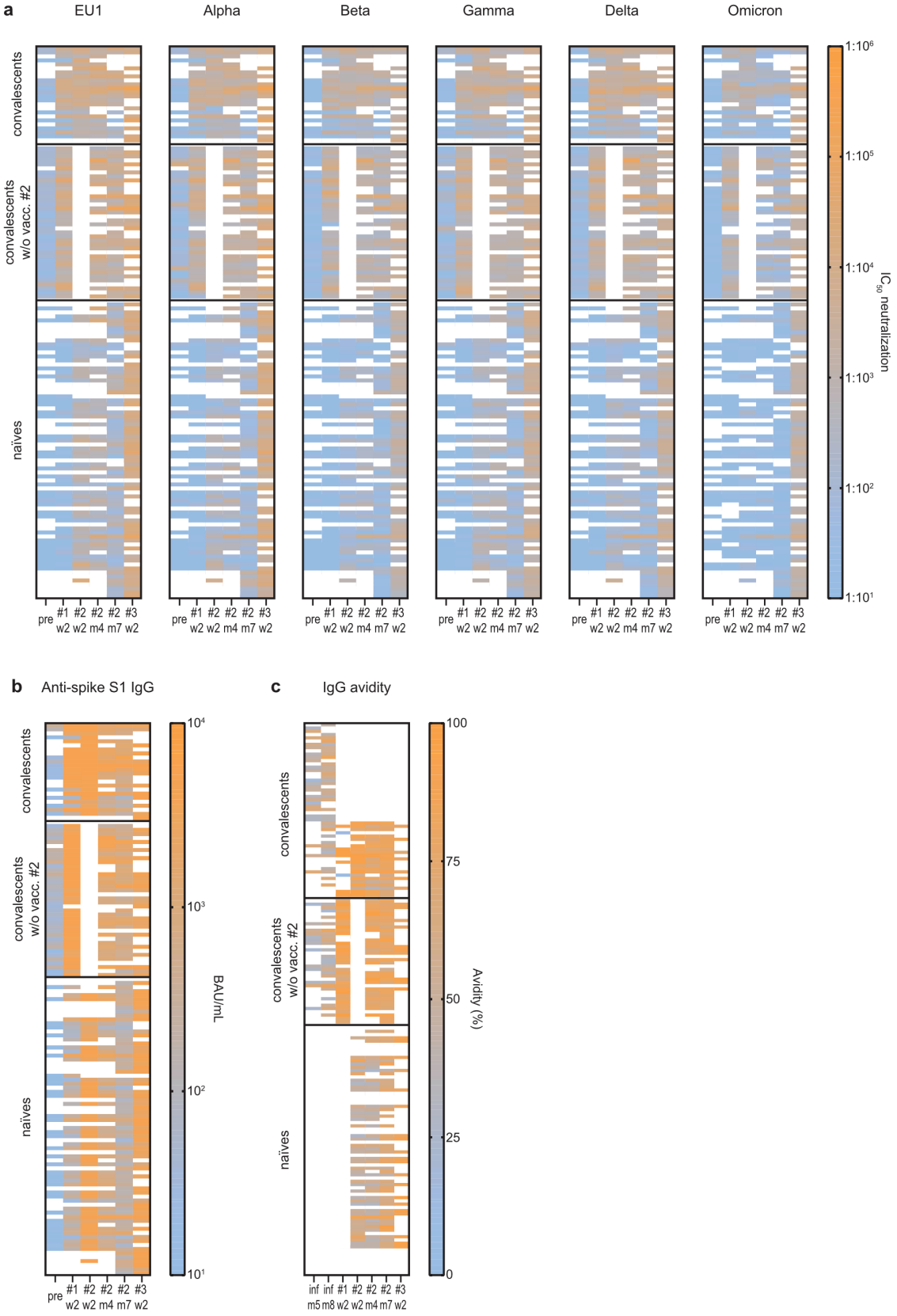
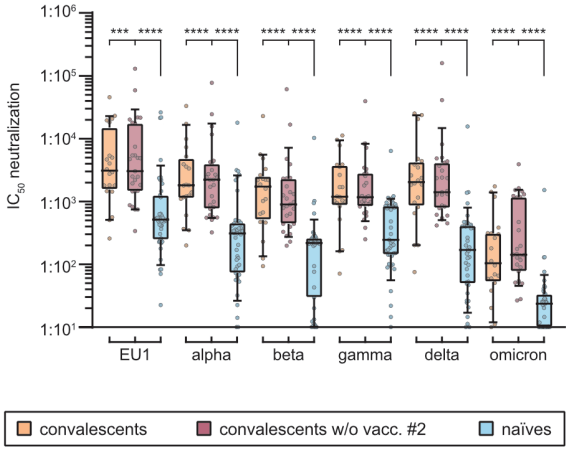
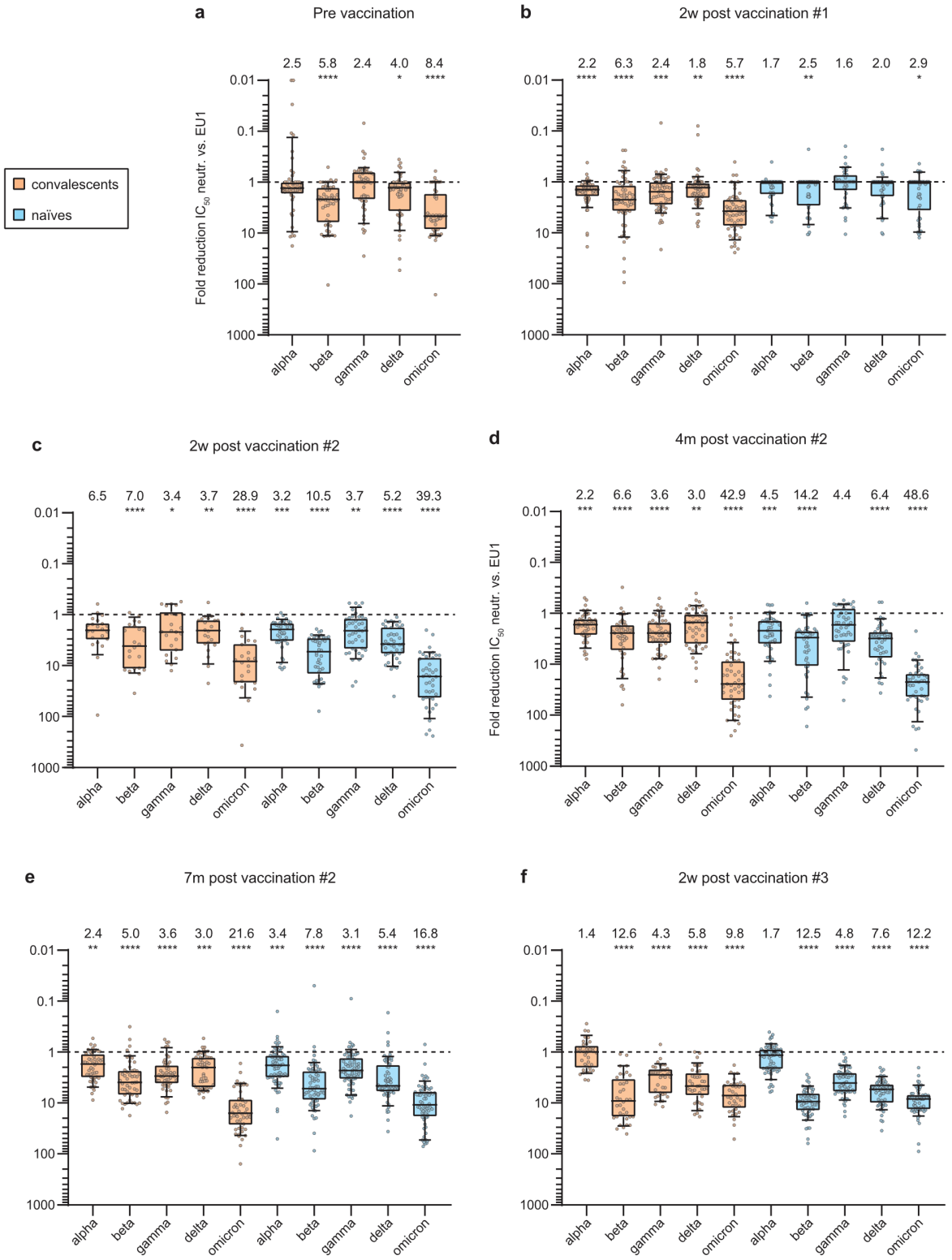


Extended Data Fig. 1 | See next page for caption.

Extended Data Fig. 1 | Heatmaps of infection neutralization capacity against SARS-CoV-2 variants, anti-SARS-CoV-2 antibody responses, and antibody avidity in naive individuals and convalescents after BNT162b2 vaccination. a,**b**, heatmaps of serum IC_{50} values for infection-neutralization capacity normalized to 10^7 viral RNA copies for serum infection-neutralization of SARS-CoV-2 variants (**a**) and IgG-type anti-spike S1 domain antibody titers (**b**) in 24 SARS-CoV-2 convalescents, 38 convalescents who did not receive vaccination #2, and 73 naive participants. **c**, heatmap of IgG-type anti-spike antibody avidity in 54 SARS-CoV-2 convalescents, 38 convalescents who did not receive the second vaccination, and 73 naive participants. For white areas within heatmaps data was not available. Abbreviations, pre – prior to first vaccination; #1 – first vaccination time point; 2 – second vaccination; #3 – third vaccination; w2 – two weeks after respective vaccination; m4 – 4 months after vaccination; m7 – 7 months after vaccination; inf. m5 – 5 months after SARS-CoV-2 infection; inf. m8 – 8 months after SARS-CoV-2 infection; BAU – binding antibody units.

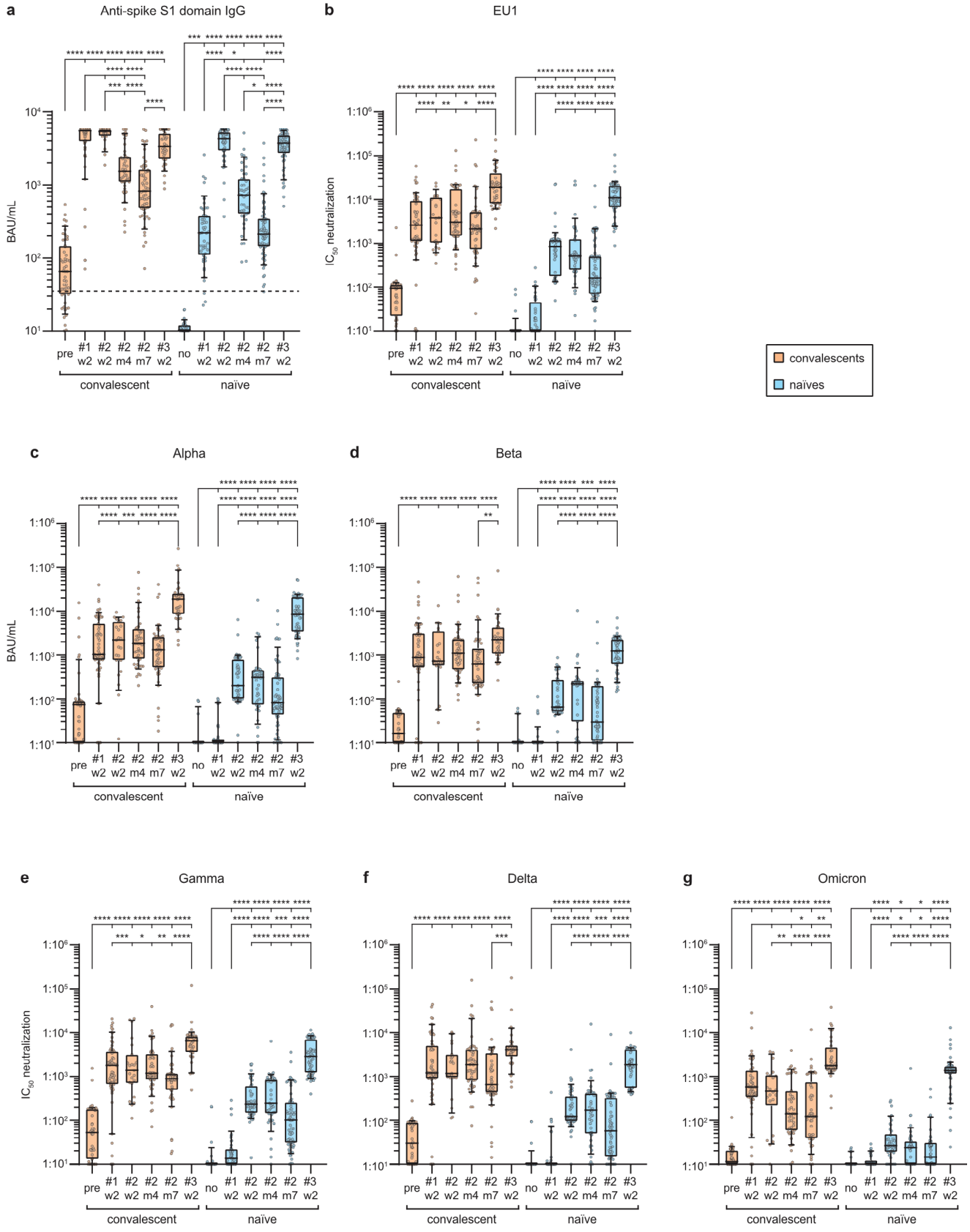


Extended Data Fig. 2 | Comparison of infection neutralization capacities for VoCs in naive individuals and convalescents 4 months after BNT162b2 vaccination. Serum IC_{50} values for infection-neutralization capacity normalized to 10^7 viral RNA copies for serum infection-neutralization of SARS-CoV-2 variants EU1 and VoCs alpha, beta, gamma, delta and omicron as box plots with median, bounds between upper and lower quartiles, and whiskers between the 10^{th} and 90^{th} percentiles for 20 SARS-CoV-2 convalescents (orange), and 43 naive participants (blue) collected 4 months after vaccination #2 and 27 convalescents who did not receive vaccination #2 collected at the same time point (red). Differences in IC_{50} values were analysed for statistical significance using the Kruskal-Wallis-test with Dunn's multiple testing correction. Connecting lines indicate statistically significant differences between groups. Absence of connecting lines or asterisks indicates absence of significance. ***P=0.0001, ****P<0.0001.



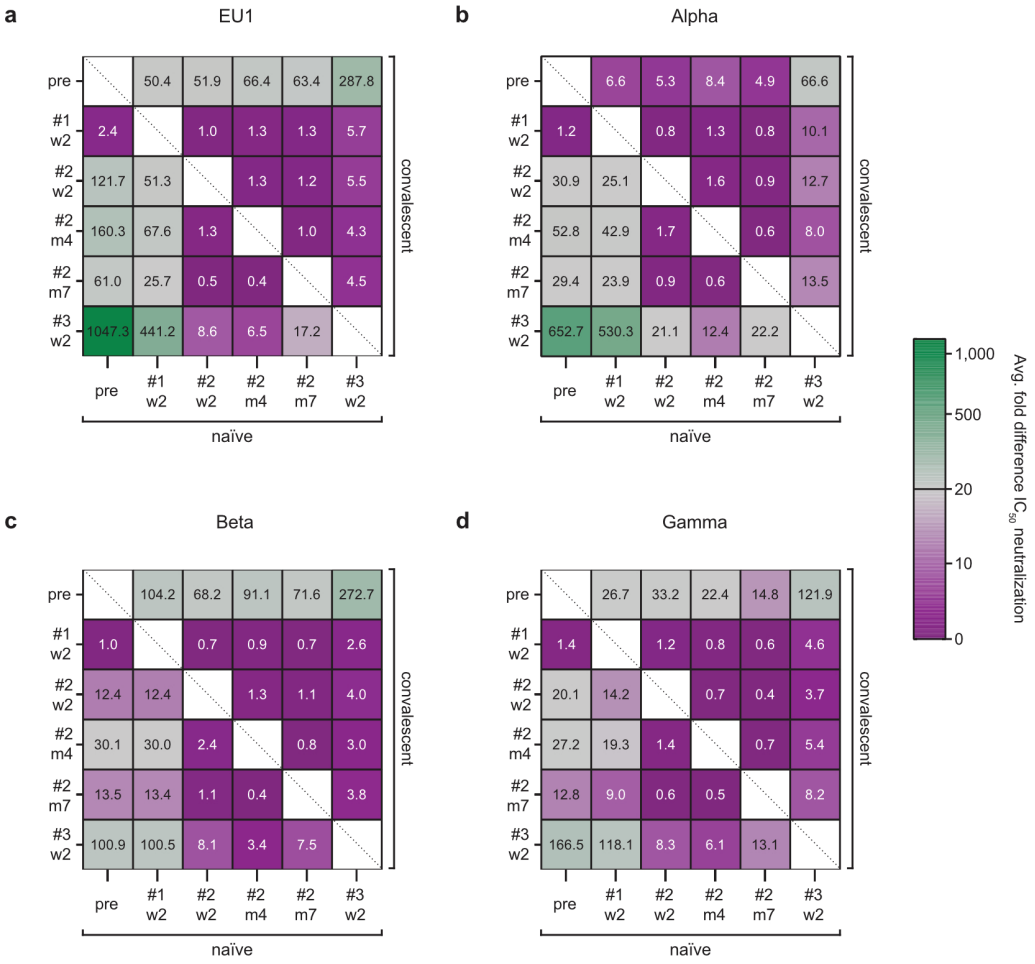
Extended Data Fig. 3 | See next page for caption.

Extended Data Fig. 3 | Comparison of infection-neutralization capacities for VoCs in naive individuals and SARS-CoV-2 convalescents after BNT162b2 vaccination. Fold-reduction of serum IC_{50} values for infection-neutralization capacity comparing the neutralization of EU1 with the VoCs depicted as box plots with median, bounds between upper and lower quartiles, and whiskers between the 10th and 90th percentiles for SARS-CoV-2 convalescents (orange) and 64 naive participants (blue). **a**, 50 SARS-CoV-2 convalescents collected prior to vaccination, $^{*}P$=0.0116. **b**, 56 convalescents, 42 naives at 2 weeks (2w) after vaccination #1, convalescent EU1 vs. gamma $^{***}P$=0.0003, delta $^{**}P$=0.0022, naive EU1 vs. beta $^{**}P$=0.0013, vs. omicron $^{*}P$=0.0121. **c**, 22 convalescents, 42 naives at 2w after vaccination #2, convalescent EU1 vs. gamma $^{*}P$=0.0496, delta $^{**}P$=0.0084, naive EU1 vs. alpha $^{***}P$=0.0001, gamma $^{**}P$=0.0055. **d**, 47 convalescents, 43 naives at 4 months (4m) after vaccination #2; **e**, 50 convalescents, and 64 naives at 7m after vaccination #2, convalescent EU1 vs. alpha $^{***}P$=0.0007, delta $^{**}P$=0.0084, naive EU1 vs. alpha $^{***}P$=0.0004. **f**, 36 convalescents, 59 naives at 2w after vaccination #3, **a**-**f**, $^{****}P$<0.0001. Differences in fold-changes were analysed for their statistical significance using the two-sided Friedman test with Dunn's multiple testing correction. Connecting lines indicate statistically significant differences between groups. Absence of connecting lines or asterisks indicates absence of significance. Numbers above boxes indicate average fold changes comparing EU1 and the respective SARS-CoV-2 VoC.

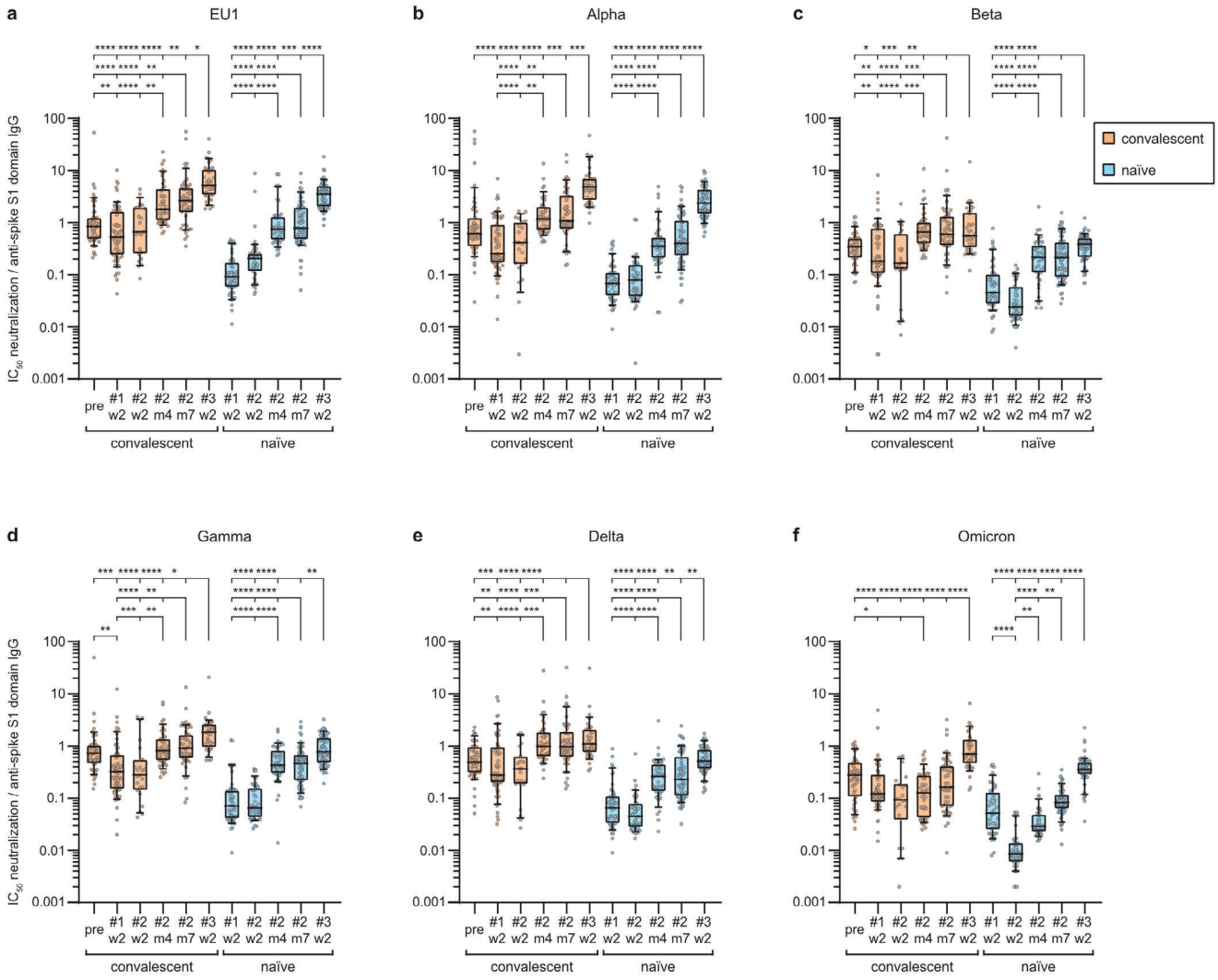


Extended Data Fig. 4 | See next page for caption.

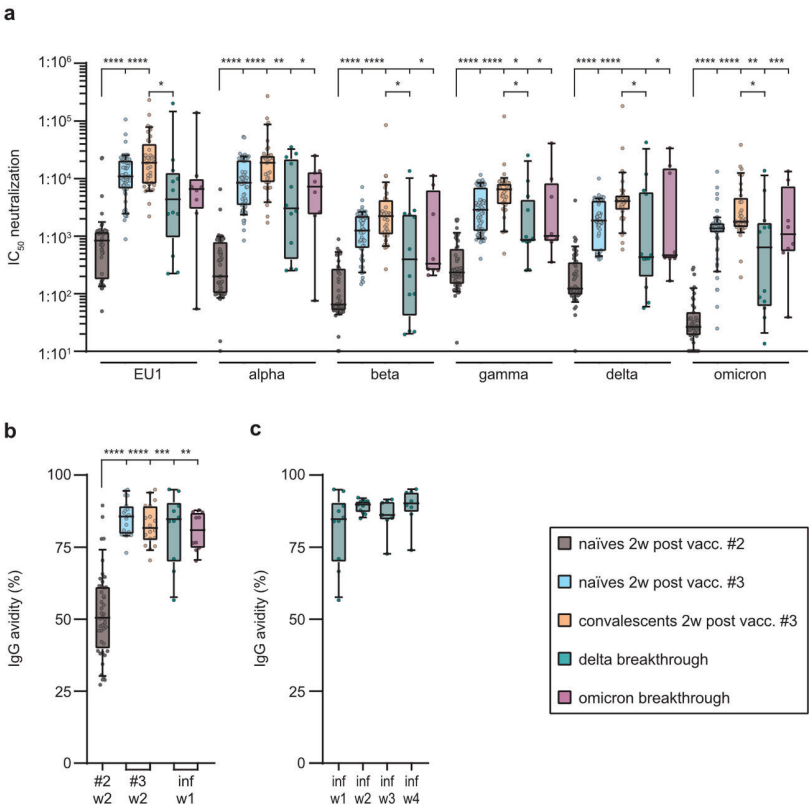
Extended Data Fig. 4 | Longitudinal comparison of infection neutralization capacities against VoCs and anti-SARS-CoV-2 spike antibody responses after BNT162b2 vaccination. a, anti-spike S1 domain antibody titers in 274 sera from 62 convalescents, and 304 sera from 73 naive participants given as binding arbitrary units (BAU)/mL, convalescent w2 after vaccination (vacc.) #2 vs. m4 vacc. #2 $^{***}P$=0.0004, naive pre-vaccination (pre) vs. w2 vacc #1 $^{***}P$=0.0002, w2 vacc. #1 vs. m4 vacc. #2 $^{*}P$=0.0181, m4 vacc. #2 vs. m7 vacc. #2 $^{*}P$=0.0123. **b**-**g**, Serum IC_{50} values for infection-neutralization capacity normalized to 10^7 viral RNA copies of SARS-CoV-2 VoCs EU1, alpha, beta, gamma, and delta in 266 / 296 (**b**-**f**) and omicron and 261 / 279 (**g**) sera from 62 convalescents / 73 naives, respectively; **b**, convalescent w2 vacc. #3 vs. w2 vacc. #2 $^{**}P$=0.0018, and vs. m4 vacc. #2 $^{*}P$=0.0108, **c**, w2 vacc. #2 vs. w2 vacc #3 $^{***}P$=0.0002, **d**, convalescent m7 vacc. #2 vs. w2 vacc. #3 $^{**}P$=0.0037, naive pre vs. m7 vacc #2 $^{***}P$=0.0036, **e**, convalescent w2 vacc. #3 vs. w2 vacc. #1 $^{***}P$=0.0006, vs. w2 vacc. #2 $^{*}P$=0.0237, and vs. m4 vacc #2 **P=0.0023, naive w2 vacc. #1 vs. m7 vacc. #2 $^{***}P$=0.0002, **f**, convalescent m7 vacc. #2 vs. w2 vacc. #3 $^{***}P$=0.0005, naive w2 vacc. #1 vs. m7 vacc. #2 $^{***}P$=0.0003, **g**, convalescent w2 vacc. #1 vs. m7 vacc. #2 $^{*}P$=0.0357, and vs. w2 vacc. #3 $^{**}P$=0.0043, w2 vacc. #2 vs. w2 vacc. #3 $^{**}P$=0.0049, naive pre vs. m4 vacc. #2 $^{*}P$=0.0197, and vs. m7 vacc. #2 $^{*}P$=0.0376, w2 vacc. #1 vs. m4 vacc. #2 $^{*}P$=0.0236, and vs. m7 vacc. #2 $^{*}P$=0.0043, **a**-**g**, $^{****}P$<0.0001. Data are shown as box plots with median, bounds between upper and lower quartiles, and whiskers between the 10th and 90th percentile for SARS-CoV-2 convalescents (orange) and naive participants (blue). Differences between time points were analysed for their statistical significance using the Kruskal-Wallis test with Dunn's multiple testing correction. Connecting lines indicate statistically significant differences between groups. Absence of connecting lines or asterisks indicates absence of significance. **a**, **f**, **g**, are also shown in Fig. 2 and were added to this extended data figure to enhance comparability. Abbreviations, pre: prior to first vaccination; #1 – first vaccination; #2 – second vaccination; #3 – third vaccination; w2 – two weeks after respective vaccination; m4 – 4 months after vaccination; m7 – 7 months after vaccination.



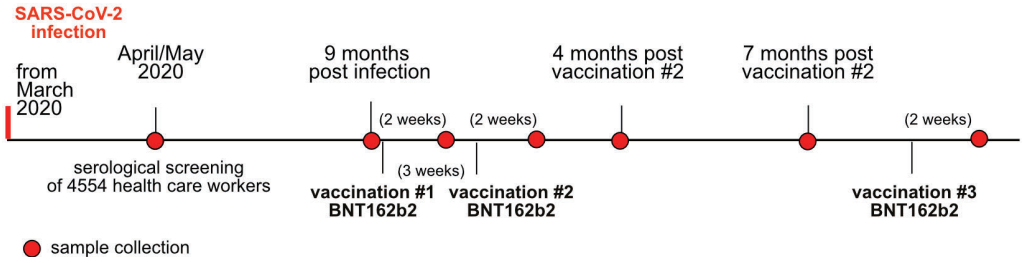
Extended Data Fig. 5 | VoC-centered heatmaps for longitudinal comparisons of infection-neutralization capacities in naive individuals and convalescents after vaccination. a-d, heatmaps illustrating average-fold changes of serum IC_{50} values for infection-neutralization capacity normalized to 10^7 viral RNA copies for serum infection-neutralization of EU1 and VoCs alpha, beta and gamma between the respective time points. Abbreviations, pre – prior to first vaccination; #1 – first vaccination; #2 – second vaccination; #3 – third vaccination; w2 – two weeks after respective vaccination; m4 – 4 months after vaccination; m7 – 7 months after vaccination.



Extended Data Fig. 6 | Longitudinal comparison of the ratios between infection neutralization capacities against VoCs and anti-SARS-CoV-2 antibodies in naive individuals and convalescents after BNT162b2 vaccination. Ratios between serum IC_{50} values for infection-neutralization capacity against VoCs and antibody titers to the S1 domain of SARS-CoV-2 spike antigen shown as box plots with median, bounds between upper and lower quartiles, and whiskers between the 10th and 90th percentiles in SARS-CoV-2 convalescents (regardless of whether or not they received vaccination #2, orange) and naive participants (blue). **a**-**e**, 263 sera from 62 convalescents, and 295 sera from 73 naives; (**f**) 258 sera from 62 convalescents, and 278 sera from 73 naives. **a**, convalescent w2 after vaccination (vacc.) #3 vs. m4 vacc. #2 $^{**}P$=0.0091, and vs. m7 vacc #2 $^{*}P$=0.0401, w2 vacc. #2 vs. m7 vacc. #2 $^{**}P$=0.0012, m4 vacc. #2 vs. pre-vaccination (pre) $^{**}P$=0.0015, and vs. w2 vacc. #2 $^{**}P$=0.0093, naive m4 vacc. #2 vs. w2 vacc. #3 $^{***}P$=0.0002. **b**, convalescent w2 vacc. #2 vs. m4 vacc. #2 $^{**}P$=0.0025, w2 vacc. #2 vs. m7 vacc. #2 $^{**}P$=0.0018, w2 vacc. #3 vs. m4 vacc. #2 $^{***}P$=0.0003, and vs. m7 vacc. #2 $^{***}P$=0.0002. **c**, convalescent m4 vacc. #2 vs. pre $^{**}P$=0.0030, and vs. w2 vacc. #2 $^{***}P$=0.0003, m7 vacc. #2 vs. pre $^{**}P$=0.0074, and vs. w2 vacc. #2 $^{***}P$=0.0008, w2 vacc. #3 vs. pre $^{*}P$=0.0250, vs. w2 vacc. #1 $^{***}P$=0.0005, and vs. w2 vacc. #2 $^{**}P$=0.0023. **d**, convalescent pre vs. w2 vacc. #1 $^{**}P$=0.0082, m4 vacc. #2 vs. w2 vacc. #1 $^{***}P$=0.0001, and vs. w2 vacc. #2 $^{**}P$=0.0064, m7 vacc. #2 vs. w2 vacc. #2 $^{**}P$=0.0017, w2 vacc. #3 vs. pre $^{***}P$=0.0006, and vs. m4 vacc. #2 $^{*}P$=0.0408, naive m7 vacc. #2 vs. w2 vacc. #3 $^{**}P$=0.0034. **e**, convalescent m4 vacc. #2 vs. pre $^{**}P$=0.0030, and vs. w2 vacc. #2 $^{***}P$=0.0003, m7 vacc. #2 vs. pre $^{**}P$=0.0052, and vs. w2 vacc. #2 $^{***}P$=0.0005, pre vs. w2 vacc. #3 $^{***}P$=0.0005, naive w2 vacc. #3 vs. m4 vacc. #2 $^{**}P$=0.0027, and vs. m7 vacc. #2 $^{**}P$=0.0032. **f**, convalescent pre vs. m4 vacc. #2 $^{*}P$=0.0340, naive w2 vacc. #2 vs. m4 vacc. #2 $^{**}P$=0.0077, m4 vacc. #2 vs. m7 vacc. #2 $^{**}P$=0.0011, **a**-**f**, $^{****}P$<0.0001. Connecting lines indicate statistically significant differences between groups. Absence of connecting lines or asterisks indicates absence of significance. Differences between time points were analysed for their statistical significance using the Kruskal-Wallis test with Dunn's multiple testing correction. The data depicted in panels (**e**, **f**) are also shown in Fig. 2 and were added to this extended data figure to enhance comparability. Abbreviations, pre: prior to first vaccination; #1 – first vaccination; #2 – second vaccination; #3 – third vaccination; w2 – two weeks after respective vaccination; m4 – 4 months after vaccination; m7 – 7 months after vaccination.



Extended Data Fig. 7 | Comparison of infection neutralization capacities for VoCs in twice BNT162b2 vaccinated individuals after breakthrough infection with delta and omicron compared to naive individuals and SARS-CoV-2 convalescents. a, serum IC_{50} values for infection-neutralization capacity normalized to 10^7 viral RNA copies of SARS-CoV-2 variants in 47 naive participants (42 for omicron) 2 weeks after vaccination #2 (dark brown), 59 naive (light blue) and 36 convalescent participants 2 weeks after vaccination #3, as well as 12 and 10 twice BNT162b2 vaccinated individuals on average 7 days after PCR-confirmed breakthrough infections with delta (green) or omicron (purple), respectively; naives 2w after vaccination (vacc.) #2 vs. naives and convalescents 2w after vacc. #3 $^{****}P<0.0001$ for all variants, vs. delta breakthrough infection for alpha $^{**}P=0.0043$, gamma $^{*}P=0.0366$, omicron $^{**}P=0.0074$, vs. omicron breakthrough for alpha $^{*}P=0.0100$, beta $^{*}P = 0.0246$, gamma $^{*}P=0.0165$, delta $^{*}P=0.0167$, omicron $^{***}P=0.0007$, convalescent 2w vacc. #2 vs. delta breakthrough for EU1 $^{*}P=0.0453$, beta $^{*}P=0.0306$, gamma $^{*}P=0.0230$, delta $^{*}P=0.0226$, omicron $^{*}P=0.0434$. (**b**) IgG-type anti-spike antibody avidities in 44 naive participants 2 weeks after vaccination #2 (dark brown), 19 naive (light blue) and 18 convalescent participants 2 weeks after vaccination #3, as well as 10 and 8 twice BNT162b vaccinated individuals on average 7 days after PCR-confirmed breakthrough infections with delta (green) or omicron (purple), respectively; naives 2w vacc. #2 vs. naives and convalescents 2w after vacc. #3 $^{****}P<0.0001$, vs. delta breakthrough $^{***}P=0.0001$, and vs. omicron breakthrough $^{**}P=0.0014$. (**c**) IgG-type anti-spike antibody avidities in twice BNT162b2 vaccinated individuals on average 7 days (n=10), 2 weeks (n=11), 3 weeks (n=7), and 4 weeks (n=8) after PCR-confirmed breakthrough infections with delta. Data are shown as box plots with median, bounds between upper and lower quartiles, and whiskers between the 10th and 90th percentiles. Differences between groups were analysed for their statistical significance using the Kruskal-Wallis test with Dunn's multiple testing correction. Connecting lines indicate statistically significant differences between groups. Absence of connecting lines or asterisks indicates absence of significance. Abbreviations, inf: after infection; #2 – second vaccination; #3 – third vaccination; w1 – 7 days after infection; w2 – two weeks after respective vaccination/infection; w3 – three weeks after infection; w4 – four weeks after infection.



Extended Data Fig. 8 | Graphical illustration of the longitudinal cohort analysis depicting time points of vaccination and blood sample collection.

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Corresponding author(s): Oliver Keppler and Ulrike Protzer

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	All data from participants were obtained after informed written consent. Clinical data from participants were collected in DIS (digital information system, University Hospital rechts der Isar, Technical University of Munich, Germany) that assures anonymization of clinical and laboratory data.
Data analysis	Data was analyzed using Prism 9.3.1. (GraphPad Software, USA)

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	All healthcare workers of a quaternary care hospital were invited using different modes of communication to participate in the study irrespective of their work environment. 4,554 were screened for SARS-CoV-2 infection after giving written informed consent. All COVID-19 convalescent individuals identified were invited to be followed up, of whom 98 agreed and were enrolled in this study. A sex-, age-, working conditions- and risk factor-matched cohort of 73 infection-naive individuals was established from the seronegative participants of the study. In total, 486 serum samples were longitudinally collected from the convalescent and naïve individuals within this cohort. In addition from a second cohort, in which we studied breakthrough infections in vaccinated individuals, sera from 15 vaccinated patients infected with SARS-CoV-2 VoC omicron, and 51 sera from 16 vaccinated patients infected with SARS-CoV-2 VoC delta were analyzed. The number of participants was tested to be suffiecient to allow a statistically significant comparison of the immune response to vaccination in convalescents vs infection-naive inidividuals by the institutional biostatistician.
Data exclusions	Six convalescent individuals were excluded because they showed a ≥8-fold increase in a surrogate assay and in IC50 neutralization, respectively, independent of vaccination indicating a recent SARS-CoV-2 re-exposure.
Replication	The assay to determine binding antibody titers was performed using a commercial, diagnostical assays that is well-validated and makes use of plate-wise calibrators, negative and positive controls. Titers were determined according to WHO standard binding units (BAU) assuring high standardization. Binding antibody titers were confirmed in a second, independent commercial assay before avidity testing. Experiments to determine antibody avidity were performed in duplicates showing low variance between results. The neutralization assay was validated previously showing low variance between results of independent experiments. Furthermore, each sample was tested in the neutralization assay at six different concentrations. Because of the low sample volumes available, experiments to determine neutralization titers were not replicated.
Randomization	4554 health care workers were screened for sub-acute/resolved COVID-19. 98 COVID-19 convalsescent participants were followed up. Naive individuals were randomly matched to the convalescent cohort according to sex, age, working conditions and other risk factors.
Blinding	Laboratory experiments and data evaluation were performed with blinded samples. De-blinding of cohorts was performed after the evaluation of all raw data.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	MDA-MB-231 (German collection of Microorganisms and Cell Cultures, Germany), Vero-E6 (American Type Culture Collection, USA)
Authentication	Cells were authenticated by short tandem repeat (STR) analysis.

Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Human research participants

Policy information about studies involving human research participants

Population characteristics	Median age was 36 (interquartile range (IQR) 29 to 53) years in naïve and 38 (IQR 29 to 53) years in convalescent participants. 65.8% naïve and 54.1% convalescent participants were female. Median age was 35 (IQR 31 to 38) years in delta- and 42 (IQR 28 to 52) years in omicron-infected participants.
Recruitment	All healthcare workers of a quaternary care hospital were invited to join an antibody testing study. 4,554 participants were recruited using E-mails, handouts and via personal communication without selection bias. Convalescents were identified to be SARS-CoV-2 antibody positive from this large-scale antibody screening. All convalescents were invited to participate in the follow-up study and all individuals who agreed to participate were included. Individuals with a possible re-exposure to SARS-CoV-2 were excluded. Naive individuals were randomly matched from the original 4,554 individuals cohort. Study participants did not receive any compension.
Ethics oversight	The study protocol was approved by the ethics committee of the Technical University Munich (TUM) (protocols 476/20, 26/21S-SR, 229/21).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	Ethics protocols of follow-up studies are: 476/20, 26/21S-SR, 229/21; no clinical trial was performed.
Study protocol	The ethics study protocols are available upon reasonable request.
Data collection	Serum samples were collected between April 2020 and December 2021 at the University Hospital rechts der Isar of the Technical University of Munich.
Outcomes	Primary and secondary outcome measures are describedin the manuscript.

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Supplementary information

Three exposures to the spike protein of SARS-CoV-2 by either infection or vaccination elicit superior neutralizing immunity to all variants of concern

In the format provided by the authors and unedited

Supplementary Table 1: Longitudinal cohort characteristics of naïve and convalescent participants (cohort 1)

Naïve individuals (cohort 1)	
participants - *n*	73
one initial vaccination	0
two initial vaccinations	73
vaccine received	BNT162b2
time span - *median days (IQR)*	
vaccination #1 to #2	21 (21 to 22)
vaccination #2 to #3	272 (266 to 282)
gender - *%*	
female	65.8
male	34.2
age - *median years (IQR)*	36 (29 to 53)

Convalescent individuals (cohort 1)	
participants - *n*	98
thereof monitored after vaccination	68
one initial vaccination	43
two initial vaccinations	25
thereof excluded due to potential SARS-CoV-2 re-exposure	6
time period of SARS-CoV-2 infection	approx. March/April 2020
hospitalizations - *n*	0
vaccine received	BNT162b2
time span - *median days (IQR)*	
vaccination #1 to #2	21 (21 to 22)
vaccination #1/#2 to #3	260 (237 to 268)
gender - *%*	
female	57.6
male	42.4
age - *median years (IQR)*	40 (29 to 54)

Supplementary Table 2: Cohort characteristics of vaccinated individuals with breakthrough infections (cohort 2)

Individuals with breakthrough infection (cohort 2)	
participants - *n*	31
delta breakthrough	16
omicron breakthrough	15
time period of SARS-CoV-2 infection	July 2021 to January 2022
hospitalizations - *n*	0
vaccines received	
2 x BNT162b2 - *n*	22
delta breakthrough	12
omicron breakthrough	10
2 x mRNA-1273 - *n*	4
delta breakthrough	2
omicron breakthrough	2
2 x ChAdOx1 - *n*	2
delta breakthrough	0
omicron breakthrough	2
1 x ChAdOx1 +1 x BNT162b2 - *n*	2
delta breakthrough	2
omicron breakthrough	0
1 x Ad26.COV2.S - *n*	1
delta breakthrough	0
omicron breakthrough	1
time span - *median days (IQR)*	
delta breakthrough	
initial vaccinations to first positive PCR result	141 (99 to 242)
first positive PCR result to sample collection	7 (5 to 7)
omicron breakthrough	
initial vaccinations to first positive PCR result	166 (146 to 194)
first positive PCR result to sample collection	10 (6 to 10)
gender - *%*	
delta breakthrough	
female	56.3
male	43.7
omicron breakthrough	
female	40.0
male	60.0
age - *median years (IQR)*	
delta breakthrough	35 (31 to 38)
omicron breakthrough	41 (28 to 49)

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