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# SARS-CoV-2 serostatus of healthcare workers in the Austrian state Vorarlberg between June 2020 and January 2021

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SARS-CoV-2 serostatus of healthcare workers in the Austrian state
Vorarlberg between June 2020 and January 2021

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Running title
SARS-CoV-2 serostatus of HCW in Austria
Structured Abstract

Objectives
Austria, and particularly its westernmost federal state Vorarlberg, developed an extremely high COVID-19 incidence rate in November 2020. Health care workers (HCW) may be at increased risk of contracting the disease within the working environment and therefore the seroprevalence in this population is of particular interest. We aimed to analyze SARS-CoV-2-specific antibody response in Vorarlberg HCW.

Design
Observational cohort study of HCW including testing at three different time points for the prevalence of anti-SARS-CoV-2 IgG antibodies specific for NP and RBD.

Setting
All five state hospitals of Vorarlberg.

Participants
A total of 395 HCW, enrolled at June 2020 (t<sub>1</sub>), two months after the end of the first wave, retested between October to November at the beginning of the second wave (t<sub>2</sub>), and again at the downturn of the second wave in January 2021 (t<sub>3</sub>).

Main outcomes
We assessed seroprevalence and associated factors, including demographic and clinical characteristics, symptoms consistent with COVID-19 infection, and infections verified by RT-PCR.

Results
At t<sub>1</sub>, 3% of HCW showed a strong IgG-specific responses to either NP or RBD. At t<sub>2</sub>, the rate had increased to 4%, and after the second wave in January 2021, 14% had a strong response, which was found to be stable for up to ten months. The amount of HCW with anti-SARS-CoV-2 IgG antibodies was 38% higher than the number of infections found by RT-PCR.

Conclusion and relevance
We found low numbers of SARS-CoV-2-seropositive HCW in a frontline setting after first wave but a very high increase during second wave. Though the seroprevalence in HCW was comparable to the general population. Our findings indicate that a realistic monitoring of SARS-CoV-2 infections would require increased surveillance and offer support for routine application of serological testing in the management of the ongoing COVID-19 pandemic.

[Keywords]
COVID-19; Public Health; Infection Control; Epidemiology; Occupational & Industrial Medicine; Clinical Chemistry
Strengths and limitations of this study

- Study participants were HCW having a high risk of becoming infected and infecting others with SARS-CoV-2.
- The study comprises data on the seroprevalence in Austria, after the first and the second wave, when Austria had one of the highest incidence rates worldwide.
- Data on antibody response are quantitative and also describe the respective stability over time.
- The study provides data for seroprevalence assessed by ELISA as well as for infections assessed by RT-PCR.
- The risk of HCW to be infected is impacted by and linked to the situation outside the hospital.

Word count

Abstract: 292
Main text: 3750 excluding references
Introduction

In March 2020 the coronavirus disease 2019 (COVID-19) was declared a global pandemic by the World Health Organization (WHO), with Europe at the time as the epicenter. The high numbers of cases and associated deaths first overwhelmed health care services in northern parts of Italy [1]. Several independent introducing events, mainly from Northern Italy have most likely contributed to clusters in Austria [2] and further accelerated the spread in many other European countries [3] during the so called first wave in March 2020. During the second and by far higher wave, peaking in Austria in November, Austria developed the highest incidence rate worldwide [4] and the federal state of Vorarlberg, despite its low degree of urbanization, reported one of the highest rates in Austria [5].

Health care workers (HCW) are on the first line of defense and have a high risk of becoming infected and infecting others with SARS-CoV-2 [6]. This has been first demonstrated in China [7] and has been confirmed in early reports from Italy, where HCW make up 9% of total cases and are over-represented amongst those affected by COVID-19 [1].

In contrast to real time reverse transcription polymerase chain reaction (RT-PCR) assays detecting SARS-CoV-2 for the initial 2-3 weeks after infection only [8], the immunoglobulin (Ig) G-specific response to SARS-CoV-2 epitopes is typically detectable in serum about two weeks after symptom onset and lasts considerably longer [9]. At least 95% of PCR-confirmed SARS-CoV-2 infected patients develop specific anti-SARS-CoV-2 antibodies [10]. The receptor binding domain (RBD) of the spike protein has meanwhile become the most common antigen used in seroconversion assays, as it has received FDA emergency approval [11] and has also been shown to correlate well with neutralizing activity [10,12–14].

This study thus investigates the dynamics of IgG-specific response against RBD and the nucleocapsid protein (NP) of SARS-CoV-2 in serial serum samples collected from 395 HCW after the first wave (June – August 2020), at the beginning of the second massive wave (October 2020), and at the downturn of the second wave (January 2021) using enzyme linked immunosorbent assay (ELISA).
Methods

Study subjects

This study comprises 395 participants of mainly Caucasian origin with a median age of 42 (min. 18 – max. 64) years working as HCW in Vorarlberg, the westernmost federal state of Austria. All participants are employed by one of the Vorarlberg state hospitals and 174 (44%) at a COVID-19-specialized hospital.

Study enrolment was voluntary and free of charge for the participants. All subjects reported to be in healthy condition. At the time of recruiting, participants completed a survey form which captured demographic information as well symptoms of COVID-19 infection in the three months prior to collection of the serum sample. Additionally, data on SARS-CoV-2-specific RT-PCR tests were collected, which had been ordered by the hospital at any suspicion of a possible infection or performed as part of routine institutional screening.

After the first wave in March 2020 and after the first hard lockdown in Austria (16th of March to 30th of April) blood samples were collected. Collection took place between 26th of June and 19th of August 2020 and is referred to as time point 1 (t1). Identical criteria were applied for the second round of sampling between 2nd October and 13th November (t2) and the third round between 7th and 20th January 2021 (t3). Thus, sampling at t2 took place mostly at the beginning of the second wave 2020 and at t3 after the second wave, during the third hard lockdown in Austria (17th November to 6th December). A summary of the study timeline is given in figure 1.

Data on 7-day incidence were obtained from the Austrian Open Government Data [15]. Only 5 out of 395 participants were missing at t2 and 24 at t3 due to end of employment, withdrawal of consent, or due to other reasons. Hence, the follow-up rate at t2 and t3 was 99 % and 94%, respectively.

Study data and laboratory analyses

Study data were collected and managed using REDCap electronic data capture tools [16,17] hosted at VIVIT. Acute SARS-CoV-2 infection was determined by virus detection through RT-PCR of nasopharyngeal swabs at the Institute of Pathology, Academic Teaching Hospital.
Feldkirch (Feldkirch, Austria). At each time point, venous blood was collected, processed, and anti-SARS-CoV-2 antibodies were detected in human serum via an ELISA specifically detecting IgGs directed against the recombinant NP RBD (5600100 Technozym, Technoclone, Vienna, Austria, [13]) according to the manufacturer’s protocol. Concentrations were calculated according to internal calibration standards using the Xlfit software package (Version 5.3.1.3, IDBS) with 1 U/mL representing 100 ng/ml of a SARS-specific antibody [18].

According to manufacturer’s protocol, values <5 U/mL were referred to as normal or background range representing the absence of SARS-CoV-2-specific antibody response. Values ≥5 U/mL were referred to as positive responses. The 5 U/mL cutoff was defined on basis of criteria suggested by the Youden index and the 99th percentile method [19]. Values ≥5 and <9 U/mL for anti-SARS-CoV-2 RBD-specific antibody response or ≥5 and <8 U/mL for anti-SARS-CoV-2 NP-specific antibody responses were referred to as a moderate positive response. Accounting for the prevalence nature of the study, a higher cut-off of ≥9 U/mL was chosen for anti-SARS-CoV-2 RBD IgG and ≥8 U/mL for anti-SARS-CoV-2 NP IgG to increase specificity, as proposed by the manufacturer and by a previous study [19]. Values ≥9 and ≥8 U/mL, respectively were thus referred to as a strong positive response. IgG concentration was measured at time points $t_1$, $t_2$, and $t_3$. Participants whose antibody levels increased between time points from background to moderate, from moderate to strong, or from background to strong response were referred to as converters. Participants with (i) a moderate or strong response at an earlier time point and (ii) no conversion during following time points and (iii) a declined or unchanged response (including also marginally increased responses not higher than 10% or 1 U/mL, respectively) were referred to as moderate or strong response decliners, respectively. The half-life of antibody response as well as the time until antibody response has dropped under the 5 U/mL threshold for seropositivity was extrapolated, assuming an exponential decline.
**Statistical analysis**

Differences in baseline characteristics were tested for statistical significance using Chi-squared tests for categorical variables, the Mann-Whitney-U tests for continuous, not normally distributed, and unpaired continuous variables, and the Wilcoxon tests for continuous, not normally distributed, and paired variables. Correlation analyses were performed calculating nonparametric Spearman rank correlation coefficients. Results are given as mean if not denoted otherwise, and p-values of 0.05 were considered significant. All statistical analyses were performed with SPSS 26.0 for Windows (IBM corp., USA), and R statistical software v. 3.5.1 (http://www.r-project.org). All values were analyzed according to complete case analysis.
Results

Seroprevalence between June 2020 and January 2021

The anti-SARS-CoV-2 specific IgGs against RBD and NP were assessed at three time points, after first wave (t₁), at the beginning of second wave (t₂), and after second wave (t₃; figure 1). The respective mean concentrations of our study participants (supplementary table 1 and supplemental table 2), the correlation of RBD- to NP- specific IgGs, as well as the proportion of seropositive subjects (5 U/mL cut-off) and in particular the seropositive subjects with a strong response (strong responder: 9 U/mL cut-off) are summarized in table 1 and figure 2 for the three time points t₁, t₂, and t₃. Overall, 73 (18%) out of all 395 HCW have been tested at least once positive at any time point (t₁, t₂, or t₃) during the study.

Change of antibody response during study

The shift of RBD- and NP-specific antibody response between time point t₁ and t₃ is depicted in supplemental figure 1 and the change is summarized in supplemental table 3. Overall, the RBD- and NP-specific IgG concentration increased during the study. Between t₁ and t₃, 44 HCW (12%) seroconverted to a strong response (t₁-t₃-strong response converter) and 6 (2%) to a moderate response (t₁-t₃-moderate response converter). Out of these 44 t₁-t₃-strong response converter, 43 converted from no response at t₁ to a strong response at t₃, and only one participant from a moderate response to a strong response. The mean increase for these 44 t₁-t₃-strong response converter was 42.3-fold for RBD- and a 43.7-fold for NP-specific antibody response; for the 6 t₁-t₃-moderate converters 3.5-fold and 2.3-fold, respectively.

Further, 19 HCW were found to have a declined antibody response between t₁ and t₃ (t₁-t₃-decliner). Of these, nine had a strong response at t₁ (t₁-t₃-strong response decliner) and ten a moderate response (t₁-t₃-moderate response decliner). The decrease of antibody response between t₁ and t₃ (5.7 months) and between t₂ and t₃ (2.8 months) is summarized in supplemental table 3. Taking into account the t₁-t₃ and t₂-t₃ time overlap, in total, 23 individuals have declined antibody responses between measurements at t₁/t₂ and t₃ during a
median time of 5.0 months. Overall, the RBD-and NP-specific antibody response of these 23 decliner has decreased by 19% per month for both. The monthly decline of antibody response was significantly correlated with the strength of response measured at t\textsubscript{1}/t\textsubscript{2} with an r of 0.706 (p<0.001) for RBD and an r of 0.887 (p<0.001) for NP (supplemental figure 2). Strong responders had a more pronounced monthly decline than moderate responder and the proportional decline between t\textsubscript{2} and t\textsubscript{3} was comparable to the one between t\textsubscript{1} and t\textsubscript{3} in spite of the shorter time span (supplemental table 3). Taking into account that exponential decline, the median half-life of RBD- and NP-specific responses were 5.5 [2.3-15.8] and 5.7 [2.2-11.2] months. In addition, the median time in which a positive antibody response (5 U/mL cut-off) for either RBD or NP can be maintained was 6.0 [1.6-19.8] months for all decliners and 10.2 [6.3-23.4] months for the strong-response decliner.

Of note, we did not find any elimination of a strong response between t\textsubscript{1} and t\textsubscript{2} or between t\textsubscript{1} and t\textsubscript{3}. In contrast, out of the mentioned 12 moderate responders at t\textsubscript{1} only 3 still had a moderate response at t\textsubscript{3}, 1 resigned, 1 converted to a strong response, and 7 did not reach the cut-off for moderate response at t\textsubscript{3}.

**Association of antibody response with RT-PCR data.**

Out of 395 HCW tested for SARS-CoV-2-specific IgGs, 249 have also been tested at least once for the presence of an acute infection with SARS-CoV-2 during the study by RT-PCR and 53 of these were positive. As mentioned above, applying ELISA, 73 out of all 395 HCW have been tested positive at least once for SARS-CoV-2-specific IgGs during the study. Thus, the number of HCW with ELISA-assessed positive antibody response is 38% higher (n=20) than infections detected by RT-PCR in the whole study population.

Taking into account only HCW who have been tested by both methods, RT-PCR and ELISA, we found that only four RT-PCR-positive HCW had no antibody response, reflecting an antibody response rate of 92% in RT-PCR-positive tested HCW. In contrast, only 73% of HCW with an antibody response have also been tested RT-PCR-positive (46/63). Regarding a strong antibody response, only 83% had been tested RT-PCR-positive (43/52).
Association of antibody response with COVID-19-symptoms and further parameters

Taking into account the survey data, HCW who had COVID-19-symptoms at $t_3$ were significantly more likely to be seropositive than asymptomatic ones (36% vs. 8% $p<0.001$), but this was not the case at $t_1$ ($p=0.193$) or $t_2$ ($p=0.645$). Further, there was no significant difference between male and female HCW being seropositive at any time point (21% vs. 18%, $p=0.518$) or between HCW with a BMI $\geq 25$ compared to those with BMI <25 (22% vs. 17%, $p=0.226$).

HCW above 40 years had a similar prevalence compared to younger ($\leq 40$ years) ones (16% vs. 18%, $p=0.603$). Participants sharing their household with children or adolescents younger than 25 years had no significantly increased risk for being seropositive compared to participants without younger persons in their households (19% vs. 14%, $p=0.202$). HCW working at a regular hospital had a slightly but not significantly lower prevalence than those at a COVID-19-specialized hospital (14% vs. 21%, $p=0.068$) and also smokers had a lower prevalence, which just failed significance (9% vs. 18%, $p=0.060$).
Discussion

Main findings

In our study the antibody response was clearly higher after the second massive wave compared to the first wave reflecting the incidence rate in Austria (figure 1 and [15]). Of note, the number of undetected SARS-CoV-2 infections during our study was quite high as only 83% of HCW with a strong antibody response, had previously been identified by RT-PCR. Moreover, a conversion to a strong response during the study was much more likely than conversion to a moderate response only and a strong response was more stable than a moderate response.

A further important finding was that we experienced no elimination of a strong response during the study: All participants with a strong response maintained a positive response during the study and, according to extrapolation, will keep it for 10 months. Similarly, the half-life of positive antibody responses was about six months for both, the RBD- and NP-specific response.

Seroprevalence after the first wave in the light of other study data on HCW

Our data revealing a 3% seroprevalence at t, after the first wave, are slightly above those from HCW in Germany [20,21] and Italy, apart from the North [22,23] being in the range of 1–2% around the same time. Higher rates of 5-6% were seen in the Veneto Region, Italy [24], Belgium [25], Norway [26], and Northern England [27]. One of the highest incidence rates of COVID-19 infections in the world were seen in the US, with a seroprevalence rate of 19% in the general population [28] and 27% in HCW at the same time [29]. Almost similar rates were found in HCW in Sweden (19%) [30] and some parts of the UK namely London (32%) [31] and Birmingham (24%) [32]. Nevertheless, these rates are still below the seropositivity rate of 67%, which has initially been estimated as threshold for community immunity against SARS-CoV-2 [33] and now has been estimated to be as high as 85% according to CDC [34].
Seroprevalence at the beginning and at the end of the second wave

A recent seroprevalence study of the general population in Austria comprising 2229 participants and collecting samples between 12th to 14th November, which took place during the second wave found neutralizing antibodies in 92 samples reflecting a seroprevalence of 4.7% [35]. This is just matching our data about the same time (t2) and thus proposes that HCW in Vorarlberg were well prepared facing the challenges by COVID-19 in the local health care system although they might have a higher chance of being infected than the general population. Passing the second wave, Austria had one of the highest incidence rates in the world [4] and the seroprevalence after the second wave has been hypothesized to be about 15% in the general population [36]. Around the same time, at t3 of our study, we found a massive increase to 14 %, having a strong antibody response. This proposes again that HCW in Vorarlberg may have had an infection rate comparable to the general population. As all HCW in Vorarlberg had the opportunity for vaccination starting on 7th January, it remains speculative whether the seroprevalence might have further increased or plateaued.

Seroconversion, protection and reinfection

Even though our study primarily aimed at observing the prevalence of seroconversion of all HCW during first and second wave of the COVID-19 pandemic, when focusing only on the subgroup of responders we found that a strong response was more stable than a moderate response.

These findings are in good alignment with the very fast increase in antibody titers and neutralization within only 10 days after symptom onset, tested with the same assay [19]. All participants who once have developed a strong response maintained a positive response, either still a strong one or at least a moderate one, during the full study time. An extrapolation, thus, suggests that these strong responders will keep their response for about ten months. This is in line with previous data of recent studies in the UK and Spain, demonstrating that SARS-CoV-2 infection-acquired immunity is present for at least six months [14,25] and suggesting that protective immunity will last up to a few years [14]. A further study in New York
City has found only a moderate decline regarding the spike protein-specific response during five months [10]. We here report a mean decline of 51% and 60% during five months for RBD- and NP-specific responses, respectively. A decrease of 17% and 31% for anti-spike IgG and anti-NP IgG titers has been reported in a study comprising 847 workers at Institute Curie in Paris during 4-8 weeks accounting rather short-lived immune responses of only 87 days for anti-spike IgG and 35 days for anti-NP IgGs, respectively [12]. Wajnberg et al. have suggested that the stability of the antibody response over time may depend on the serologic target [10] with a faster decline of NP compared to RBD. Other than NP, the spike protein is the main and potentially the only target for neutralizing antibodies [37]. It thus appears that the RBD is more suited than NP for surveillance of long-term immune response by ELISA. Nevertheless, RBD-specific IgG response as investigated in our study as well as in most others on seroprevalence is only a fragment of the very complex post-infection immunity and longevity of response.

Finally, we also have noticed one case in which a moderate antibody response at \( t_1 \) has converted to a strong response at \( t_3 \), representing a reinfection according to PCR data. That said, the number of responders at \( t_1 \) and \( t_2 \) is small compared to the initial study number and thus the conclusions (including those regarding reinfection, immunity, elimination time, and half-life) for this subgroup are limited and should be taken with care. Further limitations are mentioned in the following.

**Limitations**

This study is not a random sample of either the general population or the HCW of Vorarlberg and the infection risk of HCW is significantly impacted by the situation outside the hospital. Further, the data should be interpreted with caution, as it is possible that some of our participants which have been classified as “no response” due to a response below the assay cut-off of <5 U/mL were infected with SARS-CoV-2 a few months before sampling, and either had only a weak antibody response to start with and/or have dropped below the assay threshold since. Apart from that, our study only provides information about post-infection antibody-response and not about immunity or the chance of reinfections. In that context, it is
impossible to fully explain the nature of change of antibody-specific responses in our study, e.g. for responders of which some may be impacted by a secondary contact to the virus thus acting as kind of a booster. Furthermore, it has already been demonstrated that a NP- or spike-specific antibody response may not always be present following a proven SARS-CoV-2 infection [12]. Apart from that, a large variety of different commercial ELISAs has been used for the above-mentioned serological study data. Although IgG-specific ELISAs have been proposed to be appropriate for prevalence testing, accuracy significantly differs between different serological testing methods [38]. Finally some participants have been vaccinated during sampling at $t_3$, but in no case vaccination took place more than one week before sampling. IgG responses are generally not mounted within one week after vaccination [39], and converters at $t_3$ who have been vaccinated had responses for RBD and NP thus we preclude an effect of the RBD-based vaccine.

Given the limitations mentioned above, the antibody response is yet widely used as a surrogate for deciding whether post-infection immunity to SARS-CoV-2 exists. The antibody response in our study has proven to persist for several months. That said, our and others’ findings do not support exempting those positive for anti-SARS-CoV-2 antibodies from current infection control, other public health constraints, or the ongoing vaccination. Anyway, the current seroprevalence of HCW is far beyond any herd immunity threshold

**Conclusion**

Our findings suggest serological testing as routine application for determining and monitoring the detection rate of acute infections. It is therefore an important tool managing the ongoing COVID-19 pandemic. Given the 38% higher number of HCW with antibody response than RT-PCR-verified infections detected by current testing routine, and the at least 17% undetected infections of HCW in our hospitals indicates that a realistic monitoring of the situation would require an immediate and massively increased infection surveillance, either by routine serological, PCR-based, or other test strategies e.g. daily lateral flow tests. Apart from that, further studies are necessary to determine the long-time duration of post-infection antibody
response and immunity and compare it to vaccination data as this has major implications for
the future of the current SARS-CoV-2 pandemic and the public health system. For the
particular study participants, the ELISA may also be very helpful for determining the success
of vaccination.

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Contributorship statement
M.A. designed the study, collected data, managed the project, and wrote the manuscript. A.M.
designed the study and reviewed the manuscript. P.F. managed the project and reviewed the
manuscript. E.M.B. analyzed data and reviewed the manuscript. K.G. analyzed data and
reviewed the manuscript. M.K. designed the experimental setup and reviewed the manuscript.
M.D. designed the experimental setup and reviewed the manuscript. L.Sp. collected data and
reviewed the manuscript. B.M. collected data and reviewed the manuscript. A.V. collected data
and reviewed the manuscript. M.B. collected data and reviewed the manuscript. L.Se. collected
data and reviewed the manuscript. J.B.J. collected data and reviewed the manuscript. H.D.
designed the study and reviewed the manuscript. A.La. designed the study and reviewed the
manuscript. A.Le. designed the study, managed the project, analyzed data, and wrote the
manuscript.
Competing interest

No potential conflicts of interest relevant to this article were reported by M.A., A.M., T.W., P.F., E.M.B., K.G., M.K., M.D., L.Sp., B.M., A.V., M.B., L.Se., J.J., H.D., A.La., and A.Le..

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Data sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.
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The table summarizes the concentration of SARS-CoV-2 receptor binding domain (RBD) - and nucleocapsid protein (NP) - specific antibody response at the respective time point given as mean (with interquartile range). Correlation (r) is given together with the p-value according to spearman test. Seropositive HCW (comprising a moderate and a strong response) had a concentration of ≥ 5 U/mL for either RBD or NP-response. Seropositive with a strong response were characterized by a concentration of either ≥ 9 U/mL for RBD or ≥ 8 U/mL for NP.
Figure Legends

Figure 1: Study timeline
The figure presents the 7-day incidence per 100,000 inhabitants in Austria and in the federal state of Vorarlberg between February 2020 and January 2021. The time points of sampling (t_1, t_2, and t_3; solid black line) and lockdown (hatched line) are marked.

Figure 2: Concentration and spread of RBD- and NP-specific IgG response
SARS-CoV-2-specific anti-RBD and anti-NP-specific IgG response of study participants is depicted at study time point t_1 (A), t_2 (B), and t_3 (C). A reference range of 0-5 U/mL representing no response is separated from a moderate positive response (≥5 and <9 U/mL for anti-RBD IgG and ≥5 and <8 U/mL for anti-NP IgG) by a dashed green line and from a strong positive response (≥9 U/mL for anti-RBD and ≥8 U/mL for anti-NP) by a solid green line. The solid grey line represents a linear regression line (R^2).
figure 1

338x190mm (300 x 300 DPI)
figure 2

338x190mm (300 x 300 DPI)
Supplemental material

Supplemental table 1

| Characteristics                                      |        |
|------------------------------------------------------|--------|
| All participants; % (n)                              | 100 (395) |
| Age; years (min-max)                                 | 42 (18-64) |
| Female sex; % (n)                                    | 71 (282) |
| BMI (min-max)                                        | 25 (18-45) |
| Overweight or obese, % (n)                           | 35 (139) |
| Current smoking; % (n)                               | 18 (73) |
| Working in COVID-19-hospital; % (n)                  | 44 (174) |
| Children in household; % (n)                         | 53 (211) |
| PCR tested; % (n) / positive PCR; % (n)              | 63 (249) / 13 (53) |

Continuous data are given as mean, in the presence of a skewed distribution, mean values are given together with minimum and maximum values (min-max). Dichotomous data are given as proportion. BMI denotes body mass index and PCR polymerase chain reaction. The term children is summarizing all children or adolescents under 25 years. PCR stands for SARS-CoV-2-specific real time reverse transcription PCR.
### Supplemental table 2

#### Residence and profession

| Residence          | Vorarlberg | 364 (92.2%) |
|--------------------|------------|-------------|
|                    | out of Vorarlberg | 14 (3.5%)  |
|                    | not specified   | 17 (4.3%)  |
|                    | total          | 395 (100%) |

| Country of Birth   | Austria      | 300 (75.9%) |
|--------------------|--------------|-------------|
|                    | Germany      | 38 (9.6%)   |
|                    | Italy        | 12 (3.0%)   |
|                    | Other EU     | 11 (2.8%)   |
|                    | Outside EU   | 10 (2.5%)   |
|                    | not specified| 24 (6.1%)   |
|                    | total        | 395 (100%)  |

| Professional role  | Reception    | 10 (2.5%)  |
|--------------------|--------------|------------|
|                    | Secretarial  | 18 (4.6%)  |
|                    | Physician    | 96 (24.3%) |
|                    | Nursing/Physio | 250 (63.3%) |
|                    | Radiology    | 10 (2.5%)  |
|                    | Service      | 9 (2.3%)   |
|                    | Lab          | 1 (0.3%)   |
|                    | not specified| 1 (0.3%)   |
|                    | total        | 395 (100%) |
### Supplemental table 3

#### Seroconversion and decline of antibody response during study

| Time Interval | RBD | NP | Change of response | Change of response per month | Half-life in months |
|---------------|-----|----|--------------------|-----------------------------|-------------------|
| t1-t2 all HCW (n=371) | RBD | NP | +4.0 U/mL (335 %) | n.a. | n.a. |
| | | | +3.4 U/mL (270 %) | n.a. | n.a. |
| t1-t2-strong response converters (n=44) | RBD | NP | +35.9 U/mL (4233 %) | n.a. | n.a. |
| | | | +29.8 U/mL (4368 %) | n.a. | n.a. |
| t1-t2-moderate response converters (n=6) | RBD | NP | +4.0 U/mL (349 %) | n.a. | n.a. |
| | | | +2.6 U/mL (231 %) | n.a. | n.a. |
| all t1-t2-converters (n=50) | RBD | NP | +32.1 U/mL (3634 %) | n.a. | n.a. |
| | | | +26.5 U/mL (3611 %) | n.a. | n.a. |
| t1-t3 strong response decliners (n=9) | RBD | NP | - 7.8 U/ml (- 38 %) | - 1.5 U/mL (- 7 %) | 7.5 [4.5-215.4] |
| | | | - 11.7 U/ml (- 52 %) | - 2.1 U/mL (- 9 %) | 3.4 [2.7-11.5] |
| t1-t3 moderate response decliners (n=10) | RBD | NP | - 1.5 U/ml (- 38 %) | - 0.3 U/mL (- 7 %) | 5.6 [2.0-17.2] |
| | | | - 1.1 U/ml (- 36 %) | - 0.2 U/mL (- 6 %) | 7.6 [6.1-40.9] |
| all t1-t3-Decliners (n=19) | RBD | NP | - 4.5 U/mL (- 38 %) | - 0.8 U/mL (- 7 %) | 5.7 [3.8-17.2] |
| | | | - 6.1 U/mL (- 50 %) | - 1.1 U/mL (- 9 %) | 6.2 [2.9-17.3] |
| t2-t3 strong response decliners (n=11) | RBD | NP | - 27.8 U/ml (- 54 %) | - 11.9 U/mL (- 23 %) | 2.9 [0.9-4.6] |
| | | | - 16.3 U/ml (- 53 %) | - 6.7 U/mL (- 21 %) | 4.0 [1.5-17.6] |
| t2-t3 moderate response decliners (n=7) | RBD | NP | - 1.1 U/ml (- 23 %) | - 0.4 U/mL (- 7 %) | 11.0 [1.4-127.6] |
| | | | - 0.4 U/ml (- 18 %) | - 0.1 U/mL (- 6 %) | 10.6 [5.3-41.3] |
| all t2-t3-decliners (n=18) | RBD | NP | - 17.5 U/ml (- 52 %) | - 7.4 U/mL (- 22 %) | 3.5 [1.4-11.5] |
| | | | - 10.1 U/ml (- 51 %) | - 4.1 U/mL (- 21 %) | 5.1 [2.5-31.0] |
| all strong response decliners (n=13) | RBD | NP | - 23.3 U/mL (- 52 %) | - 9.0 U/mL (- 20 %) | 5.3 [1.8-14.5] |
| | | | - 20.9 U/mL (- 61 %) | - 6.7 U/mL (- 20 %) | 2.7 [1.8-5.1] |
| all moderate response decliners (n=10) | RBD | NP | - 1.5 U/mL (- 38 %) | - 0.3 U/mL (- 7 %) | 5.6 [2.0-17.2] |
| | | | - 1.1 U/mL (- 36 %) | - 0.2 U/mL (- 6 %) | 7.6 [6.1-40.9] |
| all decliners (n=23) | RBD | NP | - 13.8 U/mL (- 51 %) | - 5.2 U/mL (- 19 %) | 5.5 [2.3-15.8] |
| | | | - 12.3 U/mL (- 60 %) | - 3.9 U/mL (- 19 %) | 5.7 [2.2-11.2] |

The table summarizes decline as well as raise of antibody response for the respective time interval. Converters had an increase of antibody response from background to either moderate or strong. Decliners were defined as not converters and having either a decrease of a strong or moderate antibody response or no change of a strong or moderate antibody response. Median half-lives, given with interquartile range, were calculated assuming an exponential decline if applicable and are given in month until half of the initial response is lost.
Supplemental figure 1: Shift of RBD- and NP-specific IgG response during study

SARS-CoV-2-specific IgG responses of study participants at time point $t_1$ (black rhombs), are depicted ordered from high to low/background. The reference range ($< 5$ U/mL) representing no response is separated from a moderate positive response ($\geq 5$ and $< 9$ for anti-RBD and $\geq 5$ and $< 8$ for anti-NP) by a dashed green line and from a strong positive response ($\geq 9$ U/mL for anti-RBD and $\geq 8$ U/mL for anti-NP) by a solid green line. The matching responses at $t_2$ (circles), and $t_3$, (triangles) are connected by a vertical line. RBD-specific responses are represented by orange (for $t_2$) and red (for $t_3$) symbols, NP-specific responses by turquois (for $t_2$) and purple (for $t_3$) symbols.
Supplemental figure 2: Monthly decline of IgG response in correlation with baseline IgG response

The monthly decline of the SARS-CoV-2-specific response of study participants in relation to their response at baseline is depicted for anti-RBD-specific (A) and for anti-NP-specific IgGs (B). A reference range of 0-5 U/mL representing no response is separated from a moderate positive response (≥5 and <9 for anti-RBD and ≥5 and <8 for anti-NP) by a dashed green line and from a strong positive response (≥ 9 U/mL for anti-RBD and ≥ 8 U/mL for anti-NP) by a solid green line. Grey dots represent values outside the positive range and were excluded for calculation of the regression lines given as solid red and turquois lines with $R^2$ indicated.
STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

| Item No | Recommendation |
|---------|----------------|
| **Title and abstract** | 1 | (a) Indicate the study’s design with a commonly used term in the title or the abstract  
(b) Provide in the abstract an informative and balanced summary of what was done and what was found |
| **Introduction** | 2 | Explain the scientific background and rationale for the investigation being reported |
| **Objectives** | 3 | State specific objectives, including any prespecified hypotheses |
| **Methods** | 4 | Present key elements of study design early in the paper |
| **Setting** | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection |
| **Participants** | 6 | (a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up  
(b) For matched studies, give matching criteria and number of exposed and unexposed |
| **Variables** | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable |
| **Data sources/measurement** | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group |
| **Bias** | 9 | Describe any efforts to address potential sources of bias |
| **Study size** | 10 | Explain how the study size was arrived at |
| **Quantitative variables** | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why |
| **Statistical methods** | 12 | (a) Describe all statistical methods, including those used to control for confounding  
(b) Describe any methods used to examine subgroups and interactions  
(c) Explain how missing data were addressed  
(d) If applicable, explain how loss to follow-up was addressed  
(e) Describe any sensitivity analyses |
| **Results** | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed  
(b) Give reasons for non-participation at each stage  
(c) Consider use of a flow diagram |
| **Descriptive data** | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders  
(b) Indicate number of participants with missing data for each variable of interest  
(c) Summarise follow-up time (eg, average and total amount) |
| **Outcome data** | 15* | Report numbers of outcome events or summary measures over time |

Page No: 2, 4, 5-6, n.a.
Main results | 16 | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included. (b) Report category boundaries when continuous variables were categorized. (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period. | Table 2, supplement, 8-10 |

Other analyses | 17 | Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses. | 9-10, supplement |

**Discussion**

Key results | 18 | Summarise key results with reference to study objectives. | 11 |

Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias. | 13-14 |

Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence. | 11-13 |

Generalisability | 21 | Discuss the generalisability (external validity) of the study results. | 14-15 |

**Other information**

Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based. | 16 |

*Give information separately for exposed and unexposed groups.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.
SARS-CoV-2 RBD- and NP-specific antibody response of healthcare workers in the westernmost Austrian state Vorarlberg: A prospective cohort study

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SARS-CoV-2 RBD- and NP-specific antibody response of healthcare workers in the westernmost Austrian state Vorarlberg: A prospective cohort study

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Structured Abstract

Objectives

Austria, and particularly its westernmost federal state Vorarlberg, developed an extremely high incidence rate during the COVID-19 pandemic. Health care workers (HCW) worldwide are known to have an increased risk of contracting the disease within the working environment and, therefore, the seroprevalence in this population is of particular interest. We thus aimed to analyze SARS-CoV-2-specific antibody dynamics in Vorarlberg HCW.

Design

Prospective cohort study of HCW including testing at three different time points for the prevalence of anti-SARS-CoV-2 IgG antibodies specific for NP and RBD.

Setting

All five state hospitals of Vorarlberg.

Participants

A total of 395 HCW, enrolled at June 2020 (t₁), two months after the end of the first wave, retested between October to November at the beginning of the second wave (t₂), and again at the downturn of the second wave in January 2021 (t₃).

Main outcomes

We assessed weak and strong seropositivity and associated factors, including demographic and clinical characteristics, symptoms consistent with COVID-19 infection, infections verified by RT-PCR, and vaccinations.

Results

At t₁, 3% of HCW showed strong IgG-specific responses to either NP or RBD. At t₂, the rate had increased to 4%, and at t₃ to 14%. A strong response was found to be stable for up to ten months. Overall, only 55% of seropositive specimen had antibodies against both antigens RBD and NP, 29% had only RBD- and 16% only NP- specific antibodies. Compared to the number of infections found by RT-PCR, the amount of HCW being seropositive was 38% higher.

Conclusion and relevance

Serologic testing based on only one antigen implicates the risk of missing infections, thus the set of antigens should be broadened in future. The seroprevalence among participating HCW was comparable to the general population in Austria. Nevertheless, in view of undetected infections, monitoring and surveillance should be reconsidered.
[Keywords]
COVID-19; Public Health; Infection Control; Epidemiology; Occupational & Industrial Medicine; Clinical Chemistry
Stengths and limitations of this study

- The study comprises data on the seroprevalence of HCW in Austria, after the first and the second SARS-CoV-2 wave, when Austria had one of the highest incidence rates worldwide.
- The study comprises data on IgG-specific response to the viral nucleocapsid (NP) as well as to the receptor binding domain (RBD).
- Data on antibody response are quantitative and also describe the respective stability over time.
- The study provides data for seroprevalence assessed by ELISA as well as for infections assessed by RT-PCR.
- The seroprevalence assessed in this study is only based on infections and is not impacted by vaccination.

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Main text: 3979
Introduction

Since the World Health Organization (WHO) has declared COVID-19 a global pandemic in March 2020, virus spread is still unstoppable and Europe, especially Austria as an epicenter, is currently facing the fourth wave. During the second wave, peaking in Austria in November 2020, Austria developed the highest incidence rate worldwide [1] with the federal state of Vorarlberg, reporting the highest rates [2]. Health care workers (HCW) are on the first line of defense and have a high risk of becoming infected and infecting others with SARS-CoV-2 [3,4], but infection prevention in hospitals is still suboptimal [5].

In contrast to real time reverse transcription polymerase chain reaction (RT-PCR) assays detecting SARS-CoV-2 for the initial 2-3 weeks after infection only [6], the immunoglobulin (Ig) G-specific response to SARS-CoV-2 antigens is typically detectable in serum about two weeks after symptom onset and lasts considerably longer [7]. At least 95% of RT-PCR-confirmed SARS-CoV-2 infected patients develop specific anti-SARS-CoV-2 antibodies [8]. The receptor binding domain (RBD) of the spike protein, which enables binding and fusing into cell membrane, has meanwhile become the most common antigen used. It has received FDA emergency approval in seroconversion assays [9], has been shown to correlate well with neutralizing activity [8,10–12], and is the key antigen of current vaccines. The nucleocapsid protein (NP) is a multifunctional protein, which amongst others packages the viral genomic RNA and forms the helical nucleocapsid. In contrast to the spike protein and its RBD, tests that detect antibodies to NP are believed to be more sensitive [13] but are waning in the post-infection phase [14]. Apart from that, recent studies have provided information about considerably variability between anti-NP and anti-RBD enzyme linked immunosorbent assays (ELISAs) [15,16].

This present study investigates the dynamics of IgG response against SARS-CoV-2 using identically constructed ELISAs by the same manufacturer specific for RBD and NP. It therefore analyses serial serum samples collected from 395 HCW after the first wave, at the beginning of the second massive wave, and at the downturn of the second wave.
Methods

Study subjects

This prospective cohort study comprises 395 participants of mainly Caucasian origin with a median age of 42 years working as HCW in Vorarlberg, the westernmost federal state of Austria. All participants are employed by one of the state hospitals and 174 (44%) at a COVID-19-specialized hospital.

Study enrolment was voluntary and free of charge for the participants. Recruitment was initiated by informing all institutes at the respective hospitals about the study. The information has then been spread by word of mouth recruitment and bulletin boards. All subjects reported to be in healthy condition. At the time of recruiting, participants completed a survey form which captured demographic information as well as symptoms of COVID-19 infection in the three months prior to collection of the respective serum sample. Additionally, data on SARS-CoV-2-specific RT-PCR tests were collected, which had been ordered by the hospital at any suspicion of a possible infection or performed as part of routine institutional screening.

After the first wave in March 2020 and after the first full lockdown [17] in Austria (16th of March to 30th of April) blood samples were collected. Baseline collection took place between 26th of June and 19th of August 2020 and is referred to as time point 1 (t1). Identical criteria were applied for the following round of sampling between 2nd October and 13th November (t2) and between 7th and 20th January 2021 (t3). Thus, sampling at t2 took place mostly at the beginning of the second wave 2020 and at t3 after the second wave, during the third full lockdown in Austria (17th November to 6th December). All HCW in Vorarlberg had the opportunity for vaccination with Comirnaty (BNT162b2, Biontech, Pfizer) starting on 7th January. Thirty-three HCW were vaccinated ≤ 4 days before sampling at t3.

Only 5 out of 395 participants were missing at t2 and 24 at t3 due to end of employment, withdrawal of consent, or due to other reasons. Hence, the follow-up rate at t2 and t3 was 99% and 94%, respectively. A summary of the study timeline is given in figure 1.

Study data and laboratory analyses
Study data were collected and managed using REDCap electronic data capture tools [18,19] hosted at the Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT). Acute SARS-CoV-2 infection was determined by virus detection through RT-PCR of nasopharyngeal swabs at the Institute of Pathology, Academic Teaching Hospital Feldkirch (Feldkirch, Austria). At each time point, venous blood was collected, processed, and anti-SARS-CoV-2 antibodies were detected in human serum via two ELISAs specifically detecting IgGs directed against (i) RBD and (ii) NP (5600100 and 5600200 Technozym, Technoclone, Vienna, Austria [11]). Concentrations were calculated according to internal calibration standards using the Xlfit software package (Version 5.3.1.3, IDBS).

1 U/mL is representing 100 ng/mL of a SARS-specific antibody [20], and, referring to the WHO standard, is equivalent to 3.7 BAU/mL (IS 20/136) and 5.8 BAU/mL (IS 20/136) for NP and RBD, respectively. According to manufacturer’s protocol, values <5 U/mL were referred to as background range representing the absence of a SARS-CoV-2-specific antibody response. Values ≥5 U/mL were referred to as positive responses. The 5 U/mL cut-off was defined on basis of criteria suggested by the Youden index and the 99th percentile method [21]. In order to meet ongoing concerns about accuracy and cut-offs, values ≥5 and <8 U/mL for anti-SARS-CoV-2 RBD-specific and anti-SARS-CoV-2 NP-specific antibody responses were referred to as a weak positive response. Accounting for the prevalence nature of the study, a higher cut-off of ≥8 U/mL was chosen to increase specificity, as proposed by the manufacturer and by a previous study [21]. Values ≥8 U/mL were thus referred to as a strong positive response. IgG concentration was measured at time points t₁, t₂, and t₃. Participants whose antibody levels increased between time points from background levels (<5 U/mL) to a positive response or from a weak to a strong response, were referred to as converters. Participants with (i) a weak or strong response at an earlier time point and (ii) no conversion during following time points and (iii) a declined or unchanged response (including also marginally increased responses not higher than 10% or 1 U/mL, respectively) were referred to as non-converters. Antibody decay and half-life of antibody response was assumed to follow a first order exponential decline.
Statistical analysis

Differences in baseline characteristics were tested for statistical significance using Chi-squared tests for categorical variables, the Mann-Whitney-U tests for continuous, and unpaired continuous variables, and the Wilcoxon tests for continuous and paired variables. Correlation analyses were performed calculating nonparametric Spearman rank correlation coefficients.

All values were analyzed according to complete case analysis. P-values below 0.05 were considered significant. All statistical analyses were performed with SPSS 28.0 for Windows (IBM corp., USA), and R statistical software v. 3.5.1 (http://www.r-project.org).

Patient and public involvement

All participants were HCW at the respective hospitals and were involved, insomuch as they supported recruitment and conduct of the study. The study results will be shared with the participants through the hospitals’ public relations department, various media handles, and conferences.

Results

Seroprevalence between June 2020 and January 2021

The characteristics of the study participants is summarized in table 1 and supplemental table 1. The anti-SARS-CoV-2 specific IgGs against RBD and NP were assessed in 395 HCW at three time points, after first wave \( (t_1) \), at the beginning of second wave \( (t_2) \), and after second wave \( (t_3) \). Figure 1.

During the study, we collected in total 1156 specimens and performed 2312 tests, 1156 for RBD-specific and 1156 for NP-specific IgGs. The overall serum concentration of RBD and NP ranged between 0 and 200 U/mL with a median of 0.4 U/mL for both RBD and NP. The correlation of RBD- to NP- specific IgG concentration, as well as the proportion of seropositive
subjects (≥5 U/mL) and in particular the seropositive subjects with a strong response (≥8 U/mL) are summarized in table 2 and figure 2 for the three time points t₁, t₂, and t₃. Overall, 73 (18%) out of all 395 HCW have been tested at least once positive, either regarding RBD or NP, at any time point (t₁, t₂, or t₃) during the study.

Comparison of RBD- and NP-specific IgG response

Out of 1156 specimen tested 111 displayed a positive antibody response and 1045 a negative response. Out of these 111 specimen, 93 had antibodies against RBD and 79 against NP. In detail, only 61 specimen (55% of seropositive specimen) had coexisting antibodies against both antigens. The remaining 50 (45%) specimen had either only antibodies against RBD but not against NP (n=32; 29%) or against NP but not against RBD (n=18; 16%, supplemental table 2). Taking into account positive and negative test results, the concordance of NP- and RBD-specific response was 96%, the sensitivity of RBD-specific responses was 77%, and the sensitivity of NP-specific responses was 66% (table 3). This clear discrepancy referring to spread and amount of NP- and RBD-specific responses is illustrated in figure 2.

Change of antibody response during time

Overall, the number as well as the intensity of RBD- and NP-specific IgG concentration increased during the study (supplemental figure 1 and supplemental table 3). Between t₁ and t₃, 44 HCW (12%) seroconverted to a strong (≥8 U/mL) response (t₁-t₃-strong response converters) and 6 (2%) to only a weak (≥5 and <8 U/mL) response (t₁-t₃-weak response converters). Out of these 44 t₁-t₃-strong response converters, 43 converted from no response at t₁ to a strong response at t₃, and only 1 participant from an existing weak response to a strong response. The mean increase, compared to the background signal for these 44 t₁-t₃-strong response converters was 42.3-fold for RBD- and a 43.7-fold for NP-specific antibody response, and for the 6 t₁-t₃-weak converters 3.5-fold and 2.3-fold, respectively (supplemental table 3).
In contrast, 19 HCW were found to have a declined antibody response between t₁ and t₃ (t₁-t₃-decliner). Of these, 10 had a strong response at t₁ (t₁-t₃-strong response decliners) and 9 a weak response (t₁-t₃-weak response decliners).

Taking into account the t₁-t₃ and t₂-t₃ time overlap, in total, 23 individuals have declined antibody responses between t₁/t₂ and t₃ during a median time of 5.0 months (all decliners). The RBD- and NP-specific antibody response of these 23 decliners has decreased by 51% and 60%, respectively (supplemental table 3). The monthly decline of antibody response was 19% for RBD just as for NP (supplemental table 3). This decline was significantly correlated with the strength of response measured at t₁/t₂ with an r of 0.71 (p<0.001) for RBD and an r of 0.89 (p<0.001) for NP (supplemental figure 2). Strong responders had a more pronounced monthly decline than weak responders (supplemental table 3). Taking into account the exponential nature of decline, the median half-lives of RBD- (5.5 [2.3-15.8] months) and NP-specific antibody responses (5.7 [2.2-11.2] months) were comparable (supplemental table 3). In addition, the median time in which a positive antibody response (≥5 U/mL cut-off) for either RBD or NP can be maintained was 6.0 [1.6-19.8] months for all decliners and 10.2 [6.3-23.4] months for strong-response decliners.

Of note, we did not find any elimination of a strong response between t₁ and t₂ or between t₁ and t₃. In detail, every HCW who had a strong RBD-specific antibody response at t₁ or t₂ maintained a positive RBD-specific response during the study. However, three subjects with a strong NP-specific response, who also had a RBD-specific response, had lost their NP-specific responses, but maintained their RBD-specific response.

In contrast, out of 11 HCW with only a weak response at t₁, only 2 kept a weak response at t₃ (1 resigned, 1 converted to a strong response, and 7 fell beneath the cut-off for a weak response).

**Association of antibody response with RT-PCR data and vaccination**

Out of 395 HCW tested for SARS-CoV-2-specific antibodies, 249 have also been tested at least once for the presence of an acute infection with SARS-CoV-2 during the study by RT-
PCR, and 53 of these were positive. As mentioned above, applying ELISA, 73 out of 395 HCW have been tested positive at least once for SARS-CoV-2-specific antibodies during the study. Thus, the number of HCW with ELISA-assessed positive antibody response is 38% higher (n=20) than all infections detected by RT-PCR in the whole study population.

Focusing the situation at the time point of final sampling (t₃) and taking into account only HCW (n=48) who have been tested by both methods (RT-PCR and ELISA) we found that only five HCW with a RT-PCR-proven COVID-19 infection had no antibody response, reflecting an antibody response rate of 90% (43/48). Regarding RBD- and NP-specific antibody response separately, the response rate was 83% for RBD- and 73% for NP-specific response. However, only 67% had a positive response for both, RBD- as well as NP- specific, IgGs. This comes down to 50% when considering only strong responses (supplementary table 4).

The other way round, only 69% (43/62) of seropositive HCW (either with a RBD-specific or a NP-specific antibody response) at t₃ have ever been identified by RT-PCR to be infected. Regarding RBD and NP separately, RT-PCR identified 73% (40/55) of those HCW having RBD-specific IgGs and 74% (35/47) of those with NP-specific IgGs.

Apart from that, it has to be mentioned that 33 participants have been vaccinated before blood sampling at t₃. Of these, 31 were seronegative and two seropositive. One seropositive participant had a strong RBD- and a coexisting strong NP-specific IgG response, the other had only a strong NP-specific response. However, in both cases, vaccination occurred just one day before blood sampling, precluding any effect of the vaccine on the obtained data.

**Association of antibody response with COVID-19-symptoms and further parameters**

Taking into account the survey data, HCW who had COVID-19-specific symptoms at t₃ were significantly more likely to be seropositive than asymptomatic ones (36% vs. 8% p<0.001).

When comparing four categories (A-D) according to antigen-specific response, comprising HCW (A) without any response, (B) with only NP-specific response, (C) with only RBD-specific response, and (D) with both RBD-and NP-specific response, the percentage of HCW with
symptoms gradually and significantly increased (A=24.0%, B=42.9%, C=46.7%, D=77.5%; p<0.001). This demonstrates that symptoms were >3 times more common in the group having IgGs against both antigens (RBD and NP) compared to those without any IgGs. Further data comparing HCW characteristics and antigen-specific response are provided in supplementary table 5.
Discussion

Main findings

The serological immune responses after viral infection is highly variable in our study. There was a clear discrepancy between NP- and RBD-specific responses. In addition, COVID-19-specific symptoms gradually increased in line with the antigen response from no response to a NP-specific, to a RBD-specific, and to a coexisting RBD- and NP-specific response. We also found that a conversion to a strong response during the study was much more likely than a conversion to a weak response only. A further important finding was that a strong response was more stable than a weak response. We experienced no elimination of a strong response during the study: All participants with a strong response maintained a positive response during the study. The half-lives of NP- and RBD-specific responses were comparable. Finally, the number of undetected SARS-CoV-2 infections during our study was quite high, as only 83% of HCW with a strong antibody response had previously been identified by RT-PCR.

Seroprevalence in the light of other study data on HCW

Our data in HCW revealed a 3% seroprevalence (strong response) at t₁, after the first wave. This was slightly above those from HCW in Germany [22,23] being in the range of 1–2% around the same time. Higher rates of 5-6% were seen in the Northern Italy [24], Belgium [25], Norway [26], and Northern England [27], and particularly in the US, with a seroprevalence rate of 19% in the general population [28] and 27% in HCW at the same time [29].

At t₂ and t₃, when Austria was passing the second wave and had one of the highest incidence rates in the world [1], the seroprevalence in our study increased to 4% (t₂) and finally to 14% (t₃). This was just matching the seroprevalence of the general population in Austria at the same time points (t₂: 4.7% [30] and t₃: 15% [31]). Therefrom, HCW in Vorarlberg appeared to be well prepared facing COVID-19 in the local health care system, although they were initially supposed to have a higher chance of being infected than the general population.
That said, the number of HCW with a positive antibody response was 38% higher than RT-PCR-verified infections detected by current testing routines of HCW in the hospitals. Given the at least 17% undetected infections of HCW in our hospitals, one may reconsider infection surveillance.

**Limited overlap of NP- and RBD-specific IgG responses**

Currently, no vaccine used in the EU is based on the NP-antigen. Thus, the detection of NP-specific antibodies is exclusively raised by viral infection. As a consequence, NP-specific seroconversion may appear a promising tool for specifically detecting virus infection even in the context of vaccinated subjects. Our data, however, are questioning such applications as we found only a limited overlap of NP- and RBD-specific IgG responses in infected subjects. Furthermore, we also found a higher rate of symptoms in HCW with a response against both antigens than in those with a response against only a single antigen. This is in line with the magnitude of serological immune responses against SARS-CoV2 which is known to be highly variable [32]. In addition, it has also been demonstrated by others that a NP- or spike-specific antibody response may not always be present following a proven SARS-CoV-2 infection [10] or, in particular, that NP-specific antibody response is less pronounced compared to the spike protein-specific response [16].

In a recent study, the concordance between NP- and RBD-specific response of two different assay providers was only 87.5% in a UK study in 906 adults [15], which is yet beneath our data (96%). A further Canadian study testing 21676 specimen from March to August 2020 also used two different providers for detecting NP- and spike-specific IgGs and revealed a sensitivity of 73% for RBD with NP as standard [33]. This is more or less comparable to our study results, revealing 77% sensitivity, in which, however, identically constructed assays of the same provider were used. Moreover the same Canadian study suggested that the decline of NP-specific antibodies over time is substantial enough to affect the results of population seroprevalence surveys, especially in high prevalence settings [33].
We therefore conclude that looking for only a single antigen-response, as it is mainly the case with RBD, does not elucidate the real seroprevalence.

Seroconversion, protection and reinfection

When focusing on the subgroup of responders, we found that a strong response was more stable than a weak response. These findings are in good alignment with the very fast increase in antibody titers and neutralization within only 10 days after symptom onset, tested with the same assay as we did [21]. All participants who once have developed a strong response maintained a positive response, either still a strong one or at least a weak one, during the full study time. An extrapolation, thus, suggests that these strong responders will keep their response for about ten months. This is in line with previous data of recent studies in the UK and Spain, demonstrating that SARS-CoV-2 infection-acquired immunity is present for at least six months [12,25]. A further study in New York City has found only a moderate decline regarding the spike protein-specific response during five months [8]. We here report a mean decline of 51% and 60% during five months for RBD- and NP-specific responses, respectively. A decrease of 17 % and 31 % for anti-spike IgG and anti-NP IgG titers has been reported in a study comprising 847 workers at Institute Curie in Paris during 4-8 weeks accounting rather short-lived immune responses of only 87 days for anti-spike IgG and 35 days for anti-NP IgGs, respectively [10]. Wajnberg et al. have suggested that the stability of the antibody response over time may depend on the serologic target [8] with a faster decline of NP compared to RBD. That said, the magnitude of decline of NP-specific response in some studies cannot be attributed solely to the choice of NP as antigen and has been reported to be assay-specific [34].

Other than NP, the spike protein is the main and potentially the only target for neutralizing antibodies [35]. Nevertheless, RBD-specific IgG response as investigated in our study as well as in most others on seroprevalence is only a fragment of the very complex post-infection immunity and longevity of response.
Finally, we also have noticed one case in which a weak antibody response at $t_1$ has converted to a strong response at $t_3$, representing a reinfection according to PCR data. That said, the number of responders at $t_1$ and $t_2$ is small compared to the initial study number and thus the conclusions (including those regarding reinfection, immunity, elimination time, and half-life) for this subgroup are limited and should be taken with care. Further limitations are mentioned in the following.

## Limitations

This study is not a random sample of either the general population or the HCW of Vorarlberg as only HCW in hospitals have been recruited on a voluntary basis. The infection risk of HCW is significantly impacted by the situation outside the hospital. Further, the data should be interpreted with caution, as it is possible that some of our participants which have been classified as “no response” due to a response below the assay cut-off of <5 U/mL were infected with SARS-CoV-2 a few months before sampling, and either had only a weak antibody response to start with and/or have dropped below the assay threshold since. Apart from that, the present study only measured IgG and did not detect other Ig classes (e.g. IgM or IgA).

Although IgG-specific ELISAs have been proposed to be appropriate for prevalence testing, accuracy significantly differs between different serological testing methods [36]. In that context, we want to mention that a standard cut-off for BAU/mL is still lacking making a comparison of different test methods difficult. Apart from that, our study only provides information about post-infection antibody-response and not about immunity or the chance of reinfections. It is impossible to fully explain the nature of change of antibody-specific responses in our study, e.g. for responders of which some may be impacted by a secondary contact to the virus thus acting as kind of a booster. Finally, some participants have been vaccinated during sampling at $t_3$. IgG responses are not mounted before 14 days after vaccination [37] and, thus, the vaccination in our study, which took place not earlier than 4 days before sampling, can be precluded to have impacted our serologic measurements.
Given the limitations mentioned above, the antibody response is yet widely used as a surrogate for deciding whether post-infection immunity to SARS-CoV-2 exists. The antibody response in our study has proven to persist for several months. That said, our and others’ findings do not support exempting those positive for anti-SARS-CoV-2 antibodies from current infection control, other public health constraints, or the ongoing vaccination.

**Conclusion**

Serologic testing based on only one antigen implicates the risk of missing infections. We propose that the set of antigens should be broadened. Apart from the mainly used RBD, our data clearly suggest including NP in serologic routine. Further antigens e.g. the N-terminal domain (NTD) [38] or the M protein [39] may have the potential to advance serologic testing in future. In view of undetected infections represented by the higher number of HCW with antibody response than RT-PCR-verified infections detected by routine testing, monitoring of infections should be reconsidered, too. Apart from that, further studies are necessary to determine the long-time duration of post-infection antibody response in combination with vaccination approaches as this has major implications for the future fight against SARS-CoV-2 in view of current virus variants.
Ethics statements

Consent for publication

Consent was obtained from all participants.

Ethics approval

The present study conforms to the ethical guidelines of the 1975 Declaration of Helsinki and has been approved by the Ethics Committee of Vorarlberg (EK-2-4/2020). All participants gave informed consent to participate in the study before taking part.

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Contributors

ALa had the original idea. MA, AM, TW, HD, ALa, and ALe contributed to the study design and conceptualization. MA, AM, PF, and ALe managed the project. AM was responsible for ethical and regulatory submissions. ALa, ALe, and PF acquired funding. MK and MD provided experimental resources. MA, LSp, BM, AV, MB, LSe, JBJ collected data. EMB, KG, ALe analyzed data. HD is the guarantor. AM and ALe wrote the manuscript. All authors contributed to reviewing and approved the final version.

Competing interest

No potential conflicts of interest relevant to this article were reported by M.A., A.M., T.W., P.F., E.M.B., K.G., M.K., M.D., L.Sp., B.M., A.V., M.B., L.Se., J.J., H.D., A.La., and A.Le..

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Data sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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1 Tables and figures

2 Table 1

3 Characteristics

|                                      |                  |
|--------------------------------------|------------------|
| All participants; % (n)              | 100 (395)        |
| Age; years (min-max)                 | 42 (18-64)       |
| Female sex; % (n)                    | 71 (282)         |
| BMI (min-max)                        | 25 (18-45)       |
| Overweight or obese, % (n)           | 35 (139)         |
| Current smoking; % (n)               | 18 (73)          |
| Working in COVID-19-hospital; % (n)  | 44 (174)         |
| Children in household; % (n)         | 53 (211)         |
| PCR tested; % (n) / positive PCR; % (n) | 63 (249) / 13 (53) |

6 Table 1 summarizes the characteristics of all participants. Continuous data are given as mean, in the presence of a skewed distribution, mean values are given together with minimum and maximum values (min-max). Dichotomous data are given as proportion. BMI denotes body mass index and PCR polymerase chain reaction. The term children is summarizing all children or adolescents under 25 years. PCR stands for SARS-CoV-2-specific real time reverse transcription PCR.
Table 2

Antibody response during study

| participants | t₁ | t₂ | t₃ |
|--------------|----|----|----|
| all HCW | 100% (n=395) | | |
| seropositive: either RBD or NP (i) | 6% (n=24) | 4% (n=17) | 4% (n=16) |
| RBD (ii) | 18.24 (1.55-10.54) | 25.37 (5.73-13.16) | 24.32 (0.35-14.19) |
| NP (iii) | 13.45 (1.94-22.71) | 12.61 (1.21-22.11) | 19.49 (5.90-33.53) |
| RBD-NP | r=0.27, p<0.001 | r=0.78, p<0.001 | r=0.35, p<0.001 |
| all HCW | 100% (n=390) | | |
| seropositive: either RBD or NP (i) | 6% (n=25) | 5% (n=21) | 4% (n=16) |
| RBD (ii) | 35.55 (4.68-57.16) | 42.07 (7.06-88.65) | 46.36 (4.41-110.71) |
| NP (iii) | 17.04 (2.10-25.30) | 16.32 (1.82-19.65) | 25.65 (6.23-39.98) |
| RBD-NP | r=0.34, p<0.001 | r=0.68, p<0.001 | r=0.35, p<0.001 |
| all HCW | 100% (n=371) | | |
| seropositive: either RBD or NP (i) | 5% (n=17) | 3% (n=13) | 3% (n=11) |
| RBD (ii) | 64.20 (11.82-124.15) | 52.63 (3.85-120.99) | 81.04 (20.64-134.98) |
| NP (iii) | 23.86 (4.18-49.38) | 34.81 (15.45-56.97) | 40.98 (12.15-65.57) |
| RBD-NP | r=0.43, p<0.001 | r=0.19, p=0.68 | r=0.36, p=0.43 |
| all HCW | 100% (n=31) | | |
| seropositive: either RBD or NP (i) | 15% (n=55) | 13% (n=47) | 11% (n=40) |
| RBD (ii) | 32.14 (8.47-41.89) | 33.21 (8.35-41.89) | 38.74 (12.33-51.82) |
| NP (iii) | 24.44 (4.17-25.55) | 30.33 (8.91-29.91) | 32.66 (8.87-32.09) |
| RBD-NP | r=0.62, p<0.001 | r=0.50, p<0.001 | r=0.61, p<0.001 |

Table 2 summarizes the concentration of SARS-CoV-2 receptor binding domain (RBD) - and nucleocapsid protein (NP) - specific antibody response at the respective time point given as mean (with interquartile range). Correlation (r) is given together with the p-value according to spearman test. Seropositive HCW (comprising a weak and a
strong response) had a concentration of ≥ 5 U/mL for either RBD- or NP- specific response. Seropositive HCW with a strong response were characterized by a concentration of ≥ 8 U/mL for RBD or NP. Seropositive HCW were further discriminated into those with a RBD-specific response (ii), those with a NP-specific response (iii), those with either a RBD- or a NP-specific response (i) and those with both, a RBD- and a coexisting NP-specific response (iv).

Table 3

RBD- and NP-specific responses in comparison

| time point | seropositive | seropositive (strong response) |
|------------|--------------|--------------------------------|
| sensitivity of NP (=PPV for RBD) | t1 | 53% | 78% |
| | t2 | 57% | 54% |
| | t3 | 73% | 72% |
| | total | 66% | 69% |
| sensitivity of RBD (=PPV for NP) | t1 | 56% | 64% |
| | t2 | 75% | 64% |
| | t3 | 85% | 78% |
| | total | 77% | 73% |
| Concordance of NP and RBD | t1 | 96% | 98% |
| | t2 | 97% | 97% |
| | t3 | 94% | 94% |
| | total | 96% | 97% |

Table 3 summarizes the comparison between RBD- and NP- specific IgG responses of tests performed at the respective time points. Sensitivity of NP is given with RBD as standard. Sensitivity of RBD is given with NP as standard. The respective positive and negative counts are provided in the supplement (supplementary table 2). PPV = positive predictive value.

Figure Legends

Figure 1: Study timeline

The figure presents the 7-day incidence per 100,000 inhabitants in Austria and in the federal state of Vorarlberg between February 2020 and January 2021. The time points of sampling (t₁, t₂, and t₃; solid black line) and lockdown (hatched line) are marked. Data on 7-day incidence were obtained from the Austrian Open Government Data [40]. A detailed description of lockdown and public health measures in Austria is given elsewhere [17].
Figure 2: Concentration and spread of RBD- and NP-specific IgG response

A: The intensities of anti-RBD (squares) and anti-NP-specific IgG responses (triangles) of each individual subject (connected by a line) are depicted at study time point t<sub>1</sub>, t<sub>2</sub>, and t<sub>3</sub>. B: Correlation of anti-RBD and anti-NP-specific IgG response of study participants is depicted at study time point t<sub>1</sub>, t<sub>2</sub>, and t<sub>3</sub>. The solid grey line represents a linear regression line ($R^2$). The dashed green line separates positive responses ($\geq 5$ U/mL for anti-RBD and anti-NP IgG) from the background response. Values $\geq 8$ U/mL for anti-RBD and anti-NP IgG, representing a strong response, are separated by a solid green line.
figure 1

115x76mm (300 x 300 DPI)
Figure 2

84x77mm (600 x 600 DPI)
Supplemental material

Supplementary table 1

| Residence          | Vorarlberg | out of Vorarlberg | not specified | total     |
|--------------------|------------|-------------------|---------------|-----------|
| Residence          | 364 (92.2%)| 14 (3.5%)         | 17 (4.3%)     | 395 (100%)|
| Country of Birth   |            |                   |               |           |
| Austria            | 300 (75.9%)|                   |               |           |
| Germany            | 38 (9.6%)  |                   |               |           |
| Italy              | 12 (3.0%)  |                   |               |           |
| Other EU           | 11 (2.8%)  |                   |               |           |
| Outside EU         | 10 (2.5%)  |                   |               |           |
| not specified      | 24 (6.1%)  |                   |               |           |
| total              | 395 (100%) |                   |               |           |
| Professional role  |            |                   |               |           |
| Reception          | 10 (2.5%)  |                   |               |           |
| Secretarial        | 18 (4.6%)  |                   |               |           |
| Physician          | 96 (24.3%) |                   |               |           |
| Nursing/Physio     | 250 (63.3%)|                   |               |           |
| Radiology          | 10 (2.5%)  |                   |               |           |
| Service            | 9 (2.3%)   |                   |               |           |
| Lab                | 1 (0.3%)   |                   |               |           |
| not specified      | 1 (0.3%)   |                   |               |           |
| total              | 395 (100%) |                   |               |           |

Supplementary table 1 summarizes the residence and profession of all 395 HCW.
Supplementary table 2
RBD- and NP-specific IgG response during study

|                      | t1 RBD + | t1 RBD - | t2 RBD + | t2 RBD - | t3 RBD + | t3 RBD - | total RBD + | total RBD - |
|----------------------|----------|----------|----------|----------|----------|----------|------------|------------|
| NP +                 | 2.3%     | 1.8%     | 3.1%     | 1.0%     | 10.8%    | 1.9%     | 5.3%       | 1.6%       |
|                      | (9/395)  | (7/395)  | (12/390) | (4/390)  | (40/371) | (7/371)  | (61/1156)  | (18/1156)  |
| NP -                 | 2.0%     | 93.9%    | 2.3%     | 93.6%    | 4.0%     | 83.3%    | 2.8%       | 90.4%      |
|                      | (8/395)  | (371/395)| (9/390)  | (365/390)| (15/371) | (309/371)| (32/1156)  | (1045/1156)|
| NP +                 | 1.8%     | 1.0%     | 1.8%     | 1.0%     | 8.4%     | 3.2%     | 3.9%       | 1.5%       |
|                      | (7/395)  | (4/395)  | (7/390)  | (4/390)  | (31/371)| (9/371)  | (45/1156)  | (17/1156)  |
| NP -                 | 0.5%     | 96.7%    | 1.5%     | 95.6%    | 3.2%     | 86.0%    | 1.7%       | 92.9%      |
|                      | (2/395)  | (382/395)| (6/390)  | (373/390)| (12/371)| (319/371)| (20/1156)  | (1074/1156)|

Supplementary table 2 summarizes the comparison between RBD- and NP- specific IgG responses of tests performed at time points t1, t2, t3, and during the whole study (total). Seroconversion (positive response) was diagnosed at concentrations of ≥ 5 U/ml and, alternatively, at concentrations ≥ 8 U/ml when regarding a strong response only.
### Supplementary table 3

**Seroconversion and decline of antibody response during study**

|                          | Change of response | Change of response per month | Half-life in months |
|--------------------------|--------------------|------------------------------|---------------------|
|                          |                    | n.a.                         | n.a.               |
| t₁-t₃ all HCW (n=371)    | RBD                | +4.0 U/mL (335 %)            | n.a.               |
|                          | NP                 | +3.4 U/mL (270 %)            | n.a.               |
| t₁-t₃-strong response converters (n=44) | RBD | +35.9 U/mL (4233 %)          | n.a.               |
|                          | NP                 | +29.8 U/mL (4368 %)          | n.a.               |
| t₁-t₃-weak response converters (n=6) | RBD | +4.0 U/mL (349 %)            | n.a.               |
|                          | NP                 | +2.6 U/mL (231 %)            | n.a.               |
| all t₁-t₃-converters (n=50) | RBD | +32.1 U/mL (3634 %)          | n.a.               |
|                          | NP                 | +26.5 U/mL (3611 %)          | n.a.               |
| t₁-t₃-strong response decliners (n=10) | RBD | -7.4 U/ml (-38 %)            | -1.4 U/mL (-7 %)   |
|                          | NP                 | -10.5 U/mL (-52 %)           | -1.9 U/mL (-9 %)   |
| t₁-t₃ weak response-decliners (n=9) | RBD | -1.2 U/ml (-37 %)            | -0.2 U/mL (-7 %)   |
|                          | NP                 | -1.3 U/mL (-40 %)            | -0.2 U/mL (-7 %)   |
| all t₁-t₃-decliners (n=19) | RBD | -4.5 U/mL (-38 %)            | -0.8 U/mL (-7 %)   |
|                          | NP                 | -6.1 U/mL (-50 %)            | -1.1 U/mL (-9 %)   |
| t₂-t₃-strong response decliners (n=12) | RBD | -25.2 U/ml (-52 %)           | -11.9 U/mL (-25 %) |
|                          | NP                 | -14.9 U/mL (-51 %)           | -6.7 U/mL (-23 %)  |
| t₂-t₃-weak response-decliners (n=7) | RBD | -1.1 U/ml (-23 %)            | -0.4 U/mL (-7 %)   |
|                          | NP                 | -0.4 U/mL (-18 %)            | -0.1 U/mL (-6 %)   |
| all t₂-t₃-decliners (n=19) | RBD | -16.3 U/ml (-51 %)           | -7.4 U/mL (-23 %)  |
|                          | NP                 | -9.6 U/mL (-50 %)            | -4.1 U/mL (-22 %)  |
| all strong response decliners (n=13) | RBD | -23.3 U/mL (-52 %)           | -9.0 U/mL (-20 %)  |
|                          | NP                 | -20.9 U/mL (-61 %)           | -6.7 U/mL (-20 %)  |
| all weak response decliners (n=10) | RBD | -1.5 U/ml (-38 %)            | -0.3 U/mL (-7 %)   |
|                          | NP                 | -1.1 U/mL (-36 %)            | -0.2 U/mL (-6 %)   |
| all decliners (n=23)     | RBD                | -13.8 U/mL (-51 %)           | -5.2 U/mL (-19 %)  |
|                          | NP                 | -12.3 U/mL (-60 %)           | -3.9 U/mL (-19 %)  |

Supplementary table 3 summarizes decline as well as raise of antibody response for the respective time interval. Converters had an increase of antibody response from background to either weak or strong. Decliners were defined as not converters and having either a decrease of a strong or a weak antibody response or no change of a strong or weak antibody response. Median half-lives, given with interquartile range, were calculated assuming an exponential decline if applicable and are given in month until half of the initial response is lost. The decrease of antibody response between t₁ and t₃ and between t₂ and t₃ was referred to 5.7 and 2.8 months, respectively.
Supplementary table 4

|                  | participants | RBD (U/ml) | NP (U/ml) | RBD-NP correlation |
|------------------|--------------|------------|-----------|--------------------|
|                  | all HCW      | 100%       | 2.80      | 1.76               |
|                  | (n=182)      | (0.12-0.78)| (0.17-1.12)| r=0.35 p<0.001     |
|                  | seropositive | 7%         | 32.87     | 15.04              |
|                  | either RBD or NP (i) | (n=13) | (5.37-32.60) | (1.84-20.44) | r=0.27 p=0.36 |
|                  | seropositive: | 7%         | 35.39     | 14.80              |
|                  | RBD (ii)     | (n=12)     | (6.02-39.38)| (1.67-20.93) | r=0.45 p=0.14 |
|                  | seropositive: | 4%         | 44.96     | 23.56              |
|                  | NP (iii)     | (n=8)      | (9.26-104.60)| (10.22-26.94) | r=0.12 p=0.78 |
|                  | seropositive: | 4%         | 50.99     | 24.36              |
|                  | RBD and NP (iv)| (n=7) | (12.02-133.12)| (10.04-28.28) | r=0.25 p=0.59 |
|                  | seropositive: | 5%         | 45.09     | 20.95              |
|                  | (strong)     | (n=9)      | (10.18-89.63)| (8.47-25.60) | r=0.05 p=0.90 |
|                  | Seropositive: | 4%         | 50.39     | 21.33              |
|                  | (strong)     | (n=8)      | (12.45-111.38)| (7.68-26.94) | r=0.05 p=0.91 |
|                  | Seropositive: | 4%         | 49.66     | 25.94              |
|                  | (strong)     | (n=7)      | (8.35-133.12)| (10.75-28.28) | r=0.00 p=1.00 |
|                  | seropositive: | 3%         | 57.49     | 27.27              |
|                  | (strong)     | (n=6)      | (12.40-138.20)| (10.57-40.39) | r=0.03 p=0.96 |
|                  | all HCW      | 100%       | 26.62     | 24.69              |
|                  | (n=48)       | (6.75-32.10)| (4.22-21.28) | r=0.70 p=0.001    |
|                  | seropositive: | 90%        | 29.59     | 27.42              |
|                  | either RBD or NP (i) | (n=43) | (8.47-35.66)| (6.91-25.55) | r=0.59 p=0.001  |
|                  | seropositive: | 83%        | 31.60     | 28.36              |
|                  | RBD (ii)     | (n=40)     | (10.39-40.33)| (6.90-27.62) | r=0.69 p=0.001  |
|                  | seropositive: | 73%        | 33.57     | 32.88              |
|                  | NP (iii)     | (n=35)     | (9.15-49.17)| (8.86-32.82) | r=0.61 p=0.001  |
|                  | seropositive: | 67%        | 36.45     | 34.56              |
|                  | RBD and NP (iv)| (n=32) | (12.33-51.82)| (8.78-36.61) | r=0.68 p=0.001  |
|                  | seropositive: | 81%        | 31.95     | 29.81              |
|                  | either RBD or NP (i) | (n=39) | (10.82-41.89)| (7.51-28.31) | r=0.56 p=0.001  |
|                  | seropositive: | 69%        | 36.95     | 32.67              |
|                  | RBD (ii)     | (n=33)     | (12.81-50.94)| (7.14-35.34) | r=0.72 p=0.001  |
|                  | seropositive: | 63%        | 37.16     | 37.22              |
|                  | NP (iii)     | (n=30)     | (8.98-52.84)| (11.26-38.60) | r=0.63 p=0.001  |
|                  | seropositive: | 50%        | 45.34     | 43.00              |
|                  | RBD and NP (iv)| (n=24) | (16.35-53.47)| (11.00-49.32) | r=0.67 p=0.001  |

Supplementary table 4 summarizes the concentration of SARS-CoV-2 RBD- and NP-specific antibody response at time point t2 given as mean (with interquartile range) regarding their COVID-19 history proven by PCR. Out of 53 HCW with a RT-PCR-proven COVID-19 infection, 48 had also ELISA data at t1. Correlation (r) is given together with the p-value according to spearman test. Seropositive HCW (comprising a weak and a strong response) had a concentration of ≥ 5 U/mL for either RBD- or NP-specific response. Seropositivity with a strong response was characterized by a concentration of ≥ 8 U/mL (RBD and NP). Seropositive HCW were further discriminated into those with a RBD-specific response (i), those with a NP-specific response (ii), those with either a RBD or a NP-specific response (iii) and those with both, a RBD- and a coexisting NP-specific response (iv).
Supplementary table 5

|                                | Antigen specific response | p-value |
|--------------------------------|---------------------------|---------|
|                                | no (A)                    | NP only (B) | RBD only (C) | RBD & NP (D) |
| COVID-19 symptoms; %          | 24.0                      | 42.9     | 46.7         | 77.5         | <0.001     |
| Age ≥40 years; %               | 58.8                      | 71.4     | 40.0         | 60.0         | 0.78       |
| Male sex; %                   | 28.2                      | 42.9     | 20.0         | 35.0         | 0.52       |
| BMI ≥25; %                    | 34.2                      | 42.9     | 28.6         | 47.5         | 0.16       |
| Current smoking; %            | 19.7                      | 0.0      | 6.7          | 12.5         | 0.12       |
| In COVID-19-hospital; %       | 43.8                      | 42.9     | 66.7         | 55.0         | 0.07       |
| Children in household; %      | 54.1                      | 42.9     | 66.7         | 65.0         | 0.14       |

Supplementary table 5 compares characteristics of HCW in the context of antigen specific antibody response categories at t3: A = no NP- or RBD-specific antibody response; B = only NP-specific response; C = only RBD-specific response; D = NP- and RBD-specific response coexisting. BMI denotes body mass index. COVID-19 symptoms refers to characteristic symptoms reported by HCW up to 3 months before sampling at t3. The term children refers to all children or adolescents under 25 years. The p-value is given for trend A→B→C→D.
Supplementary figure 1

Supplementary figure 1: Shift of RBD- and NP-specific IgG response during study

SARS-CoV-2-specific IgG responses of study participants at time point t₁ (black rhombus), are depicted ordered from high to low/background. The reference or background range (<5 U/mL) representing no response is separated from a positive responses (≥5 U/mL) by a dashed green line and from a strong positive response (≥8 U/mL) by a solid green line. The matching responses at t₂ (circles), and t₃ (triangles) are connected by a vertical line. RBD-specific responses are represented by orange (for t₂) and red (for t₃) symbols, NP-specific responses by turquoise (for t₂) and purple (for t₃) symbols.
Supplementary figure 2: Monthly decline of IgG response in correlation with baseline IgG response

The monthly decline of the SARS-CoV-2-specific response of study participants in relation to their response at baseline is depicted for RBD-specific (A) and for NP-specific IgGs (B). The background (<5U/ml) representing no response is separated from a weak positive response (≥5 to <8 U/ml) by a dashed green line and from a strong positive response (≥ 8 U/mL) by a solid green line. Grey dots represent values outside the positive range and were excluded for calculation of the regression lines given as solid red and turquoise lines with R^2 indicated.
STROBE Statement—Checklist of items that should be included in reports of cohort studies

| Item No | Recommendation | Page No |
|---------|----------------|---------|
| **Title and abstract** | 1  
(a) Indicate the study’s design with a commonly used term in the title or the abstract  
(b) Provide in the abstract an informative and balanced summary of what was done and what was found | 1-2 |
| **Introduction** | 2  
Explain the scientific background and rationale for the investigation being reported | 5 |
| **Objectives** | 3  
State specific objectives, including any prespecified hypotheses | 5 |
| **Methods** | 4  
Present key elements of study design early in the paper | 6-7 Figure 1 |
| **Setting** | 5  
Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 6-7 Figure 1 |
| **Participants** | 6  
(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up  
(b) For matched studies, give matching criteria and number of exposed and unexposed | 6 n.a. |
| **Variables** | 7  
Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 7 |
| **Data sources/ measurement** | 8*  
For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 7 |
| **Bias** | 9  
Describe any efforts to address potential sources of bias | 11-12 |
| **Study size** | 10  
Explain how the study size was arrived at | 6 |
| **Quantitative variables** | 11  
Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 7-8 |
| **Statistical methods** | 12  
(a) Describe all statistical methods, including those used to control for confounding  
(b) Describe any methods used to examine subgroups and interactions  
(c) Explain how missing data were addressed  
(d) If applicable, explain how loss to follow-up was addressed  
(e) Describe any sensitivity analyses | 8 11-12 8 n.a. |
| **Results** | 13*  
(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed  
(b) Give reasons for non-participation at each stage  
(c) Consider use of a flow diagram | Table 1-3 n.a. n.a. |
| **Descriptive data** | 14*  
(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders  
(b) Indicate number of participants with missing data for each variable of interest  
(c) Summarise follow-up time (eg, average and total amount) | Table 1  Table 2  Figure 1, supplement |
| **Outcome data** | 15*  
Report numbers of outcome events or summary measures over time | Table 2-3 |
Main results 16  
(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included  
(b) Report category boundaries when continuous variables were categorized  
(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period  

Table 2-3, supplement, 8-12  

8-9, 11  
n.a.  

Other analyses 17  
Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses  

10-12, supplement  

Discussion  

Key results 18  
Summarise key results with reference to study objectives  

13  

Limitations 19  
Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias  

16-17  

Interpretation 20  
Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence  

13-17  

Generalisability 21  
Discuss the generalisability (external validity) of the study results  

17  

Other information  

Funding 22  
Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based  

18-19  

*Give information separately for exposed and unexposed groups.  

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.
**SARS-CoV-2 RBD- and NP-specific antibody response of healthcare workers in the westernmost Austrian state Vorarlberg: A prospective cohort study**

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**Primary Subject Heading:** Infectious diseases

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SARS-CoV-2 RBD- and NP-specific antibody response of healthcare workers in the westernmost Austrian state Vorarlberg: A prospective cohort study

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Structured Abstract

Objectives
Austria, and particularly its westernmost federal state Vorarlberg, developed an extremely high incidence rate during the COVID-19 pandemic. Health care workers (HCW) worldwide are known to have an increased risk of contracting the disease within the working environment and, therefore, the seroprevalence in this population is of particular interest. We thus aimed to analyze SARS-CoV-2-specific antibody dynamics in Vorarlberg HCW.

Design
Prospective cohort study of HCW including testing at three different time points for the prevalence of anti-SARS-CoV-2 IgG antibodies specific for NP and RBD.

Setting
All five state hospitals of Vorarlberg.

Participants
A total of 395 HCW, enrolled at June 2020 (t₁), two months after the end of the first wave, retested between October to November at the beginning of the second wave (t₂), and again at the downturn of the second wave in January 2021 (t₃).

Main outcomes
We assessed weak and strong seropositivity and associated factors, including demographic and clinical characteristics, symptoms consistent with COVID-19 infection, infections verified by RT-PCR, and vaccinations.

Results
At t₁, 3% of HCW showed strong IgG-specific responses to either NP or RBD. At t₂, the rate had increased to 4%, and at t₃ to 14%. A strong response was found to be stable for up to ten months. Overall, only 55% of seropositive specimen had antibodies against both antigens RBD and NP, 29% had only RBD- and 16% only NP- specific antibodies. Compared to the number of infections found by RT-PCR, the amount of HCW being seropositive was 38% higher.

Conclusion and relevance
Serologic testing based on only one antigen implicates the risk of missing infections, thus the set of antigens should be broadened in future. The seroprevalence among participating HCW was comparable to the general population in Austria. Nevertheless, in view of undetected infections, monitoring and surveillance should be reconsidered.
[Keywords]
COVID-19; Public Health; Infection Control; Epidemiology; Occupational & Industrial Medicine; Clinical Chemistry
Strengths and limitations of this study

- The study comprises data on the seroprevalence of HCW in Austria, after the first and the second SARS-CoV-2 wave, when Austria had one of the highest incidence rates worldwide.

- The study comprises data on IgG-specific response to the viral nucleocapsid (NP) as well as to the receptor binding domain (RBD).

- Data on antibody response are quantitative and also describe the respective stability over time.

- The study provides data for seroprevalence assessed by ELISA as well as for infections assessed by RT-PCR.

- The seroprevalence assessed in this study is only based on infections and is not impacted by vaccination.

Word count

Abstract: 299
Main text: 4005
Introduction

Since the World Health Organization (WHO) has declared COVID-19 a global pandemic, virus spread is still unstoped in Europe. During the second wave peaking in November 2020, Austria developed the highest incidence rate worldwide [1] with the federal state of Vorarlberg, reporting the highest rates [2]. Health care workers (HCW) are on the first line of defense and have a high risk of becoming infected and infecting others with SARS-CoV-2 [3,4], but infection prevention in hospitals is still suboptimal [5].

In contrast to real time reverse transcription polymerase chain reaction (RT-PCR) assays detecting SARS-CoV-2 for the initial 2-3 weeks after infection only [6], the immunoglobulin (Ig) G-specific response to SARS-CoV-2 antigens is typically detectable in serum about two weeks after symptom onset and lasts considerably longer [7]. At least 95% of RT-PCR-confirmed SARS-CoV-2 infected patients develop specific anti-SARS-CoV-2 antibodies [8]. The receptor binding domain (RBD) of the spike protein, which enables binding and fusing into cell membrane, has meanwhile become the most common antigen used. It has received FDA emergency approval in seroconversion assays [9], has been shown to correlate well with neutralizing activity [8,10–12], and is the key antigen of current vaccines. The nucleocapsid protein (NP) is a multifunctional protein, which amongst others packages the viral genomic RNA and forms the helical nucleocapsid. In contrast to the spike protein and its RBD, tests that detect antibodies to NP are believed to be more sensitive [13] but are waning in the post-infection phase [14]. Apart from that, other studies have also found a discrepancy or weak concordance between RBD- and NP-specific responses after SARS-CoV-2 infection [15,16]. However, there are up to date no data on the antibody response against RBD as well as NP using identically constructed enzyme linked immunosorbent assays (ELISAs).

The present study therefore analyses antibody dynamics, in particular IgG-specific responses to NP and RBD using identical ELISAs of the same manufacturer in serial serum samples collected from 395 HCW after the first wave, at the beginning of the second massive wave, and at the downturn of the second wave.
Methods

Study subjects

This prospective cohort study comprises 395 participants of mainly Caucasian origin with a median age of 42 years working as HCW in Vorarlberg, the westernmost federal state of Austria. All participants are employed by one of the state hospitals and 174 (44%) at a COVID-19-specialized hospital.

Study enrolment was voluntary and free of charge for the participants. Recruitment was initiated by informing all institutes at the respective hospitals about the study. The information has then been spread by word of mouth recruitment and bulletin boards. All subjects reported to be in healthy condition. At the time of recruiting, participants completed a survey form which captured demographic information as well as symptoms of COVID-19 infection in the three months prior to collection of the respective serum sample. Additionally, data on SARS-CoV-2-specific RT-PCR tests were collected, which had been ordered by the hospital at any suspicion of a possible infection or performed as part of routine institutional screening.

After the first wave in March 2020 and after the first full lockdown [17] in Austria (16th of March to 30th of April) blood samples were collected. Baseline collection took place between 26th of June and 19th of August 2020 and is referred to as time point 1 (t1). Identical criteria were applied for the following round of sampling between 2nd October and 13th November (t2) and between 7th and 20th January 2021 (t3). Thus, sampling at t2 took place mostly at the beginning of the second wave 2020 and at t3 after the second wave, during the third full lockdown in Austria (17th November to 6th December). All HCW in Vorarlberg had the opportunity for vaccination with Comirnaty (BNT162b2, Biontech, Pfizer) starting on 7th January. Thirty-three HCW were vaccinated ≤ 4 days before sampling at t3.

Only 5 out of 395 participants were missing at t2 and 24 at t3 due to end of employment, withdrawal of consent, or due to other reasons. Hence, the follow-up rate at t2 and t3 was 99% and 94%, respectively. A summary of the study timeline is given in figure 1.
Study data were collected and managed using REDCap electronic data capture tools [18,19] hosted at the Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT). Acute SARS-CoV-2 infection was determined by virus detection through RT-PCR of nasopharyngeal swabs at the Institute of Pathology, Academic Teaching Hospital Feldkirch (Feldkirch, Austria). At each time point, venous blood was collected, processed, and anti-SARS-CoV-2 antibodies were detected in human serum via two ELISAs specifically detecting IgGs directed against (i) RBD and (ii) NP (5600100 and 5600200 Technozym, Technoclone, Vienna, Austria [11]). Concentrations were calculated according to internal calibration standards using the Xlfit software package (Version 5.3.1.3, IDBS).

1 U/mL is representing 100 ng/mL of a SARS-specific antibody [20], and, referring to the WHO standard, is equivalent to 3.7 BAU/mL (IS 20/136) and 5.8 BAU/mL (IS 20/136) for NP and RBD, respectively.

According to manufacturer’s protocol, values <5 U/mL were referred to as background range representing the absence of a SARS-CoV-2-specific antibody response. Values ≥5 U/mL were referred to as positive responses. The 5 U/mL cut-off was defined on basis of criteria suggested by the Youden index and the 99\textsuperscript{th} percentile method [21]. In order to meet ongoing concerns about accuracy and cut-offs, values ≥5 and <8 U/mL for anti-SARS-CoV-2 RBD-specific and anti-SARS-CoV-2 NP-specific antibody responses were referred to as a weak positive response. Accounting for the prevalence nature of the study, a higher cut-off of ≥8 U/mL was chosen to increase specificity, as proposed by the manufacturer and by a previous study [21]. Values ≥8 U/mL were thus referred to as a strong positive response. IgG concentration was measured at time points t\textsubscript{1}, t\textsubscript{2}, and t\textsubscript{3}. Participants whose antibody levels increased between time points from background levels (<5 U/mL) to a positive response or from a weak to a strong response, were referred to as converters. Participants with (i) a weak or strong response at an earlier time point and (ii) no conversion during following time points and (iii) a declined or unchanged response (including also marginally increased responses not higher than 10% or 1 U/mL, respectively) were referred to as non-converters. Antibody decay and half-life of antibody response was assumed to follow a first order exponential decline.
Statistical analysis

Differences in baseline characteristics were tested for statistical significance using Chi-squared tests for categorical variables, the Mann-Whitney-U tests for continuous, and unpaired continuous variables, and the Wilcoxon tests for continuous and paired variables. Correlation analyses were performed calculating nonparametric Spearman rank correlation coefficients. All values were analyzed according to complete case analysis. P-values below 0.05 were considered significant. All statistical analyses were performed with SPSS 28.0 for Windows (IBM corp., USA), and R statistical software v. 3.5.1 (http://www.r-project.org).

Patient and public involvement

All participants were HCW at the respective hospitals and were involved, insomuch as they supported recruitment and conduct of the study. The study results will be shared with the participants through the hospitals' public relations department, various media handles, and conferences.

Results

Seroprevalence between June 2020 and January 2021

The characteristics of the study participants is summarized in table 1 and supplemental table 1. The anti-SARS-CoV-2 specific IgGs against RBD and NP were assessed in 395 HCW at three time points, after first wave (t₁), at the beginning of second wave (t₂), and after second wave (t₃; figure 1).

During the study, we collected in total 1156 specimens and performed 2312 tests, 1156 for RBD-specific and 1156 for NP-specific IgGs. The overall serum concentration of RBD and NP ranged between 0 and 200 U/mL with a median of 0.4 U/mL for both RBD and NP. The correlation of RBD- to NP- specific IgG concentration, as well as the proportion of seropositive
subjects (≥5 U/mL) and in particular the seropositive subjects with a strong response (≥8 U/mL) are summarized in table 2 and figure 2 for the three time points t₁, t₂, and t₃. Overall, 73 (18%) out of all 395 HCW have been tested at least once positive, either regarding RBD or NP, at any time point (t₁, t₂, or t₃) during the study.

Comparison of RBD- and NP- specific IgG response

Out of 1156 specimen tested 111 displayed a positive antibody response and 1045 a negative response. Out of these 111 specimen, 93 had antibodies against RBD and 79 against NP. In detail, only 61 specimen (55% of seropositive specimen) had coexisting antibodies against both antigens. The remaining 50 (45%) specimen had either only antibodies against RBD but not against NP (n=32; 29%) or against NP but not against RBD (n=18; 16%), supplemental table 2). Taking into account positive and negative test results, the concordance of NP- and RBD-specific response was 96%, the sensitivity of RBD-specific responses was 77%, and the sensitivity of NP-specific responses was 66% (table 3). This clear discrepancy referring to spread and amount of NP- and RBD-specific responses is illustrated in figure 2.

Change of antibody response during time

Overall, the number as well as the intensity of RBD- and NP-specific IgG concentration increased during the study (supplemental figure 1 and supplemental table 3). Between t₁ and t₃, 44 HCW (12%) seroconverted to a strong (≥8 U/mL) response (t₁-t₃-strong response converters) and 6 (2%) to only a weak (≥5 and <8 U/mL) response (t₁-t₃-weak response converters). Out of these 44 t₁-t₃-strong response converters, 43 converted from no response at t₁ to a strong response at t₃, and only 1 participant from an existing weak response to a strong response. The mean increase, compared to the background signal for these 44 t₁-t₃-strong response converters was 42.3-fold for RBD- and a 43.7-fold for NP-specific antibody response, and for the 6 t₁-t₃-weak converters 3.5-fold and 2.3-fold, respectively (supplemental table 3).
In contrast, 19 HCW were found to have a declined antibody response between t\textsubscript{1} and t\textsubscript{3} (t\textsubscript{1}-t\textsubscript{3}-decliner). Of these, 10 had a strong response at t\textsubscript{1} (t\textsubscript{1}-t\textsubscript{3}-strong response decliners) and 9 a weak response (t\textsubscript{1}-t\textsubscript{3}-weak response decliners).

Taking into account the t\textsubscript{1}-t\textsubscript{3} and t\textsubscript{2}-t\textsubscript{3} time overlap, in total, 23 individuals have declined antibody responses between t\textsubscript{1}/t\textsubscript{2} and t\textsubscript{3} during a median time of 5.0 months (all decliners). The RBD- and NP-specific antibody response of these 23 decliners has decreased by 51% and 60%, respectively (supplemental table 3). The monthly decline of antibody response was 19% for RBD just as for NP (supplemental table 3). This decline was significantly correlated with the strength of response measured at t\textsubscript{1}/t\textsubscript{2} with an r of 0.71 (p<0.001) for RBD and an r of 0.89 (p<0.001) for NP (supplemental figure 2). Strong responders had a more pronounced monthly decline than weak responders (supplemental table 3). Taking into account the exponential nature of decline, the median half-lives of RBD- (5.5 [2.3-15.8] months) and NP-specific antibody responses (5.7 [2.2-11.2] months) were comparable (supplemental table 3). In addition, the median time in which a positive antibody response (≥5 U/mL cut-off) for either RBD or NP can be maintained was 6.0 [1.6-19.8] months for all decliners and 10.2 [6.3-23.4] months for strong-response decliners.

Of note, we did not find any elimination of a strong response between t\textsubscript{1} and t\textsubscript{2} or between t\textsubscript{1} and t\textsubscript{3}. In detail, every HCW who had a strong RBD-specific antibody response at t\textsubscript{1} or t\textsubscript{2} maintained a positive RBD-specific response during the study. However, three subjects with a strong NP-specific response, who also had a RBD-specific response, had lost their NP-specific responses, but maintained their RBD-specific response.

In contrast, out of 11 HCW with only a weak response at t\textsubscript{1}, only 2 kept a weak response at t\textsubscript{3} (1 resigned, 1 converted to a strong response, and 7 fell beneath the cut-off for a weak response).

Association of antibody response with RT-PCR data and vaccination

Out of 395 HCW tested for SARS-CoV-2-specific antibodies, 249 have also been tested at least once for the presence of an acute infection with SARS-CoV-2 during the study by RT-
PCR, and 53 of these were positive. As mentioned above, applying ELISA, 73 out of 395 HCW have been tested positive at least once for SARS-CoV-2-specific antibodies during the study. Thus, the number of HCW with ELISA-assessed positive antibody response is 38% higher (n=20) than all infections detected by RT-PCR in the whole study population. Focusing the situation at the time point of final sampling (t₃) and taking into account only HCW (n=48) who have been tested by both methods (RT-PCR and ELISA) we found that only five HCW with a RT-PCR-proven COVID-19 infection had no antibody response, reflecting an antibody response rate of 90% (43/48). Regarding RBD- and NP-specific antibody response separately, the response rate was 83% for RBD- and 73% for NP-specific response. However, only 67% had a positive response for both, RBD- as well as NP- specific, IgGs. This comes down to 50% when considering only strong responses (supplementary table 4).

The other way round, only 69% (43/62) of seropositive HCW (either with a RBD-specific or a NP-specific antibody response) at t₃ have ever been identified by RT-PCR to be infected. Regarding RBD and NP separately, RT-PCR identified 73% (40/55) of those HCW having RBD-specific IgGs and 74% (35/47) of those with NP-specific IgGs.

Apart from that, it has to be mentioned that 33 participants have been vaccinated before blood sampling at t₃. Of these, 31 were seronegative and two seropositive. One seropositive participant had a strong RBD- and a coexisting strong NP-specific IgG response, the other had only a strong NP-specific response. However, in both cases, vaccination occurred just one day before blood sampling, precluding any effect of the vaccine on the obtained data.

**Association of antibody response with COVID-19-symptoms and further parameters**

Taking into account the survey data, HCW who had COVID-19-specific symptoms at t₃ were significantly more likely to be seropositive than asymptomatic ones (36% vs. 8% p<0.001). When comparing four categories (A-D) according to antigen-specific response, comprising HCW (A) without any response, (B) with only NP-specific response, (C) with only RBD-specific response, and (D) with both RBD-and NP-specific response, the percentage of HCW with
symptoms gradually and significantly increased (A=24.0%, B=42.9%, C=46.7%, D=77.5%; p<0.001). This demonstrates that symptoms were >3 times more common in the group having IgGs against both antigens (RBD and NP) compared to those without any IgGs. Further data comparing HCW characteristics and antigen-specific response are provided in supplementary table 5.
Discussion

Main findings

The study found that only 55% of seropositive specimen had IgG antibodies against both antigens RBD and NP; 29% had only RBD- and 16% only NP-specific antibodies. This clear discrepancy between NP- and RBD-specific responses confirms data in previous reports by others [15,16]. In addition, COVID-19-specific symptoms gradually increased in line with the antibody response from no response to a NP-specific, to a RBD-specific, and to a coexisting RBD- and NP-specific response. We also found that a conversion to a strong response during the study was much more likely than a conversion to a weak response only. A further important finding was that a strong response was more stable than a weak response. We experienced no elimination of a strong response during the study: All participants with a strong response maintained a positive response during the study. The half-lives of NP- and RBD-specific responses were comparable. Finally, the number of undetected SARS-CoV-2 infections during our study was quite high, as only 83% of HCW with a strong antibody response had previously been identified by RT-PCR.

Seroprevalence in the light of other study data on HCW

Our data in HCW revealed a 3% seroprevalence (strong response) at t₁, after the first wave. This was slightly above those from HCW in Germany [22,23] being in the range of 1–2% around the same time. Higher rates of 5-6% were seen in the Northern Italy [24], Belgium [25], Norway [26], and Northern England [27], and particularly in the US, with a seroprevalence rate of 19% in the general population [28] and 27% in HCW at the same time [29].

At t₂ and t₃, when Austria was passing the second wave and had one of the highest incidence rates in the world [1], the seroprevalence in our study increased to 4% (t₂) and finally to 14 % (t₃). This was just matching the seroprevalence of the general population in Austria at the same time points (t₂: 4.7% [30] and t₃: 15% [31]). Therefrom, HCW in Vorarlberg appeared to be well
prepared facing COVID-19 in the local health care system, although they were initially
supposed to have a higher chance of being infected than the general population.
That said, the number of HCW with a positive antibody response was 38% higher than RT-
PCR-verified infections detected by current testing routines of HCW in the hospitals. Given the
at least 17% undetected infections of HCW in our hospitals, one may reconsider infection
surveillance.

**Limited overlap of NP- and RBD-specific IgG responses**

Currently, no vaccine used in the EU is based on the NP-antigen. Thus, the detection of NP-
specific antibodies is exclusively raised by viral infection. As a consequence, NP-specific
seroconversion may appear a promising tool for specifically detecting virus infection even in
the context of vaccinated subjects. Our data, however, are questioning such applications as
we found only a limited overlap of NP- and RBD-specific IgG responses in infected subjects.
Furthermore, we also found a higher rate of symptoms in HCW with a response against both
antigens than in those with a response against only a single antigen. This is in line with the
magnitude of serological immune responses against SARS-CoV2 which is known to be highly
variable [32]. In addition, it has also been demonstrated by others that a NP- or spike-specific
antibody response may not always be present following a proven SARS-CoV-2 infection [10]
or, in particular, that NP-specific antibody response is less pronounced compared to the spike
protein-specific response [16].

In a recent study, the concordance between NP- and RBD-specific response of two different
assay providers was only 87.5% in a UK study in 906 adults [15], which is yet beneath our data
(96%). A further Canadian study testing 21676 specimen from March to August 2020 also used
two different providers for detecting NP- and spike-specific IgGs and revealed a sensitivity of
73% for RBD with NP as standard [33]. This is more or less comparable to our study results,
revealing 77% sensitivity, in which, however, identically constructed assays of the same
provider were used. Moreover the same Canadian study suggested that the decline of NP-
specific antibodies over time is substantial enough to affect the results of population 
seroprevalence surveys, especially in high prevalence settings [33].

We therefore conclude that looking for only a single antigen-response, as it is mainly the case 
with RBD, does not elucidate the real seroprevalence.

Seroconversion, protection and reinfection

When focusing on the subgroup of responders, we found that a strong response was more 
stable than a weak response. These findings are in good alignment with the very fast increase 
in antibody titers and neutralization within only 10 days after symptom onset, tested with the 
same assay as we did [21]. All participants who once have developed a strong response 
maintained a positive response, either still a strong one or at least a weak one, during the full 
study time. An extrapolation, thus, suggests that these strong responders will keep their 
response for about ten months. This is in line with previous data of recent studies in the UK 
and Spain, demonstrating that SARS-CoV-2 infection-acquired immunity is present for at least 
six months [12,25]. A further study in New York City has found only a moderate decline 
regarding the spike protein-specific response during five months [8]. We here report a mean 
decline of 51% and 60% during five months for RBD- and NP-specific responses, respectively.

A decrease of 17 % and 31 % for anti-spike IgG and anti-NP IgG titers has been reported in a 
study comprising 847 workers at Institute Curie in Paris during 4-8 weeks accounting rather 
short-lived immune responses of only 87 days for anti-spike IgG and 35 days for anti-NP IgGs, 
respectively [10]. Wajnberg et al. have suggested that the stability of the antibody response 
over time may depend on the serologic target [8] with a faster decline of NP compared to RBD.

That said, the magnitude of decline of NP-specific response in some studies cannot be 
attributed solely to the choice of NP as antigen and has been reported to be assay-specific 
[34].

Other than NP, the spike protein is the main and potentially the only target for neutralizing 
antibodies [35]. Nevertheless, RBD-specific IgG response as investigated in our study as well
as in most others on seroprevalence is only a fragment of the very complex post-infection
immunity and longevity of response.

Finally, we also have noticed one case in which a weak antibody response at $t_1$ has converted
to a strong response at $t_3$, representing a reinfection according to PCR data. That said, the
number of responders at $t_1$ and $t_2$ is small compared to the initial study number and thus the
conclusions (including those regarding reinfection, immunity, elimination time, and half-life) for
this subgroup are limited and should be taken with care. Further limitations are mentioned in
the following.

Limitations

This study is not a random sample of either the general population or the HCW of Vorarlberg
as only HCW in hospitals have been recruited on a voluntary basis. The infection risk of HCW
is significantly impacted by the situation outside the hospital. Further, the data should be
interpreted with caution, as it is possible that some of our participants which have been
classified as “no response” due to a response below the assay cut-off of $<5$ U/mL were infected
with SARS-CoV-2 a few months before sampling, and either had only a weak antibody
response to start with and/or have dropped below the assay threshold since. Apart from that,
the present study only measured IgG and did not detect other Ig classes (e.g. IgM or IgA).
Although IgG-specific ELISAs have been proposed to be appropriate for prevalence testing,
accuracy significantly differs between different serological testing methods [36]. In that context,
we want to mention that a standard cut-off for BAU/mL is still lacking making a comparison of
different test methods difficult. Apart from that, our study only provides information about post-
infection antibody-response and not about immunity or the chance of reinfections. It is
impossible to fully explain the nature of change of antibody-specific responses in our study,
e.g. for responders of which some may be impacted by a secondary contact to the virus thus
acting as kind of a booster. Finally, some participants have been vaccinated during sampling
at $t_3$. IgG responses are not mounted before 14 days after vaccination [37] and, thus, the
vaccination in our study, which took place not earlier than 4 days before sampling, can be precluded to have impacted our serologic measurements.

Given the limitations mentioned above, the antibody response is yet widely used as a surrogate for deciding whether post-infection immunity to SARS-CoV-2 exists. The antibody response in our study has proven to persist for several months. That said, our and others’ findings do not support exempting those positive for anti-SARS-CoV-2 antibodies from current infection control, other public health constraints, or the ongoing vaccination.

Conclusion

Serologic testing based on only one antigen implicates the risk of missing infections. We propose that the set of antigens should be broadened. Apart from the mainly used RBD, our data clearly suggest including NP in serologic routine. Further antigens e.g. the N-terminal domain (NTD) [38] or the M protein [39] may have the potential to advance serologic testing in future. In view of undetected infections represented by the higher number of HCW with antibody response than RT-PCR-verified infections detected by routine testing, monitoring of infections should be reconsidered, too. Apart from that, further studies are necessary to determine the long-time duration of post-infection antibody response in combination with vaccination approaches as this has major implications for the future fight against SARS-CoV-2 in view of current virus variants.
Ethics statements

Consent for publication

Consent was obtained from all participants.

Ethics approval

The present study conforms to the ethical guidelines of the 1975 Declaration of Helsinki and has been approved by the Ethics Committee of Vorarlberg (EK-2-4/2020). All participants gave informed consent to participate in the study before taking part.

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Contributors

ALa had the original idea. MA, AM, TW, HD, ALa, and ALe contributed to the study design and conceptualization. MA, AM, PF, and ALe managed the project. AM was responsible for ethical and regulatory submissions. ALa, ALe, and PF acquired funding. MK and MD provided experimental resources. MA, LSp, BM, AV, MB, LSe, JBJ collected data. EMB, KG, ALe analyzed data. HD is the guarantor. AM and ALe wrote the manuscript. All authors contributed to reviewing and approved the final version.

Competing interest

No potential conflicts of interest relevant to this article were reported by M.A., A.M., T.W., P.F., E.M.B., K.G., M.K., M.D., L.Sp., B.M., A.V., M.B., L.Se., J.J., H.D., A.La., and A.Le..

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Data sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Tables and figures

Table 1

Characteristics

| Characteristics               | Value |
|------------------------------|-------|
| All participants; % (n)      | 100 (395) |
| Age; years (min-max)         | 42 (18-64) |
| Female sex; % (n)            | 71 (282) |
| BMI (min-max)                | 25 (18-45) |
| Overweight or obese; % (n)   | 35 (139) |
| Current smoking; % (n)       | 18 (73) |
| Working in COVID-19-hospital; % (n) | 44 (174) |
| Children in household; % (n) | 53 (211) |
| PCR tested; % (n) / positive PCR; % (n) | 63 (249) / 13 (53) |

Table 1 summarizes the characteristics of all participants. Continuous data are given as mean, in the presence of a skewed distribution, mean values are given together with minimum and maximum values (min-max). Dichotomous data are given as proportion. BMI denotes body mass index and PCR polymerase chain reaction. The term children is summarizing all children or adolescents under 25 years. PCR stands for SARS-CoV-2-specific real time reverse transcription PCR.
| participants                  | RBD (U/mL)        | NP (U/mL)       | RBD-NP correlation |
|------------------------------|-------------------|-----------------|--------------------|
| all HCW                      | 100%              | 1.66 (0.12-0.89)| 1.40 (0.15-0.98)   |
| (n=395)                      |                   |                 | r=0.24 p<0.001     |
| seropositive: either RBD or NP | 6%                | 18.24 (1.55-10.54)| 13.45 (1.94-22.71)|
| (n=24)                       |                   |                 | r=0.27 p<0.001     |
| seropositive: RBD (i)        | 4%                | 25.37 (5.73-13.16)| 12.61 (1.21-22.11) |
| (n=17)                       |                   |                 | r=0.78 p<0.001     |
| seropositive: NP (iii)       | 4%                | 24.32 (0.35-14.19)| 19.49 (5.90-33.53) |
| (n=16)                       |                   |                 | r=0.35 p<0.001     |
| seropositive: RBD and NP (iv)| 2%                | 42.51 (9.13-66.26)| 22.60 (8.26-38.17) |
| (n=9)                        |                   |                 | r=0.23 p<0.001     |
| seropositive (strong): either RBD or NP | 3% | 30.45 (5.60-28.57) | 22.51 (8.26-34.99)  | r=0.03 p<0.001  |
| (n=13)                       |                   |                 |                   |
| seropositive (strong): RBD (i) | 2%               | 42.71 (9.13-66.26)| 20.48 (6.66-38.17) |
| (n=9)                        |                   |                 | r=0.53 p<0.001     |
| seropositive (strong): NP (iii) | 3%              | 34.38 (4.49-41.93)| 25.88 (10.69-35.71) |
| (n=11)                       |                   |                 | r=0.04 p<0.001     |
| seropositive (strong): RBD and NP (iv) | 2%          | 52.40 (10.96-90.00)| 25.19 (8.90-45.04) |
| (n=7)                        |                   |                 | r=0.14 p<0.001     |

Table 2:

Antibody response during study

Table 2 summarizes the concentration of SARS-CoV-2 receptor binding domain (RBD) - and nucleocapsid protein (NP) - specific antibody response at the respective time point given as mean (with interquartile range). Correlation (r) is given together with the p-value according to spearman test. Seropositive HCW (comprising a weak and a
strong response) had a concentration of ≥ 5 U/mL for either RBD- or NP-specific response. Seropositive HCW with a strong response were characterized by a concentration of ≥ 8 U/mL for RBD or NP. Seropositive HCW were further discriminated into those with a RBD-specific response (ii), those with a NP-specific response (iii), those with either a RBD- or a NP-specific response (i) and those with both, a RBD- and a coexisting NP-specific response (iv).

Table 3

RBD- and NP-specific responses in comparison

| time point | seropositive | seropositive (strong response) |
|------------|--------------|-------------------------------|
| sensitivity of NP (=PPV for RBD) | t1 53% | 78% |
| t2 57% | 54% |
| t3 73% | 72% |
| total 66% | 69% |
| sensitivity of RBD (=PPV for NP) | t1 56% | 64% |
| t2 75% | 64% |
| t3 85% | 78% |
| total 77% | 73% |
| Concordance of NP and RBD | t1 96% | 98% |
| t2 97% | 97% |
| t3 94% | 94% |
| total 96% | 97% |

Table 3 summarizes the comparison between RBD- and NP- specific IgG responses of tests performed at the respective time points. Sensitivity of NP is given with RBD as standard. Sensitivity of RBD is given with NP as standard. The respective positive and negative counts are provided in the supplement (supplementary table 2). PPV = positive predictive value.

Figure Legends

Figure 1: Study timeline

The figure presents the 7-day incidence per 100,000 inhabitants in Austria and in the federal state of Vorarlberg between February 2020 and January 2021. The time points of sampling (t₁, t₂, and t₃: solid black line) and lockdown (hatched line) are marked. Data on 7-day incidence were obtained from the Austrian Open Government Data [40]. A detailed description of lockdown and public health measures in Austria is given elsewhere [17].
Figure 2: Concentration and spread of RBD- and NP-specific IgG response

A: The intensities of anti-RBD (squares) and anti-NP-specific IgG responses (triangles) of each individual subject (connected by a line) are depicted at study time point t₁, t₂, and t₃. B: Correlation of anti-RBD and anti-NP-specific IgG response of study participants is depicted at study time point t₁, t₂, and t₃. The solid grey line represents a linear regression line (R²). The dashed green line separates positive responses (≥5 U/mL for anti-RBD and anti-NP IgG) from the background response. Values ≥8 U/mL for anti-RBD and anti-NP IgG, representing a strong response, are separated by a solid green line.
figure 1

115x76mm (500 x 500 DPI)
figure 2

84x77mm (818 x 818 DPI)


## Supplemental material

### Supplementary table 1

#### Residence and profession

| Residence         | Vorarlberg  | 364 (92.2%) |
|-------------------|-------------|-------------|
|                   | out of Vorarlberg | 14 (3.5%) |
|                   | not specified     | 17 (4.3%) |
|                   | total             | 395 (100%) |

| Country of Birth   | Austria       | 300 (75.9%) |
|-------------------|---------------|-------------|
|                   | Germany       | 38 (9.6%)   |
|                   | Italy         | 12 (3.0%)   |
|                   | Other EU      | 11 (2.8%)   |
|                   | Outside EU    | 10 (2.5%)   |
|                   | not specified | 24 (6.1%)   |
|                   | total         | 395 (100%)  |

| Professional role  | Reception    | 10 (2.5%)  |
|--------------------|--------------|------------|
|                    | Secretarial  | 18 (4.6%)  |
|                    | Physician    | 96 (24.3%) |
|                    | Nursing/Physio | 250 (63.3%) |
|                    | Radiology    | 10 (2.5%)  |
|                    | Service      | 9 (2.3%)   |
|                    | Lab          | 1 (0.3%)   |
|                    | not specified| 1 (0.3%)   |
|                    | total        | 395 (100%) |

Supplementary table 1 summarizes the residence and profession of all 395 HCW.
**Supplementary table 2**

**RBD- and NP-specific IgG response during study**

|                | RBD + | RBD - | RBD + | RBD - | RBD + | RBD - | RBD + | RBD - |
|----------------|-------|-------|-------|-------|-------|-------|-------|-------|
| **t1**         |       |       |       |       |       |       |       |       |
| NP +           | 2.3%  | 1.8%  | 3.1%  | 1.0%  | 10.8% | 1.9%  | 5.3%  | 1.6%  |
|                | (9/395)| (7/395)| (12/390)| (4/390)| (40/371)| (7/371)| (61/1156)| (18/1156)|
| NP -           | 2.0%  | 93.9% | 2.3%  | 93.6% | 4.0%  | 83.3% | 2.8%  | 90.4% |
|                | (8/395)| (371/395)| (9/390)| (365/390)| (15/371)| (309/371)| (32/1156)| (1045/1156)|
| **t2**         |       |       |       |       |       |       |       |       |
| NP +           | 1.8%  | 1.0%  | 1.8%  | 1.0%  | 8.4%  | 3.2%  | 3.9%  | 1.5%  |
|                | (7/395)| (4/395)| (7/390)| (4/390)| (31/371)| (9/371)| (45/1156)| (17/1156)|
| NP -           | 0.5%  | 96.7% | 1.5%  | 95.6% | 3.2%  | 86.0% | 1.7%  | 92.9% |
|                | (2/395)| (382/395)| (6/390)| (373/390)| (12/371)| (319/371)| (20/1156)| (1074/1156)|
| **t3**         |       |       |       |       |       |       |       |       |
| NP +           |       |       |       |       |       |       |       |       |
| NP -           |       |       |       |       |       |       |       |       |
| **total**      |       |       |       |       |       |       |       |       |
| RBD +          | 5.3%  | 1.6%  | 6.8%  | 8.4%  | 12.0% | 1.9%  | 6.1%  | 2.1%  |
|                | (61/1156)| (18/1156)| (120/2080)| (126/2080)| (720/1156)| (7/371)| (670/2266)| (47/2266)|

Supplementary table 2 summarizes the comparison between RBD- and NP- specific IgG responses of tests performed at time points $t_1$, $t_2$, $t_3$, and during the whole study (total). Seroconversion (positive response) was diagnosed at concentrations $\geq 5$ U/ml and, alternatively, at concentrations $\geq 8$ U/ml when regarding a strong response only.
### Supplementary table 3

Seroconversion and decline of antibody response during study

|                                               | Change of response                      | Change of response per month | Half-life in months |
|-----------------------------------------------|-----------------------------------------|------------------------------|---------------------|
|                                               |                                         | n.a.                         | n.a.                |
| t1-t3 all HCW (n=371)                         | +4.0 U/mL (335 %)                       | n.a.                         | n.a.                |
|                                               | +3.4 U/mL (270 %)                       | n.a.                         | n.a.                |
| t1-t3-strong response converters (n=44)       | +35.9 U/mL (4233 %)                     | n.a.                         | n.a.                |
|                                               | +29.8 U/mL (4368 %)                     | n.a.                         | n.a.                |
| t1-t3-weak response converters (n=6)          | +4.0 U/mL (349 %)                       | n.a.                         | n.a.                |
|                                               | +2.6 U/mL (231 %)                       | n.a.                         | n.a.                |
| all t1-t3-converters (n=50)                   | +32.1 U/mL (3634 %)                     | n.a.                         | n.a.                |
|                                               | +26.5 U/mL (3611 %)                     | n.a.                         | n.a.                |
| t1-t3-strong response decliners (n=10)        | - 7.4 U/ml (-38 %)                      | - 1.4 U/mL (-7 %)            | 7.1 [4.9-115.6]     |
|                                               | - 10.5 U/ml (-52 %)                     | - 1.9 U/mL (-9 %)            | 4.0 [2.7-23.2]      |
| t1-t3 weak response-decliners (n=9)           | - 1.2 U/ml (-37 %)                      | - 0.2 U/mL (-7 %)            | 5.5 [1.6-17.2]      |
|                                               | - 1.3 U/ml (-40 %)                      | - 0.2 U/mL (-7 %)            | 7.0 [6.1-26.0]      |
| all t1-t3-decliners (n=19)                    | - 4.5 U/ml (-38 %)                      | - 0.8 U/mL (-7 %)            | 5.7 [3.8-17.2]      |
|                                               | - 6.1 U/ml (-50 %)                      | - 1.1 U/mL (-9 %)            | 6.2 [2.9-17.3]      |
| t2-t3-strong response decliners (n=12)        | - 25.2 U/mL (-52 %)                     | - 11.9 U/mL (-25 %)          | 2.9 [0.9-4.6]       |
|                                               | - 14.9 U/ml (-51 %)                     | - 6.7 U/mL (-23 %)           | 4.0 [1.5-17.6]      |
| t2-t3-weak response-decliners (n=7)           | - 1.1 U/ml (-23 %)                      | - 0.4 U/mL (-7 %)            | 11.0 [1.4-127.6]    |
|                                               | - 0.4 U/ml (-18 %)                      | - 0.1 U/mL (-6 %)            | 10.6 [5.3-41.3]     |
| all t2-t3-decliners (n=19)                    | - 16.3 U/ml (-51 %)                     | - 7.4 U/mL (-23 %)           | 3.5 [1.4-11.5]      |
|                                               | - 9.6 U/ml (-50 %)                      | - 4.1 U/mL (-22 %)           | 5.1 [2.5-31.0]      |
| all strong response decliners (n=13)          | - 23.3 U/mL (-52 %)                     | - 9.0 U/mL (-20 %)           | 5.3 [1.8-14.5]      |
|                                               | - 20.9 U/ml (-61 %)                     | - 6.7 U/mL (-20 %)           | 2.7 [1.8-5.1]       |
| all weak response decliners (n=10)            | - 1.5 U/mL (-38 %)                      | - 0.3 U/mL (-7 %)            | 5.6 [2.0-17.2]      |
|                                               | - 1.1 U/mL (-36 %)                      | - 0.2 U/mL (-6 %)            | 7.6 [6.1-40.9]      |
| all decliners (n=23)                          | - 13.8 U/mL (-51 %)                     | - 5.2 U/mL (-19 %)           | 5.5 [2.3-15.8]      |
|                                               | - 12.3 U/mL (-60 %)                     | - 3.9 U/mL (-19 %)           | 5.7 [2.2-11.2]      |

Supplementary table 3 summarizes decline as well as raise of antibody response for the respective time interval. Converters had an increase of antibody response from background to either weak or strong. Decliners were defined as not converters and having either a decrease of a strong or a weak antibody response or no change of a strong or weak antibody response. Median half-lives, given with interquartile range, were calculated assuming an exponential decline if applicable and are given in month until half of the initial response is lost. The decrease of antibody response between t1 and t3 and between t2 and t3 was referred to 5.7 and 2.8 months, respectively.
Supplementary table 4

| No t₃ |            | RBD (U/ml) | NP (U/ml) | RBD-NP correlation |
|-------|------------|------------|-----------|---------------------|
|       | all HCW    | 100% (n=182) | 2.80 (0.12-0.78) | 1.76 (0.17-1.12) | r=0.35 p<0.001 |
|       | seropositive | 7% (n=13) | 32.87 (5.37-32.60) | 15.04 (1.84-20.44) | r=0.27 p=0.36 |
|       | seropositive: | RBD | 7% (n=12) | 35.39 (6.02-39.38) | 14.80 (1.67-20.93) | r=0.45 p=0.14 |
|       | seropositive: | NP | 4% (n=8) | 44.96 (9.26-104.60) | 23.56 (10.22-26.94) | r=0.12 p=0.78 |
|       | seropositive: | RBD and NP | 4% (n=7) | 50.39 (12.02-133.12) | 24.36 (10.04-28.28) | r=0.25 p=0.59 |
|       | seropositive (strong): | either RBD or NP | 5% (n=9) | 45.09 (10.18-89.63) | 20.95 (8.47-25.60) | r=0.05 p=1.00 |
|       | Seropositive (strong): | RBD | 4% (n=8) | 50.39 (12.45-111.38) | 21.33 (7.68-26.94) | r=0.05 p=0.91 |
|       | Seropositive (strong): | NP | 4% (n=7) | 49.66 (8.35-133.12) | 25.94 (10.75-26.28) | r=0.00 p=1.00 |
|       | seropositive (strong): | RBD and NP | 3% (n=6) | 57.49 (12.40-138.20) | 27.27 (10.57-40.39) | r=0.03 p=0.96 |
| Yes t₃ | all HCW | 100% (n=48) | 26.62 (6.75-32.10) | 24.69 (4.22-21.28) | r=0.70 p<0.001 |
|       | seropositive: | either RBD or NP | 90% (n=43) | 29.59 (8.47-35.66) | 27.42 (6.91-25.55) | r=0.59 p<0.001 |
|       | seropositive: | RBD | 83% (n=40) | 31.60 (10.39-40.33) | 28.36 (6.90-27.62) | r=0.69 p<0.001 |
|       | seropositive: | NP | 73% (n=35) | 33.57 (9.15-49.17) | 32.88 (8.86-32.82) | r=0.61 p<0.001 |
|       | seropositive: | RBD and NP | 67% (n=32) | 36.45 (12.33-51.82) | 34.56 (8.78-36.61) | r=0.68 p<0.001 |
|       | seropositive (strong): | either RBD or NP | 81% (n=39) | 31.95 (10.82-41.89) | 29.81 (7.51-28.31) | r=0.56 p<0.001 |
|       | seropositive (strong): | RBD | 69% (n=33) | 36.95 (12.81-50.94) | 32.67 (7.14-35.34) | r=0.72 p<0.001 |
|       | seropositive (strong): | NP | 63% (n=30) | 37.16 (8.98-52.84) | 37.22 (11.26-38.60) | r=0.63 p<0.001 |
|       | seropositive (strong): | RBD and NP | 50% (n=24) | 45.34 (16.35-53.47) | 43.00 (11.04-49.32) | r=0.67 p<0.001 |

Supplementary table 4 summarizes the concentration of SARS-CoV-2 RBD- and NP- specific antibody response at time point t₃ given as mean (with interquartile range) regarding their COVID-19 history proven by PCR. Out of 53 HCW with a RT-PCR-proven COVID-19 infection, 48 had also ELISA data at t₃. Correlation (r) is given together with the p-value according to spearman test. Seropositive HCW (comprising a weak and a strong response) had a concentration of ≥ 5 U/mL for either RBD- or NP-specific response. Seropositivity with a strong response was characterized by a concentration of ≥ 8 U/mL (RBD and NP). Seropositive HCW were further discriminated into those with a RBD-specific response (i), those with a NP-specific response (ii), those with either a RBD or a NP-specific response (iii) and those with both, a RBD- and a coexisting NP-specific response (iv).
Supplementary table 5 compares characteristics of HCW in the context of antigen specific antibody response categories at t₃: A = no NP- or RBD- specific antibody response; B = only NP-specific response; C = only RBD-specific response; D = NP- and RBD-specific response coexisting. BMI denotes body mass index. COVID-19 symptoms refers to characteristic symptoms reported by HCW up to 3 months before sampling at t₃. The term children refers to all children or adolescents under 25 years. The p-value is given for trend A→B→C→D.

|                                      | Antigen specific response | p-value |
|--------------------------------------|---------------------------|---------|
|                                      | no (A)                    | NP only (B) | RBD only (C) | RBD & NP (D) |
| COVID-19 symptoms; %                 | 24.0                      | 42.9      | 46.7          | 77.5          | <0.001     |
| Age ≥40 years; %                     | 58.8                      | 71.4      | 40.0          | 60.0          | 0.78       |
| Male sex; %                          | 28.2                      | 42.9      | 20.0          | 35.0          | 0.52       |
| BMI ≥25; %                           | 34.2                      | 42.9      | 28.6          | 47.5          | 0.16       |
| Current smoking; %                   | 19.7                      | 0.0       | 6.7           | 12.5          | 0.12       |
| In COVID-19-hospital; %              | 43.8                      | 42.9      | 66.7          | 55.0          | 0.07       |
| Children in household; %             | 54.1                      | 42.9      | 66.7          | 65.0          | 0.14       |
Supplementary figure 1: Shift of RBD- and NP-specific IgG response during study

SARS-CoV-2-specific IgG responses of study participants at time point t₁ (black rhombs), are depicted ordered from high to low/background. The reference or background range (<5 U/mL) representing no response is separated from a positive responses (≥5 U/mL) by a dashed green line and from a strong positive response (≥8 U/mL) by a solid green line. The matching responses at t₂ (circles), and t₃ (triangles) are connected by a vertical line. RBD-specific responses are represented by orange (for t₂) and red (for t₃) symbols, NP-specific responses by turquois (for t₂) and purple (for t₃) symbols.
Supplementary figure 2

Supplementary figure 2: Monthly decline of IgG response in correlation with baseline IgG response

The monthly decline of the SARS-CoV-2-specific response of study participants in relation to their response at baseline is depicted for RBD-specific (A) and for NP-specific IgGs (B). The background (<5 U/ml) representing no response is separated from a weak positive response (≥5 to <8 U/ml) by a dashed green line and from a strong positive response (≥ 8 U/mL) by a solid green line. Grey dots represent values outside the positive range and were excluded for calculation of the regression lines given as solid red and turquoise lines with $R^2$ indicated.
| Item No | Recommendation | Page No |
|--------|----------------|---------|
| **Title and abstract** | 1 | (a) Indicate the study’s design with a commonly used term in the title or the abstract  
(b) Provide in the abstract an informative and balanced summary of what was done and what was found | 1-2  
2 |
| **Introduction** | 2 | Explain the scientific background and rationale for the investigation being reported | 5 |
| **Objectives** | 3 | State specific objectives, including any prespecified hypotheses | 5 |
| **Methods** | 4 | Present key elements of study design early in the paper | 6-7  
Figure 1 |
| **Setting** | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 6-7  
Figure 1 |
| **Participants** | 6 | (a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up  
(b) For matched studies, give matching criteria and number of exposed and unexposed | 6  
n.a. |
| **Variables** | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 7 |
| **Data sources/measurement** | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 7 |
| **Bias** | 9 | Describe any efforts to address potential sources of bias | 11-12 |
| **Study size** | 10 | Explain how the study size was arrived at | 6 |
| **Quantitative variables** | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 7-8 |
| **Statistical methods** | 12 | (a) Describe all statistical methods, including those used to control for confounding  
(b) Describe any methods used to examine subgroups and interactions  
(c) Explain how missing data were addressed  
(d) If applicable, explain how loss to follow-up was addressed  
(e) Describe any sensitivity analyses | 8  
11-12  
8  
n.a. |
| **Results** | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed  
(b) Give reasons for non-participation at each stage  
(c) Consider use of a flow diagram | Table 1-3  
n.a.  
n.a. |
| **Descriptive data** | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders  
(b) Indicate number of participants with missing data for each variable of interest  
(c) Summarise follow-up time (eg, average and total amount) | Table 1  
Table 2  
Figure 1, supplement |
| **Outcome data** | 15* | Report numbers of outcome events or summary measures over time | Table 2-3 |
| Main results | Other analyses | 16 | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included  
(b) Report category boundaries when continuous variables were categorized  
(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | Table 2-3, supplement, 8-12  
8-9, 11 n.a.  
| | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 17 | 10-12, supplement |

| Discussion | | | | |
| Key results | 18 | Summarise key results with reference to study objectives | 13 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 16-17 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 13-17 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 17 |

| Other information | | | | |
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 18-19 |

*Give information separately for exposed and unexposed groups.

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