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

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ABSTRACT

Objectives Austria, and particularly its westernmost federal state Vorarlberg, developed an extremely high incidence rate during the COVID-19 pandemic. Healthcare workers (HCWs) 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 analyse SARS-CoV-2-specific antibody dynamics in Vorarlberg HCWs.

Design Prospective cohort study of HCWs including testing at three different time points for the prevalence of anti-SARS-CoV-2 IgG antibodies specific for nucleocapsid protein (NP) and receptor-binding domain (RBD).

Setting All five state hospitals of Vorarlberg.

Participants A total of 395 HCWs, enrolled in June 2020 (time point 1 (t_1)), 2 months after the end of the first wave, retested between October and November at the beginning of the second wave (time point 2 (t_2)) and again at the downturn of the second wave in January 2021 (time point 3 (t_3)).

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 reverse transcription PCR (RT-PCR) and vaccinations.

Results At t_1, 3% of HCWs showed strong IgG-specific responses to either NP or RBD. At t_2, the rate had increased to 4%, and at t_3 to 14%. A strong response was found to be stable for up to 10 months. Overall, only 55% of seropositive specimen had antibodies against both antigens RBD and NP; 29% had only RBD-specific and 16% only NP-specific antibodies. Compared with the number of infections found by RT-PCR, the number of HCWs being seropositive was 38% higher.

Conclusion and relevance Serological testing based on only one antigen implicates the risk of missing infections; thus, the set of antigens should be broadened in the future. The seroprevalence among participating HCWs was comparable to the general population in Austria. Nevertheless, in view of undetected infections, monitoring and surveillance should be reconsidered.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The study comprises data on the seroprevalence of healthcare workers in Austria, after the first and the second SARS-CoV-2 waves, when Austria had one of the highest incidence rates worldwide.
⇒ The study comprises data on IgG-specific response to the viral nucleocapsid protein as well as to the receptor-binding domain.
⇒ 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 reverse transcription PCR.
⇒ The seroprevalence assessed in this study is based only on infections and is not impacted by vaccination.

INTRODUCTION

Since the WHO has declared COVID-19 a global pandemic, virus spread is still unstopped 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] Healthcare workers (HCWs) are on the first line of defence 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 PCR (RT-PCR) assays detecting SARS-CoV-2 for the initial 2–3 weeks after infection

only,[6] the IgG-specific response to SARS-CoV-2 antigens is typically detectable in serum about 2 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 the cell membrane, has meanwhile become the most common antigen used. It has received Food and Drug Administration emergency approval in seroconversion assays,[9] has been shown to correlate well with neutralising activity[8 10–12] and is the key antigen of current vaccines. The nucleocapsid protein (NP) is a multifunctional protein which, among 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 postinfection phase.[14] Apart from that, other studies have also found a discrepancy or weak concordance between RBD-specific and NP-specific responses after SARS-CoV-2 infection.[15 16] However, to date, there are 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 HCWs 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-specialised 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 3 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 (16 March–30 April), blood samples were collected. Baseline collection took place between 26 June and 19 August 2020 and is referred to as time point 1 (t_1). Identical criteria were applied for the following round of sampling between 2 October and 13 November (time point 2 (t_2)) and between 7 and 20 January 2021 (time point 3 (t_3)). Thus, sampling at t_2 took place mostly at the beginning of the second wave 2020 and at t_3 after the second wave, during the third full lockdown in Austria (17 November–6 December). All HCWs in Vorarlberg had the opportunity for vaccination with Comirnaty (BNT162b2, Biontech, Pfizer) starting on 7 January. Thirty-three HCWs were vaccinated ≤4 days before sampling at t_3.

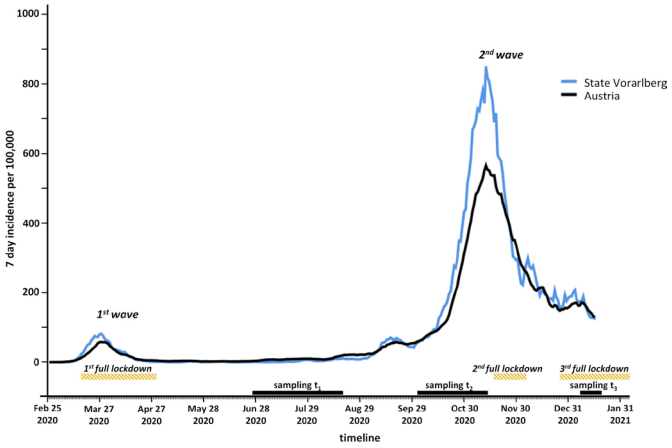

Figure 1 Study timeline. The figure presents the 7-day incidence per 100 000 inhabitants in Austria and in the federal state of Vorarlberg between February 2020 and January 2021. The time points of sampling (t_1, t_2 and t_3; solid black line) and lockdown (hatched line) are marked. Data on 7-day incidence were obtained from the Austrian open government data.[40] A detailed description of lockdown and public health measures in Austria is given elsewhere.[17] t_1, time point 1; t_2, time point 2; t_3, time point 3.

Only 5 out of 395 participants were missing at t_2 and 24 at t_3 due to end of employment, withdrawal of consent or other reasons. Hence, the follow-up rates at t_2 and t_3 were 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. 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 and processed, and anti-SARS-CoV-2 antibodies were detected in human serum via two ELISAs specifically detecting IgGs directed against (1) RBD and (2) NP (5600100 and 5600200 Technozym, Technoclone, Vienna, Austria[11]). Concentrations were calculated according to internal calibration standards using the Xlfit software package V.5.3.1.3 (IDBS).

One unit per millilitre represents 100 ng/mL of a SARS-specific antibody[20] and, referring to the WHO standard

given as binding antibody units (BAU), 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 the manufacturer's protocol, values of <5 U/mL were referred to as background range representing the absence of a SARS-CoV-2-specific antibody response. Values of ≥5 U/mL were referred to as positive responses. The 5 U/mL cut-off was defined on the 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 of ≥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 of ≥8 U/mL were thus referred to as a strong positive response. IgG concentration was measured at t_1, t_2 and t_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 (1) a weak or strong response at an earlier time point and (2) no conversion during the following time points and (3) 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 χ^2 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 non-parametric Spearman rank correlation coefficients. All values were analysed according to complete case analysis. P values below 0.05 were considered significant. All statistical analyses were performed with SPSS V.28.0 for Windows and R statistical software v. 3.5.1 (http://www.r-project.org).

Patient and public involvement

All participants were HCWs at the respective hospitals and were involved, insomuch as they supported the 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 are summarised in table 1 and online supplemental table 1. The anti-SARS-CoV-2-specific IgGs against RBD and NP were assessed in 395 HCWs at three time points, after the first wave (t_1), at the beginning of the second wave (t_2) and after the second wave (t_3, figure 1).

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)

Table 1 summarises 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. The term children summarises all children or adolescents under 25 years. PCR stands for SARS-CoV-2-specific real-time reverse transcription PCR.
BMI, body mass index.

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-specific 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 summarised in table 2 and figure 2 for t_1, t_2 and t_3. Overall, 73 (18%) out of all 395 HCWs have been tested at least once positive, either regarding RBD or NP, at any time point (t_1, t_2 or t_3) during the study.

Comparison of RBD-specific and NP-specific IgG response

Out of 1156 specimen tested, 111 displayed a positive antibody response and 1045 showed a negative response. Out of these 111 specimen, 93 had antibodies against RBD and 79 had antibodies 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%; online supplemental table 2). Taking into account positive and negative test results, the concordance of NP-specific and RBD-specific responses 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-specific and RBD-specific responses is illustrated in figure 2.

Table 2 Antibody response during study

	Participants		RBD (U/mL)	NP (U/mL)	RBD–NP correlation
t1	All HCW	100% (n=395)	1.66 (0.12–0.89)	1.40 (0.15–0.98)	r=0.24 p<0.001
	Seropositive: either RBD or NP*	6% (n=24)	18.24 (1.55–10.54)	13.45 (1.94–22.71)	r=0.27 p=0.20
	Seropositive: RBD†	4% (n=17)	25.37 (5.73–13.16)	12.61 (1.21–22.11)	r=0.78 p<0.001
	Seropositive: NP‡	4% (n=16)	24.32 (0.35–14.19)	19.49 (5.90–33.53)	r=0.35 p=0.19
	Seropositive: RBD and NP§	2% (n=9)	42.51 (9.13–66.26)	22.60 (8.26–38.17)	r=0.23 p=0.55
	Seropositive (strong): either RBD or NP*	3% (n=13)	30.45 (5.50–28.57)	22.51 (8.26–34.99)	r=−0.03 p=0.93
	Seropositive (strong): RBD†	2% (n=9)	42.71 (9.13–66.26)	20.48 (6.86–38.17)	r=0.53 p=0.14
	Seropositive (strong): NP‡	3% (n=11)	34.38 (4.49–41.93)	25.88 (10.69–35.71)	r=−0.04 p=0.89
	Seropositive (strong): RBD and NP§	2% (n=7)	52.40 (10.96–90.60)	25.19 (8.90–45.04)	r=−0.14 p=0.76
t2	All HCW	100% (n=390)	2.78 (0.04–0.84)	1.59 (0.00–0.86)	r=0.30 p<0.001
	Seropositive either RBD or NP*	6% (n=25)	35.55 (4.68–57.16)	17.04 (2.10–25.30)	r=0.34 p=0.10
	Seropositive: RBD†	5% (n=21)	42.07 (7.06–86.65)	16.32 (1.82–19.65)	r=0.68 p<0.001
	Seropositive: NP‡	4% (n=16)	46.36 (4.41–110.71)	25.65 (6.23–39.98)	r=0.35 p=0.19
	Seropositive: RBD and NP§	3% (n=12)	61.37 (9.68–125.73)	27.26 (6.23–53.17)	r=0.50 p=0.09
	Seropositive (strong): either RBD or NP*	4% (n=17)	49.78 (7.62–107.21)	23.90 (5.85–38.18)	r=0.18 p=0.49
	Seropositive (strong): RBD†	3% (n=13)	64.20 (11.82–124.15)	23.86 (4.18–49.38)	r=0.50 p=0.09
	Seropositive (strong): NP‡	3% (n=11)	52.63 (3.85–120.99)	34.81 (15.45–56.97)	r=0.43 p=0.19
	Seropositive (strong): RBD and NP§	2% (n=7)	81.04 (20.64–134.98)	40.98 (12.15–65.57)	r=0.36 p=0.43
t3	All HCW	100% (n=371)	5.17 (0.10–1.09)	4.52 (0.22–1.50)	r=0.47 p<0.001
	Seropositive: either RBD or NP*	17% (n=62)	28.69 (6.57–33.54)	23.60 (4.93–23.59)	r=0.45 p<0.001
	Seropositive: RBD†	15% (n=55)	32.14 (8.47–41.89)	24.44 (4.17–25.55)	r=0.62 p<0.001
	Seropositive: NP‡	13% (n=47)	33.21 (8.35–41.89)	30.33 (8.91–29.91)	r=0.50 p<0.001
	Seropositive: RBD and NP§	11% (n=40)	38.74 (12.33–51.82)	32.66 (8.87–32.09)	r=0.61 p<0.001
	Seropositive (strong): either RBD or NP*	14% (n=52)	33.20 (10.39–45.08)	27.57 (7.71–28.30)	r=0.35 p=0.01
	Seropositive (strong): RBD†	12% (n=43)	39.46 (13.01–49.17)	29.76 (7.00–29.91)	r=0.53 p<0.001
	Seropositive (strong): NP‡	11% (n=40)	37.22 (8.38–51.82)	34.48 (11.71–36.35)	r=0.47 p=0.002
	Seropositive (strong): RBD and NP§	8% (n=31)	47.08 (16.05–53.55)	39.53 (10.75–40.78)	r=0.56 p<0.001

Table 2 summarises the concentration of SARS-CoV-2 RBD-specific and NP-specific antibody response at the respective time point given as mean (with IQR). 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-specific or NP-specific response. Seropositive HCW were further discriminated.
*Those with either an RBD-specific or an NP-specific response.
† Those with a RBD-specific response.
‡Those with an NP-specific response.
§Those with both an RBD-specific and a coexisting NP-specific response.
HCW, healthcare worker; NP, nucleocapsid protein; RBD, receptor-binding domain.

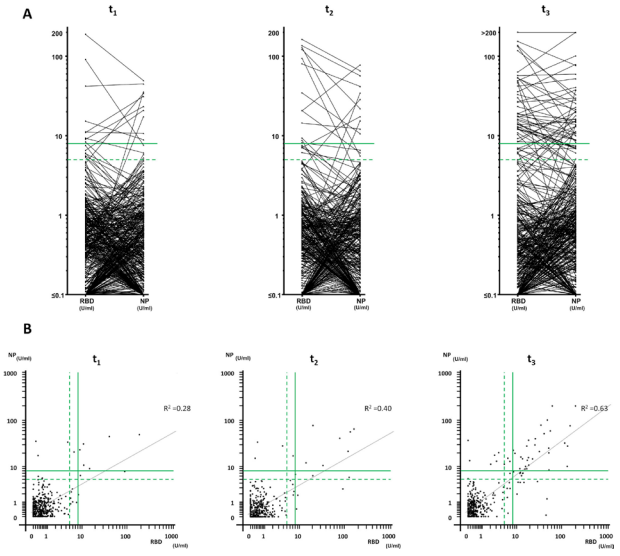

Figure 2 Concentration and spread of RBD-specific and NP-specific IgG response. (A) The intensities of anti-RBD (squares) and anti-NP-specific IgG responses (triangles) of each individual subject (connected by a line) are depicted at study time points t_1, t_2 and t_3. (B) Correlation of anti-RBD and anti-NP-specific IgG response of study participants is depicted at study time points t_1, t_2 and t_3. The solid grey line represents a linear regression line (R^2). The dashed green line separates positive responses (≥5 U/mL for anti-RBD and anti-NP IgG) from the background response. Values of ≥8 U/mL for anti-RBD and anti-NP IgG, representing a strong response, are separated by a solid green line. NP, nucleocapsid protein; RBD, receptor-binding domain; t_1, time point 1; t_2, time point 2; t_3, time point 3.

Table 3 RBD-specific and NP-specific responses in comparison

	Time point	Seropositive (%)	Seropositive (strong response, %)
Sensitivity of NP (=PPV for RBD)	t_1	53	78
	t_2	57	54
	t_3	73	72
	Total	66	69
Sensitivity of RBD (=PPV for NP)	t_1	56	64
	t_2	75	64
	t_3	85	78
	Total	77	73
Concordance of NP and RBD	t_1	96	98
	t_2	97	97
	t_3	94	94
	Total	96	97

Table 3 summarises the comparison between RBD-specific 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 (online supplemental table 2).
NP, nucleocapsid protein; PPV, positive predictive value; RBD, receptor-binding domain; t_1, time point 1; t_2, time point 2; t_3, time point 3.

Change of antibody response during time

Overall, the number as well as the intensity of RBD-specific and NP-specific IgG concentration increased during the study (online supplemental figure 1 and online supplemental table 3). Between t_1 and t_3, 44 HCWs (12%) seroconverted to a strong (≥8 U/mL) response (t_1–t_3-strong response converters) and 6 (2%) to only a weak (≥5 and <8 U/mL) response (t_1–t_3-weak response converters). Out of these 44 t_1–t_3-strong response converters, 43 converted from no response at t_1 to a strong response at t_3, and only one participant from an existing weak response to a strong response. The mean increase, compared with the background signal for these 44 t_1–t_3-strong response converters, was 42.3-fold for RBD-specific and a 43.7-fold for NP-specific antibody response, and for the 6 t_1–t_3-weak converters 3.5-fold and 2.3-fold, respectively (online supplemental table 3).

In contrast, 19 HCWs were found to have a declined antibody response between t_1 and t_3 (t_1–t_3 decliner). Of these, 10 had a strong response at t_1 (t_1–t_3-strong response decliners) and 9 had a weak response (t_1–t_3-weak response decliners).

Taking into account the t_1–t_3 and t_2–t_3 time overlap, in total, 23 individuals have declined antibody responses between t_1/t_2 and t_3 during a median time of 5 months (all decliners). The RBD-specific and NP-specific antibody responses of these 23 decliners have decreased by 51% and 60%, respectively (online supplemental table 3). The monthly decline of antibody response was 19% for RBD just as for NP (online supplemental table 3). This decline was significantly correlated with the strength of response measured at t_1/t_2 with an r of 0.71 (p<0.001) for RBD and an r of 0.89 (p<0.001) for NP (online supplemental figure 2). Strong responders had a more pronounced monthly decline than weak responders (online supplemental table 3). Taking into account the exponential nature of decline, the median half-lives of RBD-specific (5.5 (2.3–15.8) months) and NP-specific (5.7 (2.2–11.2) months) antibody responses were comparable (online 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_1 and t_2 or between t_1 and t_3. In detail, every HCW who had a strong RBD-specific antibody response at t_1 or t_2 maintained a positive RBD-specific response during the study. However, three subjects with a strong NP-specific response, who also had an RBD-specific response, had lost their NP-specific responses but maintained their RBD-specific response.

In contrast, out of 11 HCWs with only a weak response at t_1, only 2 kept a weak response at t_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 HCWs 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 previously, applying ELISA, 73 out of 395 HCWs have been tested positive at least once for SARS-CoV-2-specific antibodies during the study. Thus, the number of HCWs 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 (t_3) and taking into account only HCWs (n=48) who have been tested by both methods (RT-PCR and ELISA), we found that only five HCWs with a RT-PCR-proven COVID-19 infection had no antibody response, reflecting an antibody response rate of 90% (43/48). Regarding RBD-specific and NP-specific antibody response separately, the response rate was 83% for RBD-specific and 73% for NP-specific response. However, only 67% had a positive response for both, RBD-specific as well as NP-specific, IgGs. This comes down to 50% when considering only strong responses (online supplemental table 4).

The other way round, only 69% (43/62) of seropositive HCWs (either with an RBD-specific or an NP-specific antibody response) at t_3 have ever been identified by RT-PCR to be infected. Regarding RBD and NP separately, RT-PCR identified 73% (40/55) of those HCWs 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 t_3. Of these, 31 were seronegative and 2 were seropositive. One seropositive participant had a strong RBD-specific response and a coexisting strong NP-specific IgG response; the other had only a strong NP-specific response. However, in both cases, vaccination occurred just 1 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, HCWs who had COVID-19-specific symptoms at t_3 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 HCWs (A) without any response, (B) with only NP-specific response, (C) with only RBD-specific response and (D) with both RBD-specific and NP-specific responses, the percentage of HCWs with symptoms gradually and significantly increased (A=24.0%, B=42.9%, C=46.7% and 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 with those without any IgGs. Further data comparing HCW characteristics and antigen-specific response are provided in online supplemental 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-specific and 16% had only NP-specific antibodies. This clear discrepancy between NP-specific 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-specific and NP-specific responses. 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-specific 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 HCWs with a strong antibody response had previously been identified by RT-PCR.

Seroprevalence in the light of other study data on HCWs

Our data in HCWs revealed a 3% seroprevalence (strong response) at t_1, after the first wave. This was slightly above those from HCWs 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 USA, with a seroprevalence rate of 19% in the general population[28] and 27% in HCWs at the same time.[29]

At t_2 and t_3, 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% (t_2) and finally to 14% (t_3). This was just matching the seroprevalence of the general population in Austria at the same time points (t_2: 4.7%[30] and t_3: 15%[31]). Therefrom, HCWs in Vorarlberg appeared to be well prepared facing COVID-19 in the local healthcare 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 HCWs in the hospitals. Given the at least 17% undetected infections of HCWs in our hospitals, one may reconsider infection surveillance.

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

Currently, no vaccine used in the European Union 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-specific and RBD-specific IgG responses in infected subjects.

Furthermore, we also found a higher rate of symptoms in HCWs 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-CoV-2, which is known to be highly variable.[32] In addition, it has also been demonstrated by others that an NP-specific 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 with the spike protein-specific response.[16]

In a recent study, the concordance between NP-specific and RBD-specific responses 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 21 676 specimen from March to August 2020 also used two different providers for detecting NP-specific 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 titres and neutralisation 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 10 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 6 months.[12 25] A further study in New York City has found only a moderate decline regarding the spike protein-specific response during 5 months.[8] We here report a mean decline of 51% and 60% during 5 months for RBD-specific and NP-specific responses, respectively. A decrease of 17% and 31% for anti-spike IgG and anti-NP IgG titres 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 serological target[8] with a faster decline of NP compared with 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 neutralising 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 postinfection 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 with 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 HCWs of Vorarlberg as only HCWs in hospitals have been recruited on a voluntary basis. The infection risk of HCWs 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 (eg, 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 postinfection 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, for example, 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 serological measurements.

Given the limitations mentioned previously, the antibody response is yet widely used as a surrogate for deciding whether postinfection immunity to SARS-CoV-2 exists. The antibody response in our study has been 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

Serological 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 serological routine. Further, antigens, for example, the N-terminal domain[38] or the M protein[39] may have the potential to advance serological testing in the future. In view of undetected infections represented by the higher number of HCWs 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 postinfection 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.

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Contributors ALa had the original idea. MA, AM, TW, HD, ALa and ALe contributed to the study design and conceptualisation. 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 and JBJ collected data. E-MB, KG and ALe analysed the data. HD is the guarantor. MA and ALe wrote the manuscript. All authors contributed to reviewing the manuscript and approved the final version.

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