



COVID-19 serology in nephrology healthcare workers

Thomas Reiter · Sahra Pajenda · Ludwig Wagner · Martina Gaggl · Johanna Atamaniuk · Barbara Holzer · Irene Zimpernik · Daniela Gerges · Katharina Mayer · Christof Aigner · Robert Straßl · Sonja Jansen-Skoupy · Manuela Födinger · Gere Sunder-Plassmann · Alice Schmidt

Received: 20 January 2021 / Accepted: 9 March 2021 / Published online: 9 April 2021
 © The Author(s) 2021

Summary

Background Chronic kidney disease patients show a high mortality in cases of a severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection. Thus, information on the sero-status of nephrology personnel might be crucial for patient protection; however, limited information exists about the presence of SARS-CoV-2 antibodies in asymptomatic individuals.

Methods We examined the seroprevalence of SARS-CoV-2 IgG and IgM antibodies among healthcare workers of a tertiary care kidney center during the the first peak phase of the corona virus disease 2019 (COVID-19) crisis in Austria using an orthogonal test strategy and a total of 12 commercial nucleocapsid protein or spike glycoprotein-based assays as well as Western blotting and a neutralization assay.

Results At baseline 60 of 235 study participants (25.5%, 95% confidence interval, CI 20.4–31.5%) were

judged to be borderline positive or positive for IgM or IgG using a high sensitivity/low specificity threshold in one test system. Follow-up analysis after about 2 weeks revealed IgG positivity in 12 (5.1%, 95% CI: 2.9–8.8%) and IgM positivity in 6 (2.6%, 95% CI: 1.1–5.6) in at least one assay. Of the healthcare workers 2.1% (95% CI: 0.8–5.0%) showed IgG nucleocapsid antibodies in at least 2 assays. By contrast, positive controls with proven COVID-19 showed antibody positivity among almost all test systems. Moreover, serum samples obtained from healthcare workers did not show SARS-CoV-2 neutralizing capacity, in contrast to positive controls.

Conclusion Using a broad spectrum of antibody tests the present study revealed inconsistent results for SARS-CoV-2 seroprevalence among asymptomatic individuals, while this was not the case among COVID-19 patients.

The authors T. Reiter and S. Pajenda contributed equally to this work and share first authorship.

Data sharing statement All unidentified data are available and will be shared through request to the corresponding author.

Supplementary Information The online version of this article (<https://doi.org/10.1007/s00508-021-01848-5>) contains supplementary material, which is available to authorized users.

T. Reiter, MD · S. Pajenda, MD · L. Wagner, MD · M. Gaggl, MD, MPH · D. Gerges, MD, MSc · K. Mayer, MD · C. Aigner, MD · G. Sunder-Plassmann, MD (✉) · A. Schmidt, MD

Division of Nephrology and Dialysis, Department of Medicine III, Medical University of Vienna, Währinger Gürtel 18–20, 1090 Vienna, Austria
gere.sunder-plassmann@meduniwien.ac.at

J. Atamaniuk, MD · S. Jansen-Skoupy, MD · M. Födinger, MD
 Institute of Laboratory Diagnostics, Clinic Favoriten, Vienna Health Care Group, Vienna, Austria

B. Holzer, PhD · I. Zimpernik, DVM, PhD
 Austrian Agency for Health and Food Safety Ltd., Vienna, Austria

R. Straßl, MD
 Department of Laboratory Medicine, Division of Clinical Virology, Medical University of Vienna, Vienna, Austria

M. Födinger, MD
 Medical School, Sigmund Freud Private University, Vienna, Austria

Trial registration number CONEC, ClinicalTrials.gov number NCT04347694

Keywords Coronavirus · Pandemic · Serology test · Antibody

Introduction

Healthcare workers are at increased risk for severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) infection resulting in severe coronavirus disease 2019 (COVID-19) [1–3]. Properly used protection equipment can reduce transmission risk but direct patient contact, endotracheal intubation and contact with contagious body fluids are associated with an increased infection risk [4]. In turn, infected healthcare workers pose a significant threat to patients they care for [5]. People with a compromised immune system or on treatment with immunosuppressive drugs, such as patients with chronic kidney disease (CKD) including those on dialysis treatment or with a kidney transplant, are among the most vulnerable with respect to life-threatening infectious diseases [6–8]. Regardless of the “stay at home-stay safe” practice during the COVID-19 pandemic, they are in need of nondeferrable admission to kidney centers.

Reports on SARS-CoV-2 infections among patients with CKD showed a mortality of up to 28% in kidney transplant recipients or solid organ transplants [9–11]. Early studies from China revealed a surprisingly low mortality in dialysis patients, which contrasts with reports from the Austrian Dialysis and Transplant registry [12, 13]. On 8 May 2020 the COVID-19-specific mortality was 27% (12/44), which is comparable to the reported rate of 31% (18/59) in a recent report from the Columbia University Irving Medical Center, New York [14, 15]. It is well established that containment strategies in Austria were successful in preventing a collapse of the acute care facilities. More importantly, COVID-19-specific mortality was low as compared to other European countries [16, 17].

While reverse transcriptase polymerase chain reaction (RT-PCR) amplification of SARS-CoV-2 RNA has its utility to identify acutely infected patients, serology testing is important to identify patients that have been infected in the past. Thus, detection of SARS-CoV-2 specific antibodies is a prevalence marker in a population and can be used to measure herd immunity [18–20]. In patients suffering from COVID-19 with RT-PCR proven SARS-CoV-2 infection up to 100% tested positive for antiviral immunoglobulin G (IgG) within 3 weeks after symptom onset. Seroconversion for IgG and immunoglobulin M (IgM) occurred simultaneously or sequentially [21–25]; however, the antibody response to SARS-CoV-2 and seroprevalence among asymptomatic healthcare workers are far from clear. In this respect the SARS-CoV-2 immune status of personnel of kidney centers is of eminent importance due to the susceptibility of renal patients to COVID-19.

We aimed to examine the prevalence of SARS-CoV-2 antibodies among nephrology healthcare workers in a tertiary care, university-based hospital in Austria. We adhered to an orthogonal test strategy and used a comprehensive set of commercial laboratory tests and Western blotting, including COVID-19 controls and analysis of neutralizing antibodies [26].

Methods

Study design and participants

The COVID-19 serology in nephrology healthcare workers (CONEC, ClinicalTrials.gov no. NCT04347694) study is a longitudinal study examining the antibody response to SARS-CoV-2 among staff members of the Division of Nephrology and Dialysis, Department of Medicine III, at the Medical University of Vienna, Austria. We enrolled nurses, doctors, researchers administrators, cleaners and other staff. The study protocol includes sample collection at baseline and every 3 months thereafter for a minimum of 1 year. Serum samples were stored at -80°C before testing. At each study visit, participants filled out a questionnaire including demographic data, job title, medical history, medication, travel history since the beginning of the COVID-19 crisis in Austria, COVID-19-specific history including contact to proven COVID-19 cases, COVID-19 specific symptoms, and results of non-study-related SARS-CoV-2 laboratory tests. At baseline participants were screened for the presence of serum anti-SARS-CoV-2 IgG and IgM antibodies by means of the ImmunoDiagnostics test system (ImmunoDiagnostics, HongKong) [27]. Subjects with a borderline positive or positive initial antibody test were invited to follow-up serum antibody tests, and a SARS-CoV-2 RT-PCR, within 2–4 weeks. Employing this extended orthogonal test strategy, the ImmunoDiagnostics test was repeated and samples were also tested by a set of other commercial laboratory tests, covering nucleocapsid protein and spike glycoprotein specific tests for anti-SARS-CoV-2 IgG, IgM, and IgA, as well as by nucleocapsid protein and spike glycoprotein Western blots [26]. We used two additional anti-SARS-CoV-2 IgG enzyme-linked immunosorbent assays (ELISA) tests and a microscopy-based neutralization assay with authentic SARS-CoV-2 to confirm the presence of SARS-CoV-2 specific neutralizing or non-neutralizing antibodies in all follow-up samples that previously tested positive for any anti-SARS-CoV-2 IgG antibodies. Serum samples of five COVID-19 patients served as positive controls for all follow-up laboratory tests. We report here the baseline data of the CONEC study, which was approved by the institutional review board (IRB) at the Medical University of Vienna (unique IRB identifier: 1357/2020). All methods were performed in accordance with the relevant guidelines and regulations. All participants provided written informed consent.

Laboratory analysis

We used a set of 10 commercial serologic tests for detection of anti SARS-CoV-2 IgG (2 for antigenic target nucleocapsid protein, 2 for antigenic target spike glycoprotein), of anti-SARS-CoV-2 IgM (2 antigenic target nucleocapsid protein, 1 antigenic target spike glycoprotein), of anti-SARS-CoV-2 IgA (1 antigenic target spike glycoprotein), and of anti-SARS-CoV-2 total antibody (1 antigenic target nucleocapsid protein, 1 antigenic target spike glycoprotein) including ELISA, chemiluminescence immunoassay (CLIA), and electrochemiluminescence immunoassay (ECLIA), according to the instructions of the manufacturers, for all follow-up analyses (Supplementary material). Technical details of these tests are indicated in Supplementary Table S1. In subjects with IgG antibodies at follow-up and for COVID-19 control samples we also used two additional IgG ELISAs. Western blotting, analysis of neutralizing antibodies, and SARS-CoV-2 RT-PCR are described in the Supplementary material.

Statistical analysis

Demographic information at baseline and follow-up are given in means (standard deviation) and count (%), respectively. For the main outcome (occult immunization yes/no), results are tabulated for each time point. All prevalence estimates are presented with 95% confidence intervals approximated by the Wald method. Formal statistical testing for categorical data is done by Fisher's exact test. Continuous data are analyzed by unpaired t-tests or by non-parametric tests in cases of non-normally distributed data. *P*-values were not adjusted for multiple testing and should be interpreted exploratorily only. Data management and statistical analysis have been performed by means of MS Excel (Microsoft, Redmond, WA, USA) and R (R Core Team 2016, R Foundation for Statistical Computing, Vienna, Austria).

Results

Participants

Beginning 4 weeks after the first documented COVID-19 cases in the Austrian states of Tyrol and Vienna in late February 2020, and 2 weeks after the shutdown in our country, we approached 288 healthcare workers at the Division of Nephrology and Dialysis at the Medical University of Vienna for participation in this study. The different sections of care of our department as well as the number and occupations of study participants are indicated in Supplementary Figs. S1 and S2. A total of 235 staff members (82%) agreed to enter the study and had their first study visit during a period of about 4 weeks. The rate of participation was 94.4% among physicians, 74.4% among nurses,

90.9% among researchers, 84.6% among administrative staff and 89.2% among other staff.

Demographic and general clinical data of all participants and COVID-19-related history of these individuals is given in Table 1. Of the participants 11 (4.7%) reported contact with proven COVID-19 cases, and 36 (15.3%) with category 1 or 2 persons. A travel history outside or inside Austria was evident in 93 (39.6%)

Table 1 Demographic characteristics and COVID-19-related history

Characteristic	N= 235
Age, years (mean ± SD)	44.2 ± 11.4
Sex, female, no. (%)	165 (70.2)
BMI, kg/m ² (mean ± SD)	25.4 ± 4.7
Profession, no. (%)	
Physician	51 (21.7)
Nurse	119 (50.6)
Researcher	10 (4.2)
Administrative staff	22 (9.4)
Other staff	33 (14.0)
Comorbidities, no. (%)	
Hypertension	36 (15.3)
Diabetes	6 (2.6)
Coronary artery disease	4 (1.7)
Chronic kidney disease	2 (0.85)
Lung disease	16 (6.8)
Autoimmune disease	18 (7.7)
Cancer	5 (2.1)
Clinical symptoms, no. (%)	
Fever	10 (4.2)
Cough	34 (14.5)
Dyspnea	4 (1.7)
Gastrointestinal complaints	18 (7.7)
Loss of smell and taste	7 (3.0)
Other complaints	27 (11.5)
Smoking history, no. (%)	
Never smoked	128 (54.5)
Former smoker	53 (22.6)
Current smoker	53 (22.6)
ACEI or ARB use, no. (%)	21 (8.9)
History of influenza vaccination, no. (%)	106 (45.1)
Travel to other countries since February 2020, no. (%)	43 (18.3)
Travel within Austria since February 2020, no. (%)	50 (21.3)
SARS-CoV-2 RT-PCR before study entry, no. (%)	19 (8.1)
Positive	0
Subjects in same household, median (IQR)	1 (1–2)
SARS-CoV-2 RT-PCR positive in same household, no. (%)	0
Contact to COVID-19 patients before study entry, no. (%)	11 (4.7)
Contact to category 1 or 2 individuals before study entry, no. (%)	36 (15.3)
COVID-19 coronavirus disease 2019, BMI body mass index, SD standard deviation, ACEI angiotensin converting enzyme inhibitor, ARB angiotensin receptor blocker, SARS-CoV-2 severe acute respiratory syndrome coronavirus 2, RT-PCR reverse transcriptase-polymerase chain reaction, IQR interquartile range	

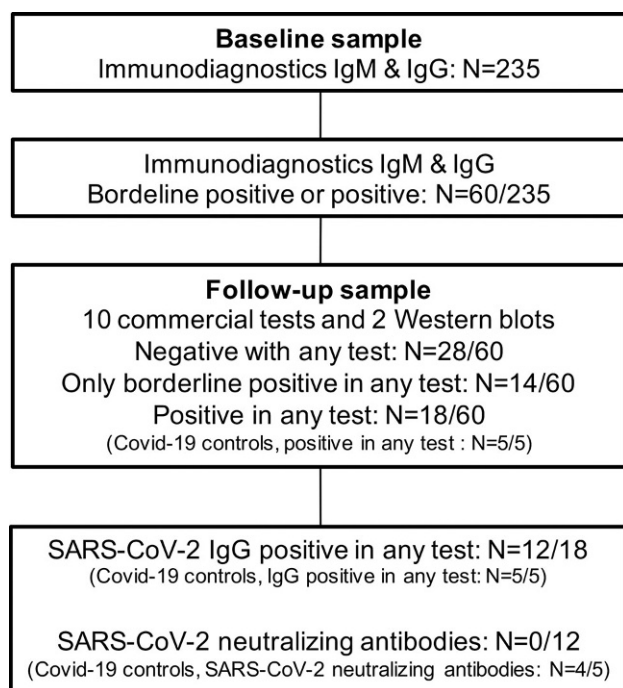


Fig. 1 Overview of main results

cases, 2 (0.9%) were in quarantine and none reported a history of COVID-19 or home isolation at baseline; however, 62 (26.4%) persons reported symptoms possibly related to undetected mild COVID-19 (Table 1) and 19 (8.1%) of these subjects reported a previous SARS-CoV-2 RT-PCR at baseline, which was negative in all cases. During the study period, a total of 313 nasopharyngeal swabs were negative in 179 (76.2%) participants (56 participants had no SARS-CoV-2 RT-PCR).

SARS-CoV-2 antibodies—baseline

Among 235 participants, we judged 60 (25.5%, 95% CI: 20.4–31.5%) at baseline to be either borderline positive or positive for anti-SARS-CoV-2 IgG and/or IgM by the ImmunoDiagnosics ELISA using a conservative threshold of an OD of 0.200 and 0.300 in at least one of two duplicates, respectively (Fig. 1). Thus, 18 (7.7%, 95% CI: 4.8–11.9%) individuals were assumed to be IgM positive, 3 others (1.3%, 95% CI: 0.03–3.9%) IgG positive, and the remaining 39 (16.6%, 95% CI: 12.4–21.9%) borderline positive for IgM and/or IgG.

SARS-CoV-2 antibodies—follow-up

All 60 follow-up participants had a serum test and the majority a SARS-CoV-2 RT-PCR within 18.5 ± 7.0 days after baseline. Their clinical characteristics are indicated in Table 2. All but five, who had no RT-PCR, tested negative for SARS-CoV-2 RNA in nasopharyngeal swabs at this point in time. Details of test results of 28 (46.7%, 95% CI: 34.6–59.1%) follow-up negative and 14 (23.3%, 95% CI: 14.3–35.6%) borderline posi-

tive participants (Fig. 1) are given in the Supplementary material.

Of the follow-up participants 18 (7.7%, 95% CI: 4.8–11.9%) of the total cohort showed a positive SARS-CoV-2 antibody result in one or more laboratory tests at follow-up (Figs. 1 and 2). Details of these 18 participants are indicated in Table 2. All clinical characteristics but smoking (odds ratio, OR: 3.82, 95% CI: 1.06–13.77, $p < 0.05$, for presence of antibodies for smokers) did not differ between follow-up positive as compared to follow-up negative or borderline positive participants at baseline or follow-up. Individual test results of these 18 subjects are shown in Supplementary Tables S2 and S3 and explained in the Supplementary material.

Of the participants 6 (2.6%, 95% CI: 1.1–5.6%) showed IgM and 2 (0.9%, 95% CI: 0.03–3.3%) showed IgA antibodies at follow-up. With respect to anti-SARS-CoV-2 IgG, 10 of 18 individuals had anti-nucleocapsid IgG and 2 had anti-spike glycoprotein IgG in at least 1 test system, resulting in an overall prevalence of anti-SARS-CoV-2 IgG of 5.1% (12/235; 95% CI: 2.9–8.8%). None of these 12 study participants had neutralizing SARS-CoV-2 antibodies, but 2 of them also tested positive for IgG by the Vircell (Vircell, Granada, Spain) and the IDvet (IDvet, Grabels, France) ELISAs (Fig. 2). At least two positive IgG antibody results were seen in 5 of 18 subjects (2.1% of the entire study cohort, 95% CI: 0.7–5.0%; all anti-nucleocapsid protein IgG; ID 65, 67, 133, 200, and 222 in Fig. 2, and Supplementary Tables S2 and S3). Only one of these individuals reported symptoms possibly related to mild COVID-19 (ID 133). The proportion of SARS-CoV-2 antibody positive subjects among the different professions is shown in Supplementary Fig. S5.

All 5 COVID-19 serum samples (case vignettes can be found in the Supplementary material) tested positive for SARS-CoV-2 antibodies in the majority of tests (mean time between first positive SARS-CoV-2 RT-PCR and serum sampling: 31.8 ± 12.2 days). Out of the five patients four had neutralizing SARS-CoV-2 antibodies and one had the mildest form of COVID-19 among positive controls presenting with joint pain (Fig. 2; Supplementary material; Supplementary Tables S2 and S3).

Discussion

Our study addresses several important issues related to COVID-19: First, the seroprevalence of anti-SARS-CoV-2 IgG antibodies among nephrology healthcare workers at the Medical University of Vienna during the infection peak in Austria was, at best, 2.1% (95% CI: 0.7–5.0%). Second, it is valid to assume that the successful containment measures taken in Austria and especially in Vienna have minimized exposure of healthcare workers at our institution to COVID-19 in the community and at the point of care as compared to

Table 2 Baseline and follow-up clinical characteristics and COVID-19-related history of 60 individuals with a borderline positive or positive SARS-CoV-2-IgG and/or -IgM ImmunoDiagnostics test at study entry, and of subgroups comprising 42 who tested negative or borderline positive at follow-up in any test system (subgroup 1), and of 18 with a positive test result in any test system at follow-up (subgroup 2)

Characteristic	All follow-up N= 60 ^a		Subgroup 1 N= 42 ^b		Subgroup 2 N= 18 ^c		<i>p</i> [*] Between BL	<i>p</i> [*] Between FU
	BL	FU ^d	BL	FU ^d	BL	FU ^d		
Age, years (±SD)	41.7 (12.6)	N/A	40.6 (12.9)	N/A	44.4 (11.7)	N/A	0.29	N/A
Female sex, no. (%)	49 (81.7)	N/A	37 (88.1)	N/A	12 (66.7)	N/A	0.07	N/A
History of influenza vaccination, no. (%)	23 (38.3)	N/A	17 (40.5)	N/A	6 (33.3)	N/A	0.77	N/A
History of autoimmune disease, no. (%)	6 (10.0)	N/A	4 (9.5)	N/A	2 (11.1)	N/A	1	N/A
Smoker, no (%)	13 (21.7)	N/A	6 (14.3)	N/A	7 (38.9)	N/A	0.046	N/A
Days between BL and FU (±SD)	N/A	18.5 (7.1)	N/A	18.8 (7.5)	N/A	17.6 (5.7)	N/A	0.48
<i>Clinical symptoms, no. (%)</i>								
Fever	1 (1.7)	0	0	0	1 (5.6)	0	1	1
Cough	12 (20.0)	5 (8.3)	7 (16.7)	3 (7.1)	5 (27.8)	2 (11.1)	0.48	0.63
Dyspnea	2 (3.3)	1 (1.7)	2 (4.8)	1 (2.4)	0	0	1	1
Gastrointestinal complaints	4 (6.7)	2 (3.3)	2 (4.8)	2 (4.8)	2 (11.1)	0	0.58	1
Loss of smell and taste	4 (6.7)	1 (1.7)	3 (7.1)	1 (2.4)	1 (5.6)	0	1	1
Travel to other countries, no. (%)	10 (16.7)	0	8 (19.0)	0	2 (11.1)	0	0.70	N/A
Travel within Austria, no. (%)	14 (23.3)	8 (13.3)	8 (19.0)	4 (9.5)	6 (33.3)	4 (22.2)	0.32	0.23
Subjects in same household, median (IQR)	1 (1–2)	1 (1–2)	2 (1–2)	1.5 (1–2)	1 (1–2)	1 (1–2)	0.29	0.40
SARS-CoV-2 RT-PCR positive, no. (%)	0	0	0	0	0	0	N/A	N/A
Contact to COVID-19 patients, no. (%)	4 (6.7)	0	2 (4.8)	0	2 (11.1)	0	0.58	N/A
Contact to category 1 or 2 individuals, no. (%)	7 (11.7)	0	5 (11.9)	0	2 (11.1)	0	1	N/A

COVID-19 coronavirus disease 2019, SARS-CoV-2 severe acute respiratory syndrome-coronavirus-2, BL baseline, FU follow-up, SD standard deviation, RT-PCR reverse transcriptase-polymerase chain reaction, IQR interquartile range, N/A not applicable

**p*-values have been determined by means of Student's T-test, Wilcoxon rank-sum test, and Fisher's exact test, as appropriate

^aPhysicians 12 (20%), nurses 28 (46.7%), research staff 2 (3.3%), administration 5 (8.3%), other staff 13 (21.7%)

^bPhysicians 8 (19.0%), nurses 19 (45.2%), research staff 2 (4.8%), administration 3 (7.1%), other staff 10 (23.8%)

^cPhysicians 4 (22.2%), nurses 9 (50%), research staff none, administration 2 (11.1%), other staff 3 (16.7%)

^dNumbers refer to time period between baseline and follow-up

other countries. Third, commercially available laboratory tests, including ELISA, CLIA, and ECLIA, may fail to uniformly detect potential low-level immune response to SARS-CoV-2 in asymptomatic subjects or mild disease, or differentially cross-react as false positives.

The analytical specificity of a laboratory test is reflected by the positive predictive value (PPV), which depends not only on the sensitivity and specificity of the test but also on the disease prevalence. Requiring a PPV of at least 90%, the analytical specificity of a test should ideally exceed 99.9%, which is influenced by the presence of autoimmune diseases, heterophilic antibodies, or antibodies to other coronaviruses [28, 29]. At follow-up, we found no effect of influenza vaccination or history of autoimmune disease on COVID-19 serostatus; however, there were more smokers among antibody positives. This finding may be related to the preference of nicotine for the ACE2-SARS-CoV-2 complex that reduces SARS-CoV-2 virulence by interfering with the spike protein [30].

Overall, serologic tests based on spike glycoprotein appear to distinguish between emerging and endemic coronaviruses, whereas assays based on the nucleocapsid protein can serve as a marker of recent infec-

tion but might be expected to cross-react more with endemic coronaviruses [31]. Test reactivity thresholds used to define a positive result can be adjusted to optimize the trade-off between sensitivity and specificity. With higher thresholds, sensitivity decreases as cases with low serum antibody levels are categorized as negative, but specificity improves as low amounts of non-specific antibody are no longer considered positive [31]. In our study, we used a low threshold for IgM and IgG ELISAs at baseline to account for higher sensitivity, accepting low specificity as demonstrated by follow-up examinations.

A meta-analysis of 38 studies covering 7848 individuals confirmed that tests using the spike glycoprotein are more sensitive than nucleocapsid-based tests. The IgG tests performed better compared to IgM tests with higher sensitivity at later time points after the onset of symptoms. Combined IgG and IgM tests performed better in terms of sensitivity than measuring either antibody alone. All methods yielded high specificity with some tests reaching levels around 99% [32]; however, statistically the PPV varies widely and can be as low as 30–50% in low prevalence settings [33].

Rigorous containment strategies may also reduce the prevalence of anti-SARS-CoV-2 in different pop-

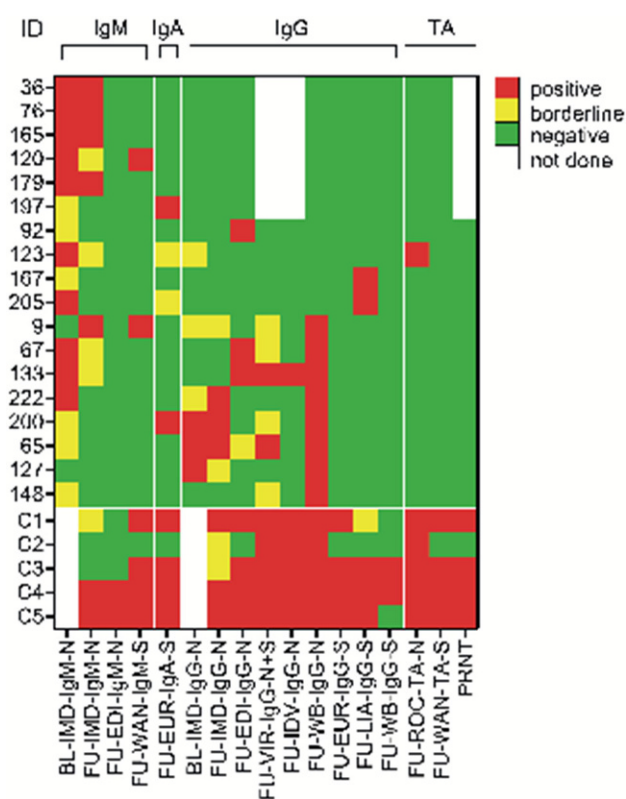


Fig. 2 Baseline and follow-up SARS-CoV-2 antibody test results of 18 study participants positive in at least one test system at follow-up and of 5 COVID-19 patients. The laboratory results of five COVID-19 patients, indicated by C1–C5, are shown in the bottom lines corresponding to the follow-up test results of study participants. TA total antibody, BL baseline, IMD ImmunoDiagnostics, HongKong, N SARS-CoV-2 nucleocapsid protein, FU follow-up, EDI Epitope Diagnostics Inc., San Diego, CA, USA, WAN Beijing Wantai Biological Pharmacy Enterprise Co., Ltd., Beijing, China, S SARS-CoV-2 spike glycoprotein, EUR Euroimmun Medizinische Labordiagnostika AG, Lübeck, Germany, VIR Vircell, Granada, Spain, IDV IDvet, Grabels, France, WB Western blot, LIA Liaison, DiaSorin S.p.A, Saluggia, Italy, ROC Roche Diagnostics Deutschland GmbH, Mannheim, Germany, PRNT plaque reduction neutralization test

ulations at the cost of an early development of herd immunity. Travel restrictions and other control measures reduced COVID-19 transmission early last year in China [34]. Later, it was estimated that among 11 European countries, the national lockdown had the greatest effect on the reproduction number R_t among nonpharmacologic interventions including school closure, avoidance of public events, social distancing, and self-isolation. As such, Austria (Supplementary Fig. S6) and Norway had the lowest infection rate in this analysis [17]. At the beginning of this study the R_t in Vienna was 2.0 (95% CI: 1.87–2.14) and further decreased thereafter. The implementation of public interventions affects the case number after about 2 weeks, and employment of econometric techniques showed that policy changes in 6 countries across the globe averted 530 million infections [35, 36].

The low seroprevalence of COVID-19 antibodies in nephrology healthcare workers in our institution reflects successful measures taken to prevent transmission/infection by the city of Vienna and the Medical University of Vienna. Taken together, this minimized exposure risk to COVID-19 for our staff at work (Supplementary Figs. S7 and S8).

The prevalence of asymptomatic cases among SARS-CoV2 infected patients is assumed to be 40–45% [37]. In our cohort one of five participants considered to be IgG positive showed symptoms potentially related to SARS-CoV-2 exposure. In contrast, significant exposure to COVID-19 cases resulted in a seroprevalence of 17.4% and 44% among healthcare workers in the USA and in China, respectively [38, 39]. Other studies in high-risk settings, however, showed a low seroprevalence among hospital staff [40–44]. These surveys utilized only one test system and none of these studies employed an orthogonal strategy, confirming borderline positive or positive samples in an independent follow-up serum sample using other laboratory tests.

The extended orthogonal test strategy of the present study, including 12 different commercial tests, Western blots, and a neutralization test, has potentially allowed for an increase in sensitivity/specificity for confirmation of seropositivity among some individuals. Assuming true seropositivity in 5 of 18 healthcare workers, with positive IgG titers in at least 2 of the commercial or in-house test systems at follow-up (uniform nucleocapsid protein IgG in all five cases, also pointing to potential cross-reaction), suggests that single antibody tests do not enable correct detection of true seroconversion and have no acceptable sensitivity and/or specificity in largely asymptomatic and SARS-CoV-2 RT-PCR negative individuals. This is nicely shown by the rag-rug pattern of seroconversion in Fig. 2. Of note, nonexposed healthy subjects harbor pre-existing SARS-CoV-2 cross-reactive T cells, specific for a huge array of SARS-CoV-2 antigens, suggesting some potential for pre-existing immunity in the population [45]. This finding matches, at least in part, with the presence of SARS-CoV-2 antibodies in nonexposed individuals.

In contrast, all 5 COVID-19 serum samples in our study showed SARS-CoV-2 IgM and IgG antibodies in more than 2 test systems (Fig. 2). Of the five samples four had neutralizing antibodies, whereas none of the serum samples of the IgG positive study participants showed SARS-CoV-2 neutralizing capacity (Fig. 2; Supplementary Table S3). The differential development of antibody response to nucleocapsid protein and spike glycoprotein antigens among patients with proven COVID-19 and the study participants is in support of more cross-reaction than anti-SARS-CoV-2 seroconversion in healthcare workers enrolled in this study.

A potential limitation to this study lies in the lack of unambiguous COVID-19 cases among study partic-

ipants, which was not expected to be the case at the beginning of this study in March 2020. We also did not do a formal laboratory test performance analysis. This is largely counterbalanced by the strength of this study, namely the orthogonal test strategy with confirmation in separate serum samples using a very broad range of SARS-CoV-2 antibody tests.

In summary, our study demonstrates that single antibody tests are not reliable to assess the SARS-CoV-2 immune response in mostly asymptomatic individuals. This finding has important implications for testing cohorts with a low COVID-19 prevalence to determine whether herd immunity has been reached. Containment strategies by the City of Vienna and the Medical University of Vienna proved to be extremely effective given the very low seroprevalence in a cohort of high-risk healthcare workers during the peak of the pandemic crisis in Austria; however, caution has still to be taken, since healthcare workers are prone to COVID-19 infections and transmission to patients.

Acknowledgements We are indebted to Miriam Kunst, Karin Voith, and Gabriele Holzmann for support in sample collection and data management.

Funding No financial funding was granted for this work.

Author Contribution TR, SP, AS and GSP designed the work, recruited participants, collected data and wrote the draft of the manuscript. DG, KM and CA recruited participants, collected samples and contributed to the draft. RS, SP and LW analyzed the samples. MG did the statistical analysis of the data. BH, IZ, JA and SJS analyzed the samples and contributed with critical input on the interpretation of the results. MF contributed to the draft of the manuscript and its revision. All authors approved the manuscript for submission.

Funding Open access funding provided by Medical University of Vienna.

Conflict of interest T. Reiter, S. Pajenda, L. Wagner, M. Gaggl, J. Atamaniuk, B. Holzer, I. Zimprnik, D. Gerges, K. Mayer, C. Aigner, R. Straßl, S. Jansen-Skoupy, M. Födinger, G. Sunder-Plassmann, and A. Schmidt declare that they have no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Koh D. Occupational risks for Covid-19 infection. *Occup Med.* 2020;70(1):3–5.
- Berlin DA, Gulick RM, Martinez FJ. Severe Covid-19. *N Engl J Med.* 2020;383(25):2451–60.
- Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA.* 2020;323(11):1061–9.
- Chou R, Dana T, Buckley DI, Selph S, Fu R, Totten AM. Epidemiology of and risk factors for Coronavirus infection in health care workers: a living rapid review. *Ann Intern Med.* 2020;173(2):120–36.
- Cho JH, Kang SH, Park HC, Kim DK, Lee SH, Do JY, et al. Hemodialysis with cohort isolation to prevent secondary transmission during a COVID-19 outbreak in Korea. *J Am Soc Nephrol.* 2020;31(7):1398–408.
- Fishman JA. Infection in solid-organ transplant recipients. *N Engl J Med.* 2007;357(25):2601–14.
- Atlas of end-stage renal disease in the United States. *Am J Kidney Dis.* 2010;55(1):S182–S8.
- Saran R, Robinson B, Abbott KC, Agodoa LY, Albertus P, Ayanian J, et al. US renal data system 2016 annual data report: Epidemiology of kidney disease in the United States. *Am J Kidney Dis.* 2017;69(3 Suppl 1):A7–A8.
- Akalin E, Azzi Y, Bartash R, Seethamraju H, Parides M, Hemmige V, et al. Covid-19 and Kidney Transplantation. *N Engl J Med.* 2020;382(25):2475–7.
- Fernández-Ruiz M, Andrés A, Loínaz C, Delgado JF, López-Medrano F, San Juan R, et al. COVID-19 in solid organ transplant recipients: a single-center case series from Spain. *Am J Transplant.* 2020;20(7):1849–58.
- Pereira MR, Mohan S, Cohen DJ, Husain SA, Dube GK, Ratner LE, et al. COVID-19 in solid organ transplant recipients: Initial report from the US epicenter. *Am J Transplant.* 2020;20(7):1800–8.
- Xiong F, Tang H, Liu L, Tu C, Tian JB, Lei CT, et al. Clinical characteristics of and medical interventions for COVID-19 in hemodialysis patients in Wuhan, China. *J Am Soc Nephrol.* 2020;31(7):1387–97.
- Wang R, Liao C, He H, Hu C, Wei Z, Hong Z, et al. COVID-19 in hemodialysis patients: a report of 5 cases. *Am J Kidney Dis.* 2020;76(1):141–3.
- Österreichisches Dialyse- und Transplantregister. Kummulative Anzahl der SARS-CoV-2 Infektionen [pdf-File]. Österreichisches Dialyse- und Transplantregister. 2020. <https://www.nephrologie.at/wp-content/uploads/COVID-19-OEDTR-080520.pdf> (Created 8 May 2020). Accessed 19 May 2020.
- Valeri AM, Robbins-Juarez SY, Stevens JS, Ahn W, Rao MK, Radhakrishnan J, et al. Presentation and Outcomes of Patients with ESKD and COVID-19. *J Am Soc Nephrol.* 2020;31(7):1409–15.
- CNN. On GPS: Austria's coronavirus response. 2020. <https://edition.cnn.com/videos/tv/2020/04/19/exp-gps-0419-kurz-on-reopening-austrias-economy.cnn2020>. Accessed 19 Apr 2020.
- Flaxman S, Mishra S, Gandy A, Unwin HJT, Mellan TA, Coupland H, et al. Estimating the effects of non-pharmaceutical interventions on COVID-19 in Europe. *Nature.* 2020;584(7820):257–61.
- Sethuraman N, Jeremiah SS, Ryo A. Interpreting diagnostic tests for SARS-CoV-2. *JAMA.* 2020;323(22):2249–51.
- Krammer F, Simon V. Serology assays to manage Covid-19. *Science.* 2020;368(6495):1060–1.
- Lee CY, Lin RTP, Renia L, Ng LFP. Serological approaches for Covid-19: epidemiologic perspective on surveillance and control. *Front Immunol.* 2020;11:879.

21. Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med*. 2020;26(6):845–8.
22. Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Muller MA, et al. Virological assessment of hospitalized patients with Covid-19. *Nature*. 2020;581(7809):465–9.
23. Okba NMA, Müller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM, et al. Severe acute respiratory syndrome Coronavirus 2-specific antibody responses in Coronavirus disease patients. *Emerg Infect Dis*. 2020;26(7):1478–88.
24. Sun B, Feng Y, Mo X, Zheng P, Wang Q, Li P, et al. Kinetics of SARS-CoV-2 specific IgM and IgG responses in Covid-19 patients. *Emerg Microbes Infect*. 2020;9(1):940–8.
25. To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis*. 2020;20(5):565–74.
26. CDC. Interim guidelines for Covid-19 antibody testing 2020.. <https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html> (Created 23 Mar 2020). Accessed 17 May 2020.
27. Guo L, Ren L, Yang S, Xiao M, Chang D, Yang F, et al. Profiling early humoral response to diagnose novel Coronavirus disease (COVID-19). *Clin Infect Dis*. 2020;71(15):778–85.
28. Diamandis P, Prassas I, Diamandis EP. Antibody tests for Covid-19: drawing attention to the importance of analytical specificity. *Clin Chem Lab Med*. 2020;58(7):1144–5.
29. Jiang S, Hillyer C, Du L. Neutralizing antibodies against SARS-CoV-2 and other human coronaviruses. *Trends Immunol*. 2020;41(5):355–9.
30. Kumar S, Kumar SA, Wei H. A computational insight of the improved nicotine binding with ACE2-SARS-CoV-2 complex with its clinical impact. *arXiv:2004.14943*. 2020. pp. 1–11.
31. Cheng MP, Yansouni CP, Basta NE, Desjardins M, Kanjilal S, Paquette K, et al. Serodiagnostics for severe acute respiratory syndrome-related Coronavirus 2 : a narrative review. *Ann Intern Med*. 2020;173(6):450–60.
32. Kontou PI, Braliou GG, Dimou NL, Nikolopoulos G, Bagos PG. Antibody tests in detecting SARS-CoV-2 infection: a meta-analysis. *Diagnostics*. 2020;10(5):319.
33. Mathur G, Mathur S. Antibody testing for Covid-19. *Am J Clin Pathol*. 2020;154(1):1–3.
34. Prem K, Liu Y, Russell TW, Kucharski AJ, Eggo RM, Davies N, et al. The effect of control strategies to reduce social mixing on outcomes of the Covid-19 epidemic in Wuhan, China: a modelling study. *Lancet Public Health*. 2020;5(5):e261–e70.
35. Hsiang S, Allen D, Annan-Phan S, Bell K, Bolliger I, Chong T, et al. The effect of large-scale anti-contagion policies on the COVID-19 pandemic. *Nature*. 2020;584(7820):262–7.
36. Dehning J, Zierenberg J, Spitzner FP, Wibral M, Neto JP, Wilczek M, et al. Inferring change points in the spread of COVID-19 reveals the effectiveness of interventions. *Science*. 2020;369(6500):eabb9789.
37. Oran DP, Topol EJ. Prevalence of asymptomatic SARS-CoV-2 infection : a narrative review. *Ann Intern Med*. 2020;173(5):362–7.
38. Chen Y, Tong X, Wang J, Huang W, Yin S, Huang R, et al. High SARS-CoV-2 antibody prevalence among health-care workers exposed to COVID-19 patients. *J Infect*. 2020;81(3):420–6.
39. Hains DS, Schwaderer AL, Carroll AE, Starr MC, Wilson AC, Amanat F, et al. Asymptomatic seroconversion of immunoglobulins to SARS-coV-2 in a pediatric dialysis unit. *JAMA*. 2020;323(23):2424–5.
40. Brandstetter S, Roth S, Harner S, Buntrock-Döpke H, Toncheva AA, Borchers N, et al. Symptoms and immunoglobulin development in hospital staff exposed to a SARS-CoV-2 outbreak. *Pediatr Allergy Immunol*. 2020;31(7):841–7.
41. Korth J, Wilde B, Dolff S, Anastasiou OE, Krawczyk A, Jahn M, et al. SARS-CoV-2-specific antibody detection in healthcare workers in Germany with direct contact to Covid-19 patients. *J Clin Virol*. 2020;128:104437.
42. Madsen T, Levin N, Niehus K, Law K, Mayer J, Chapman M, et al. Prevalence of IgG antibodies to SARS-CoV-2 among emergency department employees. *Am J Emerg Med*. 2020;38(12):2752.
43. Xu X, Sun J, Nie S, Li H, Kong Y, Liang M, et al. Seroprevalence of immunoglobulin M and G antibodies against SARS-CoV-2 in China. *Nat Med*. 2020;26(8):1193–5.
44. Steensels D, Oris E, Coninx L, Nuyens D, Delforge ML, Vermeersch P, et al. Hospital-wide SARS-coV-2 antibody screening in 3056 staff in a tertiary center in Belgium. *JAMA*. 2020;324(2):195–7.
45. Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, et al. Targets of T cell responses to SARS-coV-2 Coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell*. 2020;181(7):1489–501.e15.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.