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Comparison of the prevalence of SARS-CoV-2 nucleoprotein antibodies in healthcare workers and an unselected adult and paediatric all-comer patient population: insights from a longitudinal study of healthcare workers and concurrent serial cross-sectional studies of patients at an academic medical centre in Austria

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ABSTRACT

Objectives This study aimed to estimate and compare the prevalence of the virus-specific antibodies against the SARS-CoV-2 nucleoprotein antigen (anti-SARS-CoV-2 N) in healthcare workers and an all-comer paediatric and adult patient population.

Design, setting and participants A longitudinal study enrolling healthcare professionals and concurrent serial cross-sectional studies of unselected all-comer patients were conducted at an Austrian academic medical centre. Healthcare workers were tested at enrolment and after 1, 2, 3, 6 and 12 months. The cross-sectional studies in patients were conducted at three time periods, which roughly coincided with the times after the first, second and third wave of SARS-CoV-2 in Austria (ie, 24 August–7 September 2020; 8–22 February 2021 and 9–23 November 2021). Anti-SARS-CoV-2 N antibodies were measured using a sandwich electrochemiluminescence assay (Roche).

Results In total, 2735 and 9275 samples were measured in 812 healthcare workers (median age: 40 years, 78% female) and 8451 patients (median age: 55 years, 52% female), respectively. Over the entire study period, anti-SARS-CoV-2 N antibodies were detected in 98 of 812 healthcare workers, resulting in a seroprevalence of 12.1% (95% CI 10.0% to 14.5%), which did not differ significantly (p=0.63) from that of the all-comer patient population at the end of the study period (407/3184; 12.8%, 95% CI 11.7% to 14.0%). The seroprevalence between healthcare workers and patients did not differ significantly at any time and was 1.5-fold to 2-fold higher than the number of confirmed cases in Austria throughout the pandemic. In particular, there was no significant difference in the seroprevalence between paediatric and adult patients at any of the tested time periods.

Conclusion Throughout the pandemic, healthcare staff and an adult and paediatric all-comer patient population had similar exposure to SARS-CoV-2.

Trial registration number ClinicalTrials.gov Identifier: NCT04407429.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ A large number of all-comer adult and paediatric patients (n=8451) and healthcare workers (n=812) were enrolled at a single tertiary medical centre in Austria, which had one of the highest numbers of PCR tests conducted per capita.
⇒ Because of the slow recruitment, the planned number of healthcare workers was not achieved.
⇒ However, because the true seroprevalence in our sample was lower than anticipated, this study remained well powered and provided estimates with narrow CIs.

BACKGROUND

The global spread of the SARS-CoV-2 represents the worst pandemic crisis since the 1918 'Spanish flu' influenza pandemic and has demanded rapid responses from national healthcare providers and governments. Containment measures, which ranged from

indoor masking, social distancing and the prohibition of crowd gatherings, to school closures, limiting freedom of movement and all the way to full lockdowns, were based primarily on the number of confirmed new cases with the goal of estimating the trajectory of hospitalisation and overburdening of healthcare systems. In accordance with the WHO recommendations, Austria established a comprehensive testing strategy in the winter of 2020, with one of the highest numbers of PCR tests conducted per capita worldwide aimed at tracking the spread of the virus and limiting transmission.[1 2] Despite these efforts, undertested populations, including children and underserved populations, as well as false-negative results due to sampling errors can result in erroneously low numbers. In addition, asymptomatic carriers may inadvertently contribute to the spread of the disease. Hence, serosurveys are critical to determining SARS-CoV-2 exposure and enabling population-level surveillance including estimating the number of unreported infections, which directly impacts the proper scale of necessary containment measures. Virus-specific antibodies against the SARS-CoV-2 nucleoprotein antigen (anti-SARS-CoV-2 N) are usually detectable 10–14 days after exposure to SARS-CoV-2 and may persist at least for several months.[3] Detection of these antibodies is largely specific for a previous infection, as they are not produced in response to vaccination with the currently used mRNA, vector or peptide vaccines.

Individuals seeking medical care in tertiary referral centres frequently belong to a vulnerable patient population. Moreover, a proportion of these patients may not develop immunity after infection or immunisation because of their underlying disease or concurrent treatments, such as immunosuppressive therapy, and are therefore at higher risk of hospitalisations and death. Studies have suggested that patients requiring healthcare might avoid medical services during the pandemic due to concerns about infection with SARS-CoV-2, which might further contribute to higher death rates.[4–8] Therefore, knowledge of the exposure risk of SARS-CoV-2 among healthcare workers and patients in hospitals is critical. However, only limited data on change in SARS-CoV-2 exposure over time, with a direct comparison of adult and paediatric patients versus healthcare workers, are available.

The present study aimed to estimate and compare the prevalence of anti-SARS-CoV-2 N antibodies in healthcare workers and an all-comer paediatric and adult patient population at a tertiary academic medical centre in Austria.

METHODS

Study design

The Vienna-versus-Virus study (ClinicalTrials.gov Identifier: NCT04407429) was designed as a prospective, observational study to assess the seroprevalence of SARS-CoV-2 antibodies in healthcare workers and an all-comer patient population at Vienna General Hospital and the Medical University of Vienna. The present study consisted of two parts: a longitudinal study enrolling healthcare professionals and serial cross-sectional studies of unselected all-comer patients.

Vienna General Hospital is a tertiary referral and academic medical centre that was designated as a primary non-COVID-19 hospital at the onset of the pandemic. As such, Vienna General Hospital was contracted to provide care for non-COVID-19 related emergencies (except for dedicated COVID-19 classification units responsible for screening and transferring patients to designated COVID-19 hospitals) or to provide enhanced intensive care medicine. Therefore, patients admitted to the ward were typically required to have no signs of active SARS-CoV-2 infection and a negative SARS-CoV-2 PCR test. In addition, hospital containment policies in Austria included regular PCR testing of healthcare workers and testing of hospitalised patients on admission, visitor restrictions and the wearing of face masks for healthcare workers and visitors.

Healthcare workers

Eligible healthcare workers were physicians, nursing staff, midwives, medical-technical assistants (ie, medical, therapeutic and diagnostic healthcare staff, and medical and nursing assistants) and administrative personnel with patient contact. Serial blood samples were collected at enrolment and after 1, 2, 3, 6 and 12 months. Biomaterial was processed and stored according to standard operating procedures in an ISO 9001:2015-certified environment by the biobank facility of the Medical University of Vienna.[9] Information on demographic characteristics, the professional environment and health status, including comorbidities, clinical symptoms and possible exposure to SARS-CoV-2 were obtained through electronic questionnaires.

Unselected all-comer patient population

All patients who received medical care at Vienna General Hospital and the Medical University of Vienna with available residual serum samples (which were collected based on the clinical indication by the patients' treating physician) were included consecutively at three different time periods (period A: 24 August–7 September 2020; period B: 8 February–22 February 2021; period C: 9 November–23 November 2021). These time points roughly coincided with the times after the first, second and third waves of SARS-CoV-2 in Austria.

Antibody testing was done using leftover diagnostic serum samples. The results were linked with the electronic health records at the individual patient level (including patients' demographics, medical history, available echocardiograms and laboratory measurements).

Anti-SARS-CoV-2 N antibody testing

Anti-SARS-CoV-2 N antibodies were measured using a sandwich electrochemiluminescence assay on cobas

e602 modular analyzers (Elecsys Anti-SARS-CoV-2, Roche Diagnostics, Rotkreuz, Switzerland) at the Department of Laboratory Medicine of the Medical University of Vienna (with a quality management system accredited according to ISO 15189:2012). Anti-SARS-CoV-2 N antibodies are detected after natural infection with SARS-CoV-2, whereas antibodies against the SARS-CoV-2 spike arise as a response to both effective SARS-CoV-2 vaccination and infection.

In brief, this binding assay uses SARS-CoV-2 nucleoprotein antigens, which are either biotinylated or ruthenylated. Anti-SARS-CoV-2 N antibodies (IgG, IgA and IgM) present in patient sera are captured by these antigens in a double-antigen sandwich. The biotinylated antigen keeps the circulating antibodies attached to magnetic microparticles via biotin/streptavidin interaction within the measuring cell, whereas the ruthenium complex bound to the second antigen emits an electrochemiluminescence signal that rises with the antibody concentration. This assay comes with excellent analytical sensitivity (>99%) and specificity (>99%) for samples taken >14 days after symptom onset.[10] A cut-off index (COI) ≥1.0 indicates the presence of anti-SARS-CoV-2 antibodies. The reported intermediate precision in positive samples was 2.3%–6.5%.

Statistics

We used the median and IQR to summarise continuous variables and counts and frequencies for categorical data. Comparisons between groups were made using the Mann-Whitney U test for continuous variables and the χ^2 test for categorical variables. We calculated the seroprevalence and 95% CIs according to Wilson's score method. All statistical analyses were done using R (version 4.1.2, Foundation for Statistical Computing, Vienna, Austria). A two-sided p value of 0.05 was considered significant for all tests.

Sample size considerations

At the time that the study was designed at the onset of the pandemic, we did not know the pandemic's trajectory and assumed that 20% of healthcare workers and 19% of the subjects in the patient population would have detectable antibodies. A sample size of 2908 healthcare workers (after correction for a finite population size of 40000 healthcare workers in Austria), and 3017 patients was required to estimate the expected

Table 1 Baseline characteristics of healthcare workers stratified by presence of anti-SARS-CoV-2 N antibodies

Characteristic	Overall n=812	Anti-SARS- CoV-2 N antibodies n=98	No anti- SARS-CoV-2 N antibodies n=714	P value
Age	40 (30–51)	38 (28–52)	40 (30–51)	0.95
Female sex	633 (78%)	76 (78%)	557 (78%)	0.92
BMI (kg/m^2)	23.8 (21.5–27.5)	25.1 (22.4–29.3)	23.7 (21.5–27.1)	0.014
Occupation				0.058
Physician	163 (20%)	12 (12%)	151 (21%)	
Nursing staff	370 (46%)	56 (57%)	314 (44%)	
Medical technician	208 (26%)	21 (21%)	187 (26%)	
Administrative personnel	71 (8.7%)	9 (9.2%)	62 (8.7%)	
Working hours per week	40 (35–40)	40 (35–40)	40 (35–40)	0.68
Working setting				0.044
Inpatient	413 (51%)	61 (62%)	352 (49%)	
Outpatient	269 (33%)	27 (28%)	242 (34%)	
Laboratory	130 (16%)	10 (10%)	120 (17%)	
Smoking	161 (20%)	12 (12%)	149 (21%)	0.045
Physical activity >2× per week	416 (51%)	49 (50%)	367 (51%)	0.79
Any medication	279 (34%)	38 (39%)	241 (34%)	0.33
Pneumococcal immunisation	123 (15%)	18 (18%)	105 (15%)	0.34
Influenza immunisation	341 (42%)	32 (33%)	309 (43%)	0.046
Number of people living in household				0.021
<3	579 (71%)	63 (64%)	516 (72%)	
3–4	210 (26%)	28 (29%)	182 (25%)	
>4	23 (2.8%)	7 (7.1%)	16 (2.2%)	
Households with children <10 years	163 (24%)	18 (22%)	145 (25%)	0.59

Continuous data are reported as medians (25th–75th percentile).
BMI, body mass index.

Table 2 Baseline characteristics of the unselected all-comer patient population stratified by presence of anti-SARS-CoV-2 N antibodies

Characteristic	Overall n=**8451**	**Anti-SARS-CoV-2 N antibodies n=658**	No anti-SARS-CoV-2 N antibodies n=**7793**	**P value**
Age	55 (39–67)	49 (33–62)	55 (40–68)	<0.001
Female sex	4369 (52%)	370 (56%)	3999 (51%)	0.016
Outpatients	6392 (76%)	520 (79%)	5872 (75%)	0.035
Diabetes	987 (12%)	68 (10%)	919 (12%)	0.26
Hypertension	2515 (30%)	143 (22%)	2372 (30%)	<0.001
Coronary artery disease	1283 (15%)	74 (11%)	1209 (16%)	0.003
Heart failure	753 (8.9%)	47 (7.1%)	706 (9.1%)	0.10
PAD	238 (2.8%)	11 (1.7%)	227 (2.9%)	0.065
Atrial fibrillation	713 (8.4%)	45 (6.8%)	668 (8.6%)	0.12
CKD	1532 (18%)	86 (13%)	1446 (19%)	<0.001
Stroke	512 (6.1%)	31 (4.7%)	481 (6.2%)	0.13
Pneumonia/COPD	737 (8.7%)	49 (7.4%)	688 (8.8%)	0.23
Liver disease	533 (6.3%)	29 (4.4%)	504 (6.5%)	0.037
Cancer	2075 (25%)	114 (17%)	1961 (25%)	<0.001
BMI (kg/m^2)	26.3 (23.2–30.4)	25.3 (21.8–30.3)	26.4 (23.3–30.4)	0.45
eGFR (mL/min/1.73 m^2)	86 (64–106)	93 (70–117)	85 (63–106)	<0.001
Serum creatinine (mg/dL)	0.86 (0.71–1.11)	0.80 (0.66–1.03)	0.87 (0.71–1.11)	<0.001
Total cholesterol (mg/dL)	174 (143–207)	175 (142–207)	174 (143–207)	0.81
LDL-C (mg/dL)	92 (67–120)	96 (70–123)	92 (67–120)	0.10
Triglycerides (mg/dL)	110 (78–159)	111 (75–162)	109 (78–158)	0.91
WCC (10^9/L)	7.9 (6.0–10.6)	7.8 (5.9–10.4)	7.9 (6.1–10.6)	0.34
hsCRP (mg/L)	0.6 (0.2–3.0)	0.6 (0.2–3.2)	0.6 (0.2–3.0)	0.29
Hemoglobin A1c (%)	5.70 (5.30–6.20)	5.60 (5.27–6.30)	5.70 (5.30–6.20)	0.19
NT-proBNP (pg/mL)	438 (118–1821)	254 (62–1297)	458 (123–1862)	<0.001

Continuous data are reported as medians (25th–75th percentile).

BMI, body mass index; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C reactive protein; LDL-C, Low-density lipoprotein cholesterol; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; PAD, peripheral artery disease; WBC, white cell count.

proportion with 1.4% absolute precision and 95% confidence. This sample size would also provide >80% power to detect a difference of 3% between the two cohorts (assuming a prevalence of 20% in healthcare workers). However, recruitment of healthcare workers was stopped early before reaching the planned sample size due to slow enrolment and a substantially lower observed prevalence of SARS-CoV-2 infections than anticipated (a smaller sample size is required to provide estimates with the same accuracy when the true seroprevalence is lower).

Patient and public involvement

The present study did not involve healthcare workers and patients in the design, conduct, reporting or dissemination plans. However, healthcare workers were informed about their antibody testing result.

RESULTS

Study population

In total, 812 healthcare workers and 8451 unselected all-comer patients were included in the present study. The healthcare workers' median age was 40 years (25th–75th percentiles 30–51 years), and 633 (78%) were female (table 1).

The majority of healthcare staff were employed at the Department of Internal Medicine (24%) and worked on wards (51%). Compared with healthcare workers, the patient population was older (55 years, 25th–75th percentiles 39–67 years) and had a balanced sex ratio (n=4369, 52%). Among the included patient population, 25% had a history of cancer, 15% had coronary artery disease, 12% diabetes and 9% heart failure (table 2).

Most of the characteristics of the patient population were similar across the different time periods. However, the

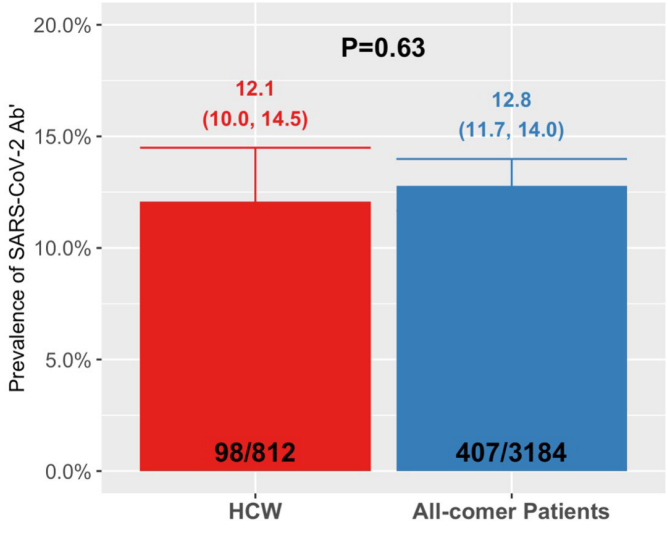

Figure 1 Prevalence of anti-SARS-CoV-2 N antibodies in healthcare workers over the entire study period versus an unselected all-comer patient population tested at the end of the study. HCW, healthcare workers.

proportion of patients <18 years (1.4% vs 5.3%) and the proportion of inpatients (15% vs 27%) were significantly lower in patients recruited in the last cross-sectional analysis in November 2021 than in patients who were enrolled during earlier periods (online supplemental table 1).

Seroprevalence in healthcare workers and in an unselected all-comer patient population

A total of 2735 and 9275 samples were assayed from 812 healthcare workers and 8451 patients, respectively. Throughout the study period, anti-SARS-CoV-2 N antibodies we measured in 98 of 812 healthcare workers, resulting in a seroprevalence of 12.1% (95% CI 10.0% to 14.5%), which was not significantly different from the cross-sectional analysis in the all-comer patient population at the end of the study period (407/3184; 12.8%, 95% CI 11.7% to 14.0%, p=0.63; figure 1).

Among healthcare workers with anti-SARS-CoV-2 N antibodies, the median relative reduction in SARS-CoV-2 COI was 26.8% and 50.4% after 3 and 6 months, respectively (figure 2). Four individuals fell below the cut-off for positivity (ie, below 1.0) (2 individuals after 2 months, 1 individual after 3 months and 1 after 6 months), though they retained a COI >0.50. One individual who turned negative after 6 months experienced reinfection.

Regarding the specific time periods during the study, between 29 May and 7 September 2020 (period A), 16 of 568 healthcare workers and 60 of 3010 patients had detectable anti-SARS-CoV-2 N antibodies, resulting in a seroprevalence of 2.8% (95% CI 1.7% to 4.5%) and 2.0% (95% CI 1.6% to 2.6%), respectively (figure 3). In the subsequent period (from 8 September 2020 to 22 February 2021), we measured anti-SARS-CoV-2 N antibodies in 36 of 498 healthcare workers and 288 of the 3082 patients, yielding a seroprevalence of 7.2% (95% CI 5.3% to 9.8%) and 9.3% (95% CI 8.4% to 10.4%), respectively. In the final testing period (from 23 February 2021 to 23 November 2021), we measured anti-SARS-CoV-2 N antibodies in 98 of 812 healthcare workers (14.9%, 95% CI 12.2% to 18.1%) and in 407 of 3184 (12.8%, 95% CI 11.7% to 14.0%, figure 2). The seroprevalence of healthcare workers and patients did not differ significantly at any of the individual time periods (all p>0.10). However, the proportion of both healthcare workers and patients with detectable anti-SARS-CoV-2 N antibodies was significantly higher than the proportion of confirmed cases in Austria's general population at all time periods (7 September 2020: 0.3%; 22 February 2021: 5.0%; 23 November 2021: 12.2%; all p<0.01).[11] These findings remained consistent when implementing a lower threshold (ie, COI >0.165) of the SARS-CoV-2 assay (online supplemental figure 1).

Seroprevalence in pediatric patients

In total, 371 paediatric patients (median age 13 years, 25th–75th percentiles 9–16 years; 56% female) were included. The seroprevalence in paediatric patients was 1.8% (95% CI 0.6% to 5.1%; n/n=3/170), 13.5% (95% CI 9.0% to 19.7%; n/n=21/156) and 11.1% (95% CI 4.8% to 23.5%, n/n=5/45) between 24 August and 7 September 2020 (period A), 8 February and 22 February 2021 (period B) and 9 November 2021–23 November 2021 (period C), respectively (figure 4). The median age of paediatric patients with anti-SARS-CoV-2 N antibodies was 13 (25th–75th percentile 8–14 years) and 48% were female (online supplemental figure 2). There was no significant difference in the seroprevalence between paediatric and adult patients at any time period tested (all p values >0.09).

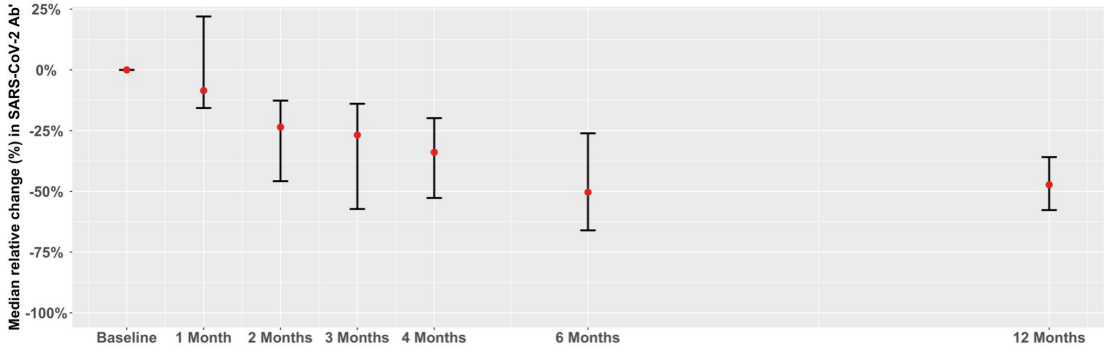

Figure 2 Median relative reduction in anti-SARS-CoV-2 N nucleoprotein antibody cut-off index over time.

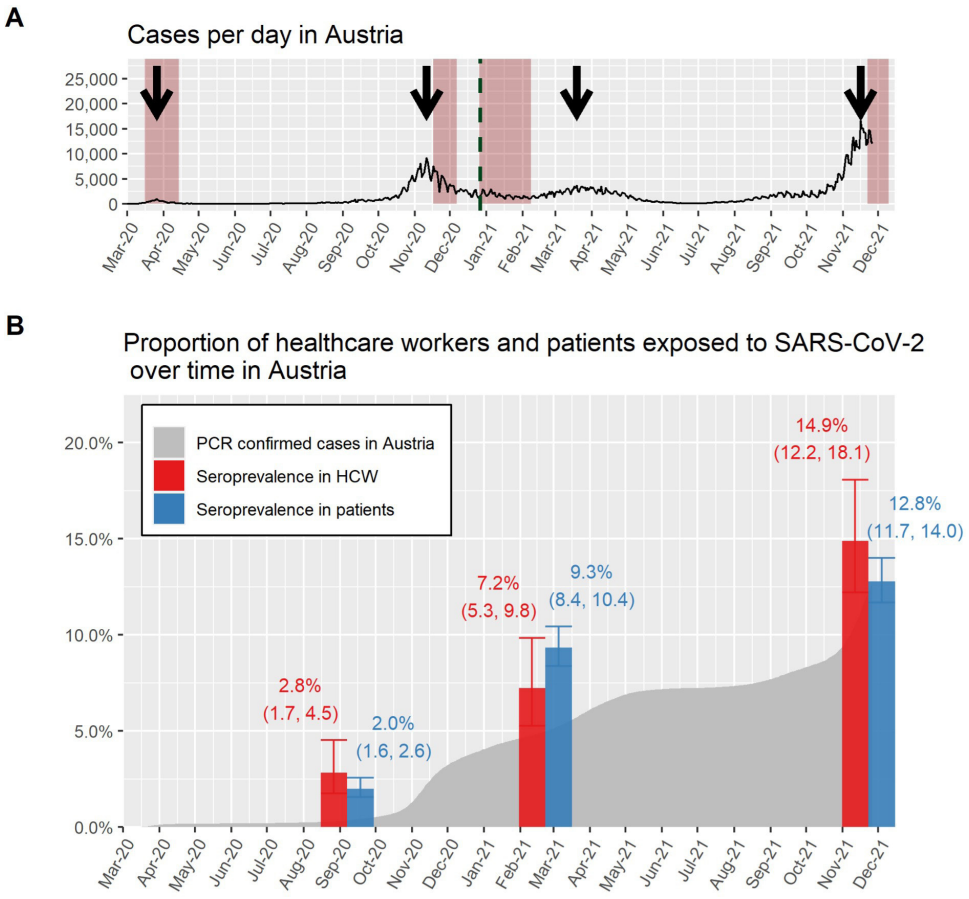

Figure 3 Prevalence of anti-SARS-CoV-2 N antibodies in healthcare workers (HCWs) versus an unselected all-comer patient population tested and the number of confirmed cases in Austria. Panel A: confirmed PCR cases per day in Austria. The lockdown time periods in Austria are highlighted in red. The black arrows indicate the peaks of each wave during the pandemic. Panel B: prevalence of anti-SARS-CoV-2 N antibodies in healthcare workers (HCWs) and an unselected all-comer patient population and the cumulative number of confirmed cases in Austria.

Anti-SARS-CoV-2 N antibody response after positive PCR results in patients

In total, 27 220 PCR tests were done ≥14 days before laboratory testing in 4872 patients. Overall, 299 positive PCR tests were recorded in 108 patients of which 91 (84.3%) patients had detectable antibodies. Among patients with a previous positive PCR test and detectable antibodies, the median time between the first documented positive PCR test and antibody testing was 146 days (25th–75th percentiles 67–298 days; figure 5).

Clinical characteristics of individuals with anti-SARS-CoV-2 N antibodies

Healthcare workers with detectable anti-SARS-CoV-2 N antibodies were similar to those without them in their measured baseline characteristics but were more likely to live in larger households (table 1). In contrast, the prevalence of detectable anti-SARS-CoV-2 N antibodies in patients was associated with a better overall health status, as reflected in their younger age (49 vs 56), and lower rates of cancer, hypertension, coronary artery disease and chronic kidney disease (table 2, online supplemental table 2. There was no significant difference across the hospital departments (all p values >0.14; online supplemental figure 3 or in the proportion of inpatients versus outpatients with and without anti-SARS-CoV-2 N antibodies across the tested time periods (all p values >0.29, online supplemental figure 4. Healthcare workers with detectable anti-SARS-CoV-2 N antibodies reported a wide range of symptoms online supplemental figure 5. Only 13 (13%) individuals were completely asymptomatic, and 26 (26.5%) were oligosymptomatic and reported only one or two symptoms online supplemental figure 6.

DISCUSSION

In this large-scale study of healthcare workers and patients, we found a similar proportion of anti-SARS-CoV-2 N antibodies in healthcare workers and an unselected all-comer patient population that was nearly 1.5-fold to two-fold higher than the reported number of confirmed cases in Austria throughout the pandemic. Moreover, at any time during the pandemic, the seroprevalence was similar in adult and paediatric patients.

These findings have important clinical implications: first, seroprevalence estimates provide a more accurate assessment of the true SARS-CoV-2 infection rate than reported case numbers would allow, because mild or

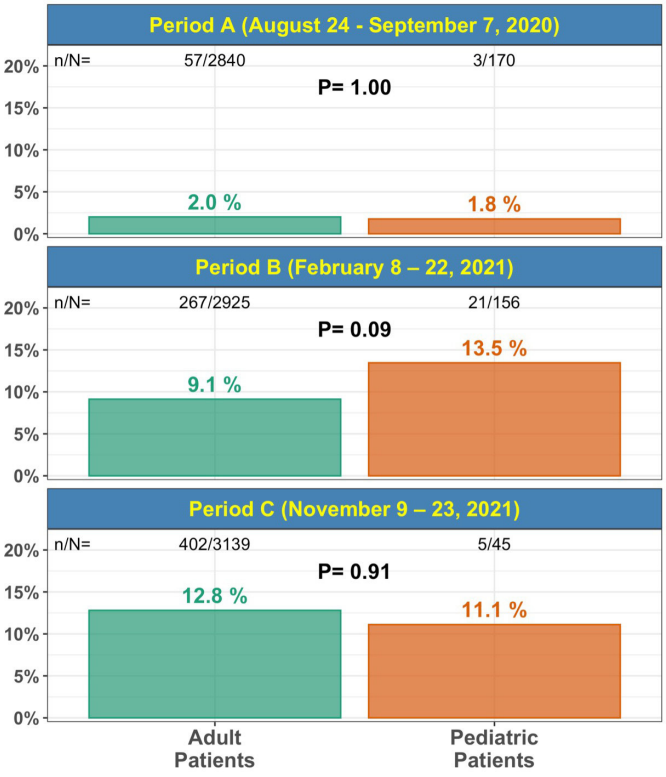

Figure 4 Prevalence of anti-SARS-CoV-2 N antibodies in paediatric (ie, <18 years) and adults patients across the tested time periods.

asymptomatic infections may be missed. Despite extensive per capita PCR testing in Austria, which is among the most frequently performed worldwide,[1] and comprehensive containment policies that resulted in a lower number of confirmed cases compared with other European countries,[12] the infection rate in our study was 1.5-fold to two-fold higher than the number of confirmed cases in Austria throughout the pandemic. However, it is worth noting that only few healthcare workers were asymptomatic or oligosymptomatic.

The present findings suggest that healthcare workers and patients have a similar risk of exposure to SARS-CoV-2. Patients with versus without anti-SARS-CoV-2 N antibodies were younger and had fewer comorbidities, reflecting higher activity in life, suggesting that the true infection rate in the general population is likely even higher. Because of differences in containment policies, availability of personal protective equipment, access to healthcare and testing regimen across countries, substantial regional heterogeneity in the seroprevalence and the number of confirmed COVID-19 cases has been reported.[13–18] Exemplary, a sero-survey conducted during the first wave in New York suggested that case-based surveillance underestimated the number of infections by a factor of 10 during that time.[13] The results of the present study thus imply that the established hospital containment policies, including regular PCR testing of healthcare workers and testing of hospitalised patients on admission, visitor restrictions and the wearing of face masks for healthcare workers and visitors masking were effective.

Second, these findings indicate greater transmission in children than had previously been assumed.[15] This knowledge is essential because vaccination had not been approved for children <5 years old.[19]

High rates of transmission pose other dangers as well: in addition to acute SARS-CoV-2 infections, children and adults can develop long-term sequelae, such as fatigue,[20] impaired exercise capacity, cognitive disturbances and changes in brain structure,[21] and cardiovascular complications,[22] including arrhythmias, inflammatory heart disease, heart failure and thromboembolic disease. Although patients with more severe COVID-19 during the acute phase of the infection were more likely to develop significant health problems, these risks and burdens were evident even among not hospitalised individuals.[22] Even if the proportion of individuals who develop postacute sequelae of SARS CoV-2 infection is small, the absolute numbers will be staggering due to the high global burden and will likely have a significant impact on both healthcare systems and the economy. At this time, it is unclear how long these conditions may persist, and

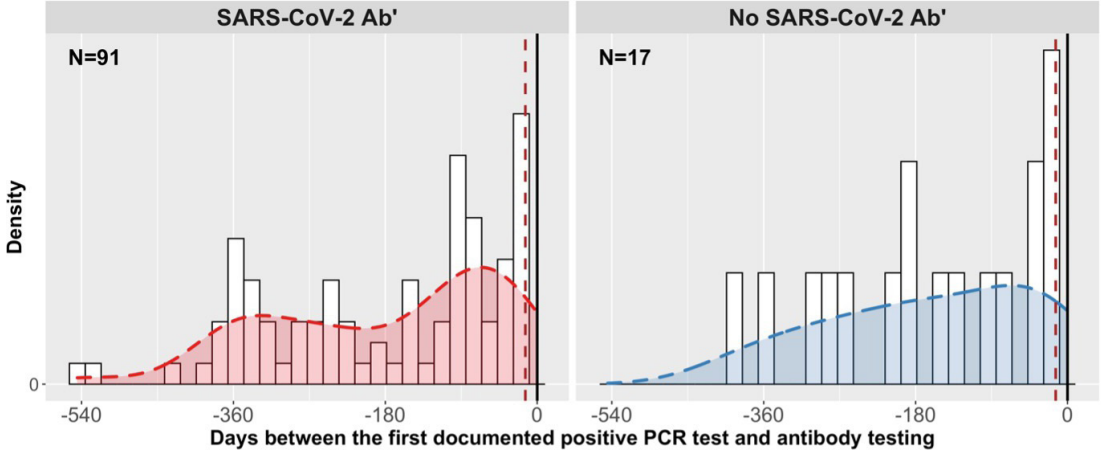

Figure 5 Distribution between the time of the first documented positive PCR test and antibody testing in patients with (left panel) and without (right panel) detectable anti-SARS-CoV-2 N antibodies.

there is currently no treatment available. As a result of the increasing infection rates, future research will need to focus on postacute sequelae of SARS CoV-2 prevention and mitigation strategies.

The assay used in the present study uses anti-SARS-CoV-2 nucleoprotein antibodies that represent previous infection (but not antibodies that develop after successful vaccination). However, at this time, it is not yet clear how long the antibodies persist after infection. We noted a linear reduction in SARS-CoV-2 COI with an approximately 50% relative reduction at 6 months. Similar findings have been reported by other groups.[23 24] Although this assay has excellent sensitivity and specificity and its results have been shown to correlate well with neutralising antibodies,[10 25–28] this observation should be, however, interpreted with caution as the magnitude of the measured result above the cut-off may not be a reliable indication of the total amount of antibody present in the sample.[27 28]

Overall, these results shed light on infection rates in specific populations, including children, and thus provide deeper insight into overall immunity status by complementing information about vaccination rates and confirmed cases, ultimately informing policies and strategies to contain the spread of the virus.[29]

Limitations

We acknowledge several limitations of this study. First, because of slow recruitment, we did not achieve the initially planned number of healthcare workers. However, because the true seroprevalence in our sample was much lower than anticipated, this study remained well powered and provided estimates with narrow CIs. Although data suggest that antibody presence to be associated with natural immunity,[29–34] individual immunological responses to SARS-CoV-2 infection can vary significantly. Our study cannot determine whether the presence of anti-SARS-CoV-2 N antibodies serve a surrogate for immunity. Furthermore, testing from different manufacturers may yield different results. Due to the descriptive nature of this analysis, no adjustments for multiple testing were performed.

CONCLUSIONS

Throughout the pandemic up until the end of 2021, healthcare staff and an all-comer patient population at a tertiary academic medical centre had similar exposure to SARS-CoV-2 that was nearly 1.5-fold to twofold higher than the reported number of confirmed cases in Austria. Infection rates did not differ between adults and children over the entire study period. These findings emphasise the need for research that focuses on preventing and mitigating long-term complications of SARS-CoV-2.

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Contributors CH and TAZ conceived of the study design. CJB, CN, ESS, MR and TS participated in the study design. ESS and TS provided statistical expertise. CJB, TP and HH provided laboratory expertise. MR, CN, FJ, EA and FH coordinated and implemented the study and participated in all aspects of its conduct. TAZ performed the statistical analysis, created the figures, wrote the first draft of the manuscript, holds the grant, and is the guarantor of the work. All authors interpreted the data, revised the manuscript and approved the final manuscript.

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Competing interests MR, CN, CJB, ESS, TS, FJ, EA, FH, HH, TP and CH report no conflicts of interest for this work. Outside of the submitted work, CJB reports honoraria for serving on advisory boards and speaker honoraria from Amgen, Daiichi-Sankyo, Novartis, SOBI; board member of Technoclone. ESS reports research grants from the US National Health Institutes (NIH), the European Commission's Program Horizon 2020, the Austrian Science Funds (FWF) and the Austrian Research Promotion Agency (FFG). HH reports grants from Glock Health Science and Research, BlueSky Immunotherapies and the Austrian Ministry of Education Science and Research. TAZ reports research grants from the Austrian Science Funds and the German Research Foundation, honoraria for serving on advisory boards from Boehringer Ingelheim, personal fees from Alkem Metabolics, AstraZeneca, Boehringer Ingelheim and Sun Pharmaceutical Industries and educational grants from Eli Lilly and Company.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained directly from patient(s)

Ethics approval This study involves human participants and was approved by ethics committee of the Medical University of Vienna (EK 1387/2020).All healthcare workers provided written informed consent. According to the institutional review board, no informed consent from the patient population was required to perform antibody testing using leftover diagnostic serum samples.

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Data availability statement Data are available on reasonable request. Per the Data Clearing House of the Medical University of Vienna, the collected data contain special categories of personal data (ie, 'sensitive' data, eg, employees' health information) and cannot be anonymised. Therefore, public data sharing has been prohibited by the Data Clearing House. However, on reasonable request for the purpose of reproducibility, data can be provided after verification and contract establishment through the Data Clearing House at the Medical University of Vienna. Any study groups wishing to study additional research questions are encouraged to address the corresponding author for collaboration.

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REFERENCES

1 Hasell J, Mathieu E, Beltekian D, *et al*. A cross-country database of COVID-19 testing. *Sci Data* 2020;7:345.
2 World Health Organization. Contact tracing in the context of COVID-19, 2021. Available: https://apps.who.int/iris/bitstream/handle/10665/339128/WHO-2019-nCoV-Contact_Tracing-2021.1-eng.pdf?sequence=24&isAllowed=y [Accessed 03 June 2022].
3 Wajnberg A, Amanat F, Firpo A, *et al*. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. *Science* 2020;370:1227–30.
4 De Luca G, Verdoia M, Cercek M, *et al*. Impact of COVID-19 pandemic on mechanical reperfusion for patients with STEMI. *J Am Coll Cardiol* 2020;76:2321–30.
5 Mafham MM, Spata E, Goldacre R, *et al*. COVID-19 pandemic and admission rates for and management of acute coronary syndromes in England. *Lancet* 2020;396:381–9.
6 Sulzgruber P, Krammel M, Aigner P, *et al*. An increase in acute heart failure offsets the reduction in acute coronary syndrome during coronavirus disease 2019 (COVID-19) outbreak. *ESC Heart Fail* 2021;8:782–3.
7 Tejada Meza H, Lambea Gil Álvaro, Saldaña AS, *et al*. Impact of COVID-19 outbreak on ischemic stroke admissions and in-hospital mortality in north-west Spain. *Int J Stroke* 2020;15:755–62.
8 Wadhera RK, Shen C, Gondi S, *et al*. Cardiovascular Deaths During the COVID-19 Pandemic in the United States. *J Am Coll Cardiol* 2021;77:159–69.
9 Haslacher H, Gerner M, Hofer P, *et al*. Usage data and scientific impact of the prospectively established fluid BioResources at the hospital-based MedUni Wien Biobank. *Biopreserv Biobank* 2018;16:477–82.
10 Elecsys® Anti-SARS-CoV-2. *Package insert V30; material numbers 09203095190 and 09203079190*, 2020: 2020–7.
11 AGES/EMS. Ages dashboard COVID19. 2021. Available: https://covid19-dashboard.ages.at/ [Accessed 13 Jan 2022].
12 World Health Organization. Coronavirus (COVID-19) Dashboard. Available: https://covid19.who.int [Accessed 25 Nov 2022].
13 Stadlbauer D, Tan J, Jiang K, *et al*. Repeated cross-sectional sero-monitoring of SARS-CoV-2 in New York City. *Nature* 2021;590:146–50.
14 Pritsch M, Radon K, Bakuli A, *et al*. Prevalence and risk factors of infection in the representative COVID-19 cohort Munich. *Int J Environ Res Public Health* 2021;18:3572.
15 Boey L, Roelants M, Merckx J, *et al*. Age-Dependent seroprevalence of SARS-CoV-2 antibodies in school-aged children from areas with low and high community transmission. *Eur J Pediatr* 2022;181:571–8.
16 Boehme KW, Kennedy JL, Snowden J, *et al*. Pediatric SARS-CoV-2 seroprevalence in Arkansas over the first year of the COVID-19 pandemic. *J Pediatric Infect Dis Soc* 2022;11:248–56.
17 Wachter F, Regensburger AP, Peter AS. Continuous monitoring of SARS-cov-2 seroprevalence in children using residual blood samples from routine clinical chemistry. *Clin Chem Lab Med* 2022;60:941–51.
18 Vette KM, Machalek DA, Gidding HF, *et al*. Seroprevalence of severe acute respiratory syndrome coronavirus 2-specific antibodies in Australia after the first epidemic wave in 2020: a national survey. *Open Forum Infect Dis* 2022;9:ofac002.
19 Molteni E, Canas LS, Kläser K. Vaccination against SARS-cov-2 in UK school-aged children and young people decreases infection rates and reduces COVID-19 symptoms. *The Lancet Regional Health - Europe* 2022;22272176
20 Carfì A, Bernabei R, Landi F, *et al*. Persistent symptoms in patients after acute COVID-19. *JAMA* 2020;324:603–5.
21 Douaud G, Lee S, Alfaro-Almagro F, *et al*. SARS-CoV-2 is associated with changes in brain structure in UK Biobank. *Nature* 2022;604:697–707.
22 Xie Y, Xu E, Bowe B, *et al*. Long-Term cardiovascular outcomes of COVID-19. *Nat Med* 2022;28:583–90.
23 Carreño JM, Mendu DR, Simon V, *et al*. Longitudinal analysis of severe acute respiratory syndrome coronavirus 2 seroprevalence using multiple serology platforms. *iScience* 2021;24:102937.
24 Ortiz AT, Torrente FF, Twigg A, *et al*. The influence of time on the sensitivity of SARS-cov-2 serological testing. *Res Sq* 2022;
25 Perkmann T, Perkmann-Nagele N, Breyer M-K, *et al*. Side-By-Side comparison of three fully automated SARS-CoV-2 antibody assays with a focus on specificity. *Clin Chem* 2020;66:1405–13.
26 Tang MS, Hock KG, Logsdon NM, *et al*. Clinical performance of the Roche SARS-CoV-2 serologic assay. *Clin Chem* 2020;66:1107–9.
27 Meyer B, Torriani G, Yerly S, *et al*. Validation of a commercially available SARS-cov-2 serological immunoassay. *Clin Microbiol Infect* 2020;26:1386–94.
28 Olbrich L, Castelletti N, Schälte Y, *et al*. Head-to-head evaluation of seven different seroassays including direct viral neutralisation in a representative cohort for SARS-cov-2. *J Gen Virol* 2021;
29 Lumley SF, O'Donnell D, Stoesser NE, *et al*. Antibody status and incidence of SARS-CoV-2 infection in health care workers. *N Engl J Med* 2021;384:533–40.
30 Addetia A, Crawford KHD, Dingens A, *et al*. Neutralizing antibodies correlate with protection from SARS-CoV-2 in humans during a fishery vessel outbreak with a high attack rate. *J Clin Microbiol* 2020;58:e02107–20.
31 Pray IW, Gibbons-Burgener SN, Rosenberg AZ, *et al*. COVID-19 Outbreak at an Overnight Summer School Retreat - Wisconsin, July-August 2020. *MMWR Morb Mortal Wkly Rep* 2020;69:1600–4.
32 Harvey RA, Rassen JA, Kabelac CA, *et al*. Association of SARS-CoV-2 seropositive antibody test with risk of future infection. *JAMA Intern Med* 2021;181:672–9.
33 Hall VJ, Foulkes S, Charlett A, *et al*. SARS-cov-2 infection rates of antibody-positive compared with antibody-negative health-care workers in england: a large, multicentre, prospective cohort study (SIREN). *Lancet* 2021;397:1459–69.
34 Pouwels KB, Pritchard E, Matthews PC, *et al*. Effect of delta variant on viral burden and vaccine effectiveness against new SARS-cov-2 infections in the UK. *Nat Med* 2021;27:2127–35.