IgG Against Human β-Coronavirus Spike Proteins Correlates with SARS-CoV-2 anti-Spike IgG Responses and COVID-19 Disease Severity

Jiong Wang MD[1], Dongmei Li PhD[2], Andrew Cameron PhD[3], Qian Zhou MD[1], Alexander WiltseBS[1,5], Jennifer Nayak MD PhD[4], Nicole D. Pecora MD PhD[3,6], and Martin S. Zand MD PhD[1,2*]

[1]Department of Medicine, Division of Nephrology, University of Rochester Medical Center, Rochester, NY USA; [2]Clinical and Translational Science Institute, University of Rochester Medical Center, Rochester, NY, USA; [3]Clinical Microbiology, Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester, NY, USA; [4]Department of Pediatrics, Division of Infectious Diseases, University of Rochester Medical Center, Rochester, NY, USA; [5]Current institute: University of Maryland Medical Center, WA, USA [6]Current institute: Brigham and Women's Hospital, Harvard University, Boston, MA, USA

[*]**Corresponding Author:** Martin S. Zand MD PhD

University of Rochester Medical Center Clinical and Translational Science Institute

265 Crittendon Boulevard - Rm. 1.207 Rochester NY, 14642, USA

Email: martin zand@urmc.rochester.edu

Summary: A rapid and robust increase in SARS-COV-2 anti-S2 subunit IgG antibodies after infection is highly associated with disease severity. This is likely due to pre-existing cross-reactive humoral immunity against human *β*-coronaviruses OC43 and HKU1, leading to early, non-neutralizing IgG production

ABSTRACT

Background: A protective SARS-COV-2 (SARS2) antibody response is crucial to decrease morbidity and mortality from severe COVID-19 disease and for vaccine efficacy. The effects of pre-existing anti-human coronavirus (HCoV) antibodies on the SARS2-specific IgG responsesand severity of disease are currently unclear.

Methods: We profiled anti-spike (S), S1, S2, RBD IgG antibodies against SARS2 and six HCoVs using a multiplex assay (mPLEX-CoV) with serum samples from SARS2 infection (155patients) and pre-COVID-19 (188 subjects) cohorts.

Results: Anti-S SARS2 IgG levels were significantly increased and highly correlated with IgG antibodies that recognized OC43 and HKU1 S proteins in COVID-19 patients. However, OC43 and HKU1 anti-S antibodies in sera collected pre-COVID-19 did not cross-react to SARS2 S. Moreover, these ”uni-directional” cross-reactive antibodies elicited by the SARS2 infection were distinct from the ”bi-directional” cross-reactive antibodies that recognized the homologous antigen strains, RaTG13 and SARS-CoV-1 (SARS1). Notably, high OC43 and anti-S2 antibodies were associated with a rapid and robust anti-SARS2 antibody response andincreased disease severity. In addition, a higher ratio of S2/S1-reactive antibodies developedover time in severe ICU patients.

Conclusions: Our study suggested that early and rapid OC43 S- and S2-reactive antibodiesemerging after SARS2 infection may correlate with COVID-19 disease severity.

Keywords: SARS-CoV-2, common cold human coronaviruses (HCoVs), cross-reactive antibody response, immune imprinting

INTRODUCTION

Infection with the human coronavirus (HCoV) SARS-CoV-2 (SARS2) can cause coronavirus disease (COVID-19). Most infected individuals have only mild symptoms, but some quickly develop severe pneumonia associated with high mortality[1, 2]. At the time of this study, COVID-19 has caused over 200 million known infections and over 4.5 million deaths worldwide[3]. Thus, understanding immunity to HCoVs is critical to developing vaccine strategies and therapeutic approaches.

SARS-CoV-2 belongs to the zoonotic coronaviridae family[4]. The HCoVs include SARS- CoV-1 (SARS1) and the Middle Eastern Respiratory Syndrome (MERS) virus, which is both *β*- HCoVs and can cause severe acute respiratory syndrome (SARS). Four common HCoVs in two classes, *β* (OC43, HKU1) and *α* (229E, NL63) [5], circulate in the human population, causing 30% of mild upper respiratory infections [6, 7]. Although HCoV infections occur throughout life, antibody seroconversion rates vary greatly by age and geography[6, 8-10].

The immunodominant antigens for HCoVs include the nucleocapsid (N) and the homotrimeric glycoprotein spike (S) viral protein antigens [4]. HCoVs share S- and N-protein sequence homologies and antibodies against HCoV S- and N-proteins are found in varying frequencies across all age groups [6, 7]. The SARS2 S-protein has a receptor-binding domain (RBD) on the N-terminal S1 subunit, which mediates viral binding via high-affinity interaction with the host cell surface angiotensin-converting enzyme 2 (ACE2). In contrast, the S2 subunit is responsible for virus-cell membrane fusion [11] and displays more sequence homology between HCoV strains than the S1 subunit (Fig 1A) [12, 13].

Several studies suggest an association between anti-SARS2-S IgG levels and COVID-19 disease severity [14] [1, 2] [15], raising concerns that some patterns of humoral immunity may be associated with increased morbidity and mortality during infection [16]. Less clear is the contribution of cross-reactive antibody responses to common HCoVs in

the time course and severity of initial COVID-19 disease. OC43 anti-S IgG levels correlate with an increase in both SARS2-specific IgG levels and disease severity in acutely ill COVID-19 patients [17]. In contrast, others report that immune imprinting to conserved HCoV epitopes is negatively correlated with the induction of IgG and IgM against the SARS2 S protein [18], and that primary SARS2 specific anti-S antibody responses in acutely ill patients target homologous epitopes on the S2 segment [14, 19, 20].

The goal of this study was to better understand the effect of pre-existing HCoV-reactive IgG antibodies on the development of humoral immune responses to, and the pathogenesis of, SARS2 infection. Specifically, we tested the hypothesis that pre-existing anti-OC43 S proteinand anti-SARS2 S2 segment IgG correlated with the severity of illness in hospitalized patients, as measured by Sequential Organ Failure Assessment (SOFA) scores [21]. To this end, we pro-filed anti-S and -N IgG levels against multiple HCoVs in sera from pre-COVID-19 and acute COVID-19 cohorts using a Luminex-based multiplex assay [22]. We describe high seroconversion rates of *β*-HCoVs OC43 and HKU1 anti-S antibodies in the pre-COVID era cohort and evidence to support a potential role for immune imprinting in *de novo* SARS-CoV-2 antibody responses.

METHODS

Human subjects protection

The collection of pre-COVID and COVID-19 infected subjects' serum samples, and secondary use of infant serum samples, were approved by the University of Rochester Research Subjects Review Board (RSRB protocols 00004836 and 00058437).

Pre-COVID-19 era cohort

Serum samples (n=188) from before December 2019 were obtained. In addition to healthy subjects (n-55), these included those that might create false positives for anti-

SARS2 S reactivity. These include Serum samples (n=188) from before December 2019 that were obtained. In addition to healthy subjects (n-55), these included those that might create false positives for anti-SARS2 S reactivity. These included sera from subjects positive for bacterial and viral infections (Lyme disease, syphilis, cytomegalovirus (CMV), Epstein-Barr virus (EBV), respiratory syncytial virus (RSV), influenza, autoimmune disease markers antinuclear antigen (ANA), and rheumatoid factor (RF) (Table 1). The SARS2, SARS1 anti-S, and anti-N cut-off values were set at 3 standard deviations above the negative sample mean, using serum samples from infants younger than12 months old (n=16; Supplementary Table 2).

COVID-19 Cohort

Serum samples were collected from 155 symptomatic subjects positive for SARS2 nucleic acid (Cobas SARS-CoV-2®, Roche Molecular Systems, Inc.) and SARS2 N protein antibody (Abbot Laboratories ™ ARCHITECT™ test i2000SR platform [23]) from April to June 2020. A total of 155 acute COVID-19 patients with 277 longitudinal serum samples (Days from Symptom Onset (DFSO) <=42) were divided into four cohorts by illness severity and mortality: (1) Outpatients that were never hospitalized (n=42); (2) in patients with moderate disease (n=33); (3) an ICU group (n=57); and (4) a severe COVID-19 group that died in-hospital (n=23). We quantified disease severity using the maximum daily Sequential Organ Failure Assessment (SOFA) score for all hospitalized subjects. Subject demographics were abstracted from the electronic medical record (Table 2). Serum samples were stored at 4˚C or -80˚C until analysis.

Anti-HCoV IgG multiplex assay (mPLEX-CoV)

We estimated the levels of IgG antibodies against S and N proteins of SARS2 and other HCoVs using the (mPLEX-CoV) multiplex assay [22].]. In brief, we cloned and expressed S proteins of SARS2, SARS-CoV-1, the homologous RaTG CoV (positive control), MERS, and four seasonal HCoVs (OC43, HKU1, NL63, 229E) using a baculovirus insect expression system (Supplementary Table S1), and commercial

proteins for SARS2 S1, S2, and RBD domains (Sino Biological). All proteins were coupled to Luminex beads (xMAP® Antibody Coupling kit; Luminex, Austin, TX) at 40 mole/million beads. Protein-coupled beads were incubated with 1:1000 diluted duplicate serum samples. Bound IgG was detected with PE-conjugated anti-human IgG (Southern Biotech, Birmingham, Al), and the median fluorescence intensity (MFI) was read on a Luminex MagPix (Figure 1B).

To determine the sensitivity and specificity of the mPlex-CoV assay using ROC curves for the SARS-CoV-2 anti-Spike (S) antibody with SARS2 N protein antibody Abbott™ ARCHITECTTM test as the gold standard. ROC curves were generated using the pre-COVID and PCR positive COVID era cohort samples (Mathematica V12.0.0; Wolfram Research, Inc, CA, USA).

Statistical methods

We estimated correlations between anti-HCoV and anti-SARS-CoV-2 IgG levels using Pear- son correlation coefficients. For subjects with more than 2 longitudinal samples, we used the mean IgG antibody MFI level of each HCoV strain. Immune repertoires of the pre-COVID era and COVID-19 cohorts were visualized using multidimensional scaling (MDS) analyses of anti-S and -N reactive anti-HCoV IgG levels [24]. Density plots of anti-S and N IgG HCoV repertoires of the pre-COVID era and COVID-19 cohorts were compared using the bootstrap method. Gen- generalized estimating equation (GEE) models were used to compare anti-S IgG levels among COVID19 severity groups (outpatients, inpatients, severe/ICU, death) grouped by sample days from symptom onset (0-7, 8-14, 15+). Generalized linear mixed-effects models were used to evaluate the association of maximum SOFA score with anti-S IgG levels. Statistical analyses were conducted using SAS v9.4 (SAS Institute Inc., Cary, NC) and R (R core team, 2017), and hypotheses tests were two-sided with $p<0.05$.

RESULTS

Pre-COVID-19 and COVID-19 cohorts

Pre-COVID-19 era cohort sera were collected from 188 subjects (mean age 49 years). To assess for false-positive SARS-CoV-2 anti-S results, sera were selected from pre-COVID era subjects with a history of other bacterial and viral infections (n=50), with autoantibodies (ANA, RF; n=28), and healthy subjects with no serological diagnosis (n=55) (Table 1). A positive result for SARS-CoV-2 IgG was set at 6 standard deviations above the mean anti-SARS2 (1,975) or N (1,128) protein levels in the pre-COVID era cohort. Consistent with our prior study, we found no false-positive SARS2 anti-S or -N results [23]. The COVID-19 cohort included 277 serum samples from 155 subjects (age 62±7 years) with PCR-confirmed acute SARS2 infections diagnosed between April and June 2020 (Tables 1,2) [23]. Given the limitations of sample collection, subjects in the Outpatient group did not have longitudinal samples. The cohorts were not fully matched, including uneven disease distribution across age and risk factors, as well as limitations in pre-COVID era sera availability.

Pre-COVID era sera contain high anti-S IgG levels against common human coronaviruses (HCoVs)

To determine if HCoV antibody levels changed after acute SARS2 infection, we measured anti-S and anti-N IgG levels in cohort sera against SARS2, SARS1, MERS, OC43, HKU1, 229E, NL63, and the RaTG bat coronavirus (Fig 1) [22, 25, 26]. Prior studies have shown that >75% of children >2.5 years old are HCoV seropositive [8, 9, 27]; thus, we used sera from 2-3-year-old subjects (n=11) as negative controls to compare the prevalence of HCoVs in the pre-COVID era cohort(Table S2). The adult pre-COVID era sera demonstrated a high antibody prevalence to the four common HCoVs (Fig 2C). Over 90% of pre-COVID era subjects had anti-S IgG against OC43 (92.7%) and 229E (93.6%), but less anti-N IgG against OC43 (53.2%) and 229E (16.8%) (Table S2), consistent with

previous reports [6, 8-10]. The mismatch between anti- S and anti-N IgG to HCoVs suggests that most anti-S IgG developed from distant exposure. The above results suggest that abundant anti-HCoV, but not SARS2, antibodies existed in the population prior to the pandemic.

β HCoVs anti-S IgG antibody levels associated with SARS2 anti-S in COVID-19 patients

In the acute SARS2 infected cohort, the profile of IgG levels against multiple HCoV strains showed that the infection elicited anti-S IgG against SARS2, antigenically similar CoV strains(RaTG, SARS1), and cross-reactive IgG against the *β*-HCoV OC43 and HKU1 S-proteins. In contrast, SARS2 infection did not increase anti-S IgG against genetically dissimilar *α*-HCoV (229E, NL63; Figure 2 A, B). These results were visualized using MDS (Fig S1B) [22, 25, 26, 28]. Pre-COVID-19 and COVID-19 cohorts could be graphically distinguished by anti-S, but not by anti-N, IgG immune repertoire cartography. As expected, SARS2 infection did not change anti-N IgG levels against *α*- and *β*-HCoVs.

Pre-COVID-19 era uni-directional cross-reactivity of OC43 and HKU1 anti-S IgG antibodies

SARS2 infection significantly increased anti-S IgG levels against *β*-HCoVs OC43 and HKU1, but not against the *α*-HCoVs (229E, NL63; Fig 2B). Moreover, high levels of RaTG and SARS1- reactive anti-S antibodies were still detected in both a small number of pre-COVID-19 era and a much larger number of post-COVID-19 subjects. These results suggest that before SARS2infection, OC43 and HKU1 anti-S antibodies did not cross-react with SARS2, RaTG, or SARS1 at detectable levels, despite their sequence homologies. However, SARS2 infection resulted in high levels of anti-S IgG against SARS2, SARS1, RaTG, and *β*-HCOVs, but not *α*-HCoVs. These results suggest that either SARS2 infection elicits anti-*β*-HCoV IgG with low specificity and broad cross-reactivity, or high-affinity IgG against shared epitopes, which are immunodominant in SARS2 but not OC43 or

HKU1, and are shared by RaTG and SARS1.

Elevated OC43 and HKU1 anti-S IgG levels were highly correlated with SARS2 anti-S IgG levels in serum samples of COVID-19 patients (Fig S1A), demonstrating the same conclusions (Fig 2C). If IgG antibodies cross-reacted with common epitopes across HCoVs, we would expect bi-directional binding to both S-proteins. In contrast, we found that while OC43 and HKU1 anti-S IgG antibodies were present in sera from both cohorts, pre-COVID-19 samples lacked reactivity against the SARS2 S protein (Fig 2A and B). We refer to this as "uni-directional" reactivity, which is distinct from the cross-reactivity of the sera from COVID-19 patients that bind both SARS2 and OC43 [17, 29].

OC43 and HKU1 anti-S IgG levels correlate with the rapid increase in SARS2 anti-S IgG in de novo infection

To understand the kinetics of SARS2, OC43, and HKU1 anti-S antibodies in acute COVID-19 subjects, we measured the anti-S IgG repertoire profile against all HCoVs and SARS2 in samples binned by days from symptom onset (0-31 DFSO; n=54 subjects with >2 longitudinal samples). SARS2 anti-S IgG antibodies appeared in 42% (29/69) of patients' sera within 7 DFSO. Among them, 62% (18/29) of subjects' SARS2 anti-S IgG levels peaked 8-14 DFSO (Fig 3A). Notably, serum samples from subjects with high SARS2 IgG levels also showed higher levels of OC43 anti-S antibody (Fig 3B). SARS2 anti-S IgG seroconversion occurred within 1-2 weeks of infection for most subjects. OC43 and HKU1 anti-S antibodies showed a similar pattern but less statistical significance (Fig3 A).

To analyze the correlations between the HCoV and anti-S IgG levels in the rapid-response subjects, we binned serum samples by DFSO and visualized subject antibody profiles (Fig 3B). Within-subject analysis showed a high correlation between anti-*β*-HCoV (OC43, HKU1) and anti-SARS2 spike IgG levels in all three time periods, which was not seen with the *α*-HCoVs (Fig 3C). These results demonstrate that increased OC43 (R^2=0.8184) and HKU1(R^2=0.6926) anti-S IgG levels are highly correlated with the rapid appearance of SARS-2 anti-S IgG during early infection (DFSO≤7). This data supports the

hypothesis that higher OC43 and HKU1 anti-S antibodies early in COVID-19 disease may accelerate the anti-SARS2 S IgG response. After two weeks (DFSO≥15), 95% of COVID-19 subjects developed high IgG levels against SARS2, OC43, and HKU1 S-proteins. Thus, pre-existing IgG targeting OC43 and HKU1 may also influence the *de novo* antibody response to SARS2 infection.

Anti-OC43-S IgG levels correlate with COVID-19 severity

Accumulating data demonstrates that rapid and robust increases in SARS2 S-reactive IgG correlate with disease severity and plasmablast expansion [2, 11, 15]. Broadly cross-reactive anti-SARS2 antibody responses in more severe COVID-19 patients [29], and higher OC43 anti-S antibody levels, are also associated with disease severity [17]. To our knowledge, there is no published data demonstrating this correlation between SARS2 and HCoV anti-S IgG levels and SOFA scores, a standard measure of disease severity [21].

We first profiled the anti-S IgG antibodies against SARS2 and other HCoVs across moderate to severe illness within the COVID-19 cohorts: out-patients (n=42), in-patients (n=33), ICU (survivors, n=57), and those who died (n=23). Each subject had 1–5 serum samples available within 42 days after infection, and all hospitalized subjects (inpatient, ICU, death) had maximum SOFA scores for the sampling date (Table 2). Fig 4A shows the rapid and robust rise of anti-S SARS2 IgG levels in the severe (ICU) versus moderate (in- and out-patient) disease cohorts. Mean maximum SOFA score changes were associated with a one-unit increase in subject IgG levels against HCoV spike proteins using linear mixed-effects models (Table 3). Notably, changes in SARS2 (P=0.0112) and OC43 (P=0.0047) anti-S IgG were highly associated with the mean change in maximum SOFA scores on the day of serum sampling. We also observed a delayed increase of anti-S SARS2 IgG levels in the non-survivor group compared with severe disease survivors [15]. Notably, the dynamics of OC43 anti-S IgG over the clinical course of the disease were very similar to S-reactive SARS2 IgG, with a rapid rise in antibody levels in the surviving severe patients. The weekly anti-S antibody level comparison results also showed that anti-S antibodies

against OC43 and HKU1 were significantly higher in the severe survivors than moderate patients (Fig 4B).

Rapidly elevated anti-spike S2-reactive IgG correlates with COVID- 19 severity

To further characterize cross-reactive anti-SARS2 S IgG, we evaluated binding to the S1, S2, and RBD subunits. As expected, S1, S2, and RBD-reactive IgG levels were highly correlated with the development of anti-SARS2 IgG during acute SARS2 infection (Fig 2 and 3). Interestingly, we found a higher linear correlation between anti-S compared with anti-S2 IgG levels (R^2=0.9723) than with anti-S1 (R^2 =0.825). In addition, the dynamics of IgG levels against the S1 and S2 subunits were very different. The trajectory of the S1 antibody was significantly higher in the severe disease (ICU) group with higher SOFA scores compared to the mild disease in-patient group ($P<0.05$, trend testing within the generalized estimating equation framework). However, the S2 antibody increased rapidly in the severe group, leading to an increased S2/S1 IgG ratio (Fig 4C). The S2/S1 ratio decreased over time in outpatients with mild symptoms but increased in the hospitalized inpatients, including high SOFA score ICU patients (Fig 4D).Notably, a higher level of anti-S IgG antibody against the S1 domain of SARS2 and other common cold HCoVs did not correlate with illness severity. We could not detect a higher trajectoryof anti-S1 antibody in the out-patient cohort in this study. This is an intriguing finding, which should be further investigated by a future analysis with more longitudinal data.

DISCUSSION

We report a multi-dimensional analysis of pre-COVID-19 and COVID-19 era sera using multi-plex analysis to profile S comprehensively- and N-reactive IgG against SARS2 and common HCoVs. We found a high prevalence of anti-S and -N IgG antibodies against seasonal HCoVs in the pre-COVID era, without cross-reactive binding to SARS2 S and N

proteins. This occurred despite moderate S-protein sequence homologies between SARS2 and the β-HCoVs OC43 (74.9%) and HKU1 (75.7%) [30]. Consistent with recent reports, we also found that SARS2 infection was associated with elevated anti-SARS2 S IgG and correspondingly high IgG levels against OC43 and HKU1 S proteins [15, 17, 18, 31]. This association was especially prominent in the first week after symptom onset, suggesting that pre-existing β-HCoV immunity may contribute to the initial SARS2 antibody response. This uni-directional IgG binding was distinct from the bi-directional cross-reactivity after SARS2 infection. Post-COVID, there was strong serum reactivity against RaTG13 and SARS1, which have 96.1% [4] and 81.2% homology, respectively, with SARS2 [32]. Of note, during the first week of infection, increased β-HCoV and S2-reactive IgG highly correlated with SOFA scores, illness severity, andmortality [33-35].

Our analysis found a significant correlation between COVID-19 severity and the kinetics of anti-S1 and anti-S2 IgG levels during acute infection. Early, elevated anti-S2 IgG levels were associated with higher SOFA scores and mortality. Several reports have shown that pre-existing S2-specific memory B cells [14] and $CD4^+$ T cells [36-38] contribute to an early immune response strongly biased towards the S2 domain. The high prevalence of anti-S IgGagainst the similar OC43 and HKU1 β-HCoVs described here and by others [39], suggests that imprinting may bias an early, non-neutralizing immune response towards S2 epitopes. In contrast, the slower emergence of anti-S1 and anti-RBD IgG in subjects with higher SOFA scores is consistent with the hypothesis that anti-S1 kinetics reflect a primary antibody response. It also suggests that the lag in protective anti-RBD IgG may contribute to increased COVID-19 disease severity. Whether imprinting against S1 and RBD epitopes after vaccination reverses this trend is yet to be determined. We also found uni-directional anti-S IgG binding to -HCoV S proteins from OC43 and HKU1 in pre-COVID-19 era sera.

In contrast, COVID-19 sera had strong bi-directional reactivity against OC43, HKU1, and SARS2 S proteins. The precise epitopes involved in high cross-reactivity with OC43

and HKU1 β-HCoVs in the COVID-19 sera remain unclear. One possibility is the presence of unique immunodominant epitopes on OC43 and HKU1 S proteins. Alternatively, low-prevalence memory B cells recognizing conserved sub-immunodominant epitopes during infection may produce HCoV cross-reactive antibodies, consistent with immune imprinting [18], as described with influenza infection and vaccination [2, 11, 15, 40]. Infected and vaccinated individuals acquire the ability to produce high-affinity specific antibodies against novel epitopes from new virus strains [41]. Such cross-reactive anti-HCoV antibodies have alsobeen isolated from the convalesced COVID-19 patients [29].

Relevant to both disease severity and population immunity, the above findings suggest a role for immune imprinting in SARS-CoV-2 anti-S protein IgG responses. Elevated IgG antibodies against OC43 S and SARS2 S2 early (<7 days) in acute COVID-19 subjects with high SOFA scores may be due to prior β-HCoV immune imprinting of memory B cells. Broadly cross-reactive antibodies recognizing both SARS2 and OC43 most likely developed from rapidly expanding broadly memory B cells that recognize conserved epitopes. Further study with both linear and spatial B-cell epitopes with cloned IgG from B cells during acute infection is needed to better characterize the molecular basis of the switch from uni- to bi-directional IgG binding.

Finally, this study provides direct evidence that pre-existing β-HCoV S-reactive antibodies are associated with the SARS2 specific antibody response, and the OC43 anti-S and SARS2 anti-S2 antibodies highly correlate with the COVID-19 severity. One possible mechanism for this finding is that anti-S2 IgG does not prevent receptor binding and viral fusion, enabling higher viral loads. Alternatively, complement activation by anti-spike IgG1 and IgG3 could potentiate thrombosis via complement-dependent cytotoxicity (CDC) of vascular endothelial cells [2, 42-45]. However, several caveats apply. First, while anti-RBD antibodies have been shown to correlate with viral neutralization, we did not directly measure neutralizing activity. In addition, sera were obtained for secondary analysis from clinical samples and not a longitudinal series spanning from pre- to post-infection in single

individuals. This may introduce a selection bias, where subjects with the most longitudinal samples tended to be those with severe COVID-19, limiting our ability to detect the significant impact of pre-existing OC43 antibodies in mild illness. Similarly, while we observed a trend of delayed development of SARS2 S-reactive IgG in the non-survivor group [15, 31], we could not isolate the effects of OC43, and HKU1 antibodies have on specific antibody development. Future, prospective, longitudinal studies would help address some of these questions.

CONCLUSION

Our study suggests that a rapid and robust increase in anti-S2 subunit IgG antibodies after SARS2 infection may be a marker of disease severity. This quick *de novo* response may be due to immune imprinting of pre-existing *β*-HCoVs cross-reactive memory B cells or plasmablasts, which may, in turn, lead to non-neutralizing IgG production. These findings may impact future vaccine development strategies targeting new emerging SARS-CoV-2 variants.

NOTES

Competing interests

There are no competing interests.

Author contributions statement

JW, NDP, and MSZ conceived the investigation plan designed the experiments and analytic methods. JW, QZ, AW, and AC carried out the experiments. JW, DL, and MSZ analyzed the data. JW, MSZ, QZ, DL, AW, JN, and AC wrote and edited the manuscript.

Funding

This work was supported by the National Institutes of Health Institute of Allergy, Immunology and Infectious Diseases grant R21 AI138500 and R01 AI129518 (MZ, JW), and the University of Rochester Clinical and Translational Science Award UL1 TR002001 from the National Center for Advancing Translational Sciences of the National Institutes of Health (DL, MZ), and the Rochester Vaccine Fellowship (AC). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. None of the above funders had any role in study design, data collection, analysis, the decision to publish, or preparation of the manuscript.

Acknowledgments

The authors thank the anonymous reviewers for their valuable suggestions. Finally, we would like to thank Dr. Shannon Hilchey, Samantha King, and Eric Mendleson for their critical reading of the manuscript.

REFERENCE

1. Dan JM, Mateus J, Kato Y, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science **2021**.

2. Zohar T, Alter G. Dissecting antibody-mediated protection against SARS-CoV-2. Nat Rev Immunol **2020**; 20:392-4.

3. WHO. WHO Coronavirus Disease (COVID-19) Dashboard, **2021**.

4. Andersen KG, Rambaut A, Lipkin WI, Holmes EC, Garry RF. The proximal origin of SARS-CoV-2. Nat Med **2020**; 26:450-2.

5. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol **2019**; 17:181-92.

6. Lim YX, Ng YL, Tam JP, Liu DX. Human Coronaviruses: A Review of Virus-Host Interactions. Diseases **2016**; 4.

7. Tyrrell DA, Bynoe ML. Cultivation of a Novel Type of Common-Cold Virus in Organ Cultures. Br Med J **1965**; 1:1467-70.

8. Dijkman R, Jebbink MF, El Idrissi NB, et al. Human coronavirus NL63 and 229E seroconversion in children. J Clin Microbiol **2008**; 46:2368-73.

9. Zeng ZQ, Chen DH, Tan WP, et al. Epidemiology and clinical characteristics of human coronaviruses OC43, 229E, NL63, and HKU1: a study of hospitalized children with acute respiratory tract infection in Guangzhou, China. Eur J Clin Microbiol Infect Dis **2018**; 37:363-9.

10. Dobano C, Santano R, Jimenez A, et al. Immunogenicity and crossreactivity of antibodies to the nucleocapsid protein of SARS-CoV-2: utility and limitations in seroprevalence and immunity studies. Transl Res **2021**.

11. Zost SJ, Gilchuk P, Case JB, et al. Potently neutralizing and protective human antibodies against SARS-CoV-2. Nature **2020**; 584:443-9.

12. Pan Y, Li X, Yang G, et al. Serological immunochromatographic approach in diagnosis with SARS-CoV-2 infected COVID-19 patients. J Infect **2020**; 81:e28-e32.

13. Lan J, Ge J, Yu J, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature **2020**; 581:215-20.

14. Ng KW, Faulkner N, Cornish GH, et al. Preexisting and de novo humoral immunity to SARS-CoV-2 in humans. Science **2020**; 370:1339-43.

15. Zohar T, Loos C, Fischinger S, et al. Compromised Humoral Functional Evolution Tracks with SARS-CoV-2 Mortality. Cell **2020**; 183:1508-19 e12.

16. Beretta A, Cranage M, Zipeto D. Is Cross-Reactive Immunity Triggering COVID-19 Immunopathogenesis? Front Immunol **2020**; 11:567710.

17. Guo L, Wang Y, Kang L, et al. Cross-reactive antibody against human coronavirus OC43 spike protein correlates with disease severity in COVID-19 patients: a retrospective study. Emerg Microbes Infect **2021**; 10:664-76.

18. Aydillo T, Rombauts A, Stadlbauer D, et al. Immunological imprinting of the antibody response in COVID-19 patients. Nat Commun **2021**; 12:3781.

19. Hicks J, Klumpp-Thomas C, Kalish H, et al. Serologic Cross-Reactivity of SARS-CoV-2 with Endemic and Seasonal Betacoronaviruses. J Clin Immunol **2021**; 41:906-13.

20. Ladner JT, Henson SN, Boyle AS, et al. Epitope-resolved profiling of the SARS-CoV-2 antibody response identifies cross-reactivity with endemic human coronaviruses. Cell Rep Med **2021**; 2:100189.

21. Angus DC, Seymour CW, Coopersmith CM, et al. A Framework for the Development and Interpretation of Different Sepsis Definitions and Clinical Criteria. Crit Care Med **2016**; 44:e113-21.

22. Wang J, Li D, Zhou Q, Wiltse A, Zand MS. Antibody Mediated Immunity to SARS-CoV-2 and Human Coronaviruses: Multiplex Beads Assay and Volumetric Absorptive Microsampling to Generate Immune Repertoire Cartography. Frontiers in Immunology **2021**; 12:2681.

23. Cameron A, Porterfield CA, Byron LD, et al. A multiplex microsphere IgG assay for SARS-CoV-2 using ACE2-mediated inhibition as a surrogate for neutralization. J Clin Microbiol **2020**.

24. Zand MS, Wang J, Hilchey S. Graphical Representation of Proximity Measures for Multidimensional Data: Classical and Metric Multidimensional Scaling. Math J **2015**; 17.

25. Wang J, Hilchey SP, Hyrien O, et al. Multi-Dimensional Measurement of Antibody-Mediated Heterosubtypic Immunity to Influenza. PLoS One **2015**; 10:e0129858.

26. Wang J, Hilchey SP, DeDiego M, et al. Broad cross-reactive IgG responses elicited by adjuvanted vaccination with recombinant influenza hemagglutinin (rHA) in ferrets and mice. PLoS One **2018**; 13:e0193680.

27. Dijkman R, Jebbink MF, Gaunt E, et al. The dominance of human coronavirus OC43 and NL63 infections in infants. J Clin Virol **2012**; 53:135-9.

28. Wang J, Li D, Perry S, et al. Broadly Reactive IgG Responses to Heterologous H5 Prime-Boost Influenza Vaccination Are Shaped by Antigenic Relatedness to Priming Strains. mBio **2021**:e0044921.

29. Shrock E, Fujimura E, Kula T, et al. Viral epitope profiling of COVID-19 patients reveals cross-reactivity and correlates of severity. Science **2020**.

30. Cueno ME, Imai K. Structural Comparison of the SARS CoV 2 Spike Protein Relative to Other Human-Infecting Coronaviruses. Front Med (Lausanne) **2020**; 7:594439.

31. Kaplonek P, Wang C, Bartsch Y, et al. Early cross-coronavirus reactive signatures of humoral immunity against COVID-19. Sci Immunol **2021**; 6:eabj2901.

32. Zhu Y, Yu D, Han Y, et al. Cross-reactive neutralization of SARS-CoV-2 by serum antibodies from recovered SARS patients and immunized animals. Sci Adv **2020**; 6.

33. Hejazi ME, Malek Mahdavi A, Navarbaf Z, et al. Relationship between chest CT scan findings with SOFA score, CRP, comorbidity, and mortality in ICU patients with COVID-19. Int J Clin Pract **2021**; 75:e14869.

34. Yang Z, Hu Q, Huang F, Xiong S, Sun Y. The prognostic value of the SOFA score in patients with COVID-19: A retrospective, observational study. Medicine (Baltimore) **2021**; 100:e26900.

35. Su C, Xu Z, Hoffman K, et al. Identifying organ dysfunction trajectory-based subphenotypes in critically ill patients with COVID-19. Sci Rep **2021**; 11:15872.

36. Zhao J, Zhao J, Mangalam AK, et al. Airway Memory CD4(+) T Cells Mediate Protective Immunity against Emerging Respiratory Coronaviruses. Immunity **2016**; 44:1379-91.

37. Mateus J, Grifoni A, Tarke A, et al. Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans. Science **2020**; 370:89-94.

38. Weiskopf D, Schmitz KS, Raadsen MP, et al. Phenotype and kinetics of SARS-CoV-2-specific T cells in COVID-19 patients with acute respiratory distress syndrome. Sci Immunol **2020**; 5.

39. Nguyen-Contant P, Embong AK, Kanagaiah P, et al. S Protein-Reactive IgG and Memory B Cell Production after Human SARS-CoV-2 Infection Includes Broad Reactivity to the S2 Subunit. mBio **2020**; 11.

40. Rydyznski Moderbacher C, Ramirez SI, Dan JM, et al. Antigen-Specific Adaptive Immunity to SARS-CoV-2 in Acute COVID-19 and Associations with Age and Disease Severity. Cell **2020**; 183:996-1012 e19.

41. Turner JS, Zhou JQ, Han J, et al. Human germinal centres engage memory and naive B cells after influenza vaccination. Nature **2020**; 586:127-32.

42. Liu F, Han K, Blair R, et al. SARS-CoV-2 Infects Endothelial Cells In Vivo and In Vitro. Front Cell Infect Microbiol **2021**; 11:701278.

43. Rambaldi A, Gritti G, Mico MC, et al. Endothelial injury and thrombotic microangiopathy in COVID-19: Treatment with the lectin-pathway inhibitor narsoplimab. Immunobiology **2020**; 225:152001.

44. Manolis AS, Manolis TA, Manolis AA, Papatheou D, Melita H. COVID-19 Infection: Viral Macro- and Micro-Vascular Coagulopathy and Thromboembolism/Prophylactic and Therapeutic Management. J Cardiovasc Pharmacol Ther **2021**; 26:12-24.

45. Colling ME, Kanthi Y. COVID-19-associated coagulopathy: An exploration of mechanisms. Vasc Med **2020**; 25:471-8.

Table 1. The study cohorts.

Cohort		N (Male/Female)	Age (Mean/Median)
Pre-COVID	Cytomegalovirus	10 (4/6)	52/55
	Epstein-Barr(EB) virus	10 (4/6)	55/509
	Respiratory syncytial virus (RSV)	10(6/4)	51/55
	Lyme disease	10(2/8)	49/52
	Syphilis	10(4/6)	43/55
	Autoimmune disease (ANA)	19(9/10)	45/52
	Autoimmune disease (RF)	9(5/4)	45/43
	Acute viral respiratory illness	55(20/25)	48/62
	Other *b*	55(22/23)	55/58
	Total	188 (80/108)	49/52
COVID	Out-Patients	42(19/23	68/70
	In-Patients	33(19/14)	68/66
	ICU	57(25/32)	67/67
	Death	23(11/12)	74/67
	Total	155(88/73)	68/67

Note: Each pre-COVID cohort subject had one sample

ANA (antinuclear antigen-positive sera)

RF (rheumatoid factor positive sera)

b Samples with no serological diagnosis

Table 2. Demographics of COVID-19 cohort and subgroups of acute CoVID-19 patients.

	Out-Patient	In-patient	ICU(Survivor)	Death(Non-survivor)	Total
Subjects (N)	42	33	57	23	155
Age (Mean/Median)	69/70	69/70	67/67	70/68	
Male/Female	23/19	13/20	28/29	10/13	
Max SOFA Score*	0	2.12±3.12	5.48±3.90	6.78±3.84	
Median SOFA	0	1	5	7	
Serum Sample (N)	47	45	162	23	277
DFSO=0-7	15	14	36	9	74
DFSO=8-14	12	7	42	2	63
DFSO >14	17	13	72	12	114
UNK	3	11	12	0	26

Note: Some COVID-19 cohort subjects have longitude samples, total serum samples number is 277 (n=277).

* Mean±STD

DFSO: Days From Symptom Onset (Days)

UNK: Samples without DFSO information

Table 3. The correlation coefficients (r, Spearman correlation) and mean maximum SOFA score changes associated with one unit increase in HCoV anti-S IgG within the COVID-19 cohort.

	Spearman Correlation		Max SOFA change	
	r	P-value	Mean	P-value
SARS2-S	0.2249	**0.0041**	0.26	**0.0112**
OC43	0.2326	**0.0030**	0.40	**0.0465**
HKU1	0.2453	**0.0017**	0.33	0.1312
229E	-0.0136	0.8644	-0.48	0.1268
MNL63	0.0663	0.4036	-0.07	0.7989
SARS2-S1	0.2679	**0.0006**	0.18	0.0504
SARS2-RBD	0.2415	**0.0020**	0.23	0.0777
SARS2-S2	0.2301	**0.0033**	0.27	**0.0125**

Figure 1

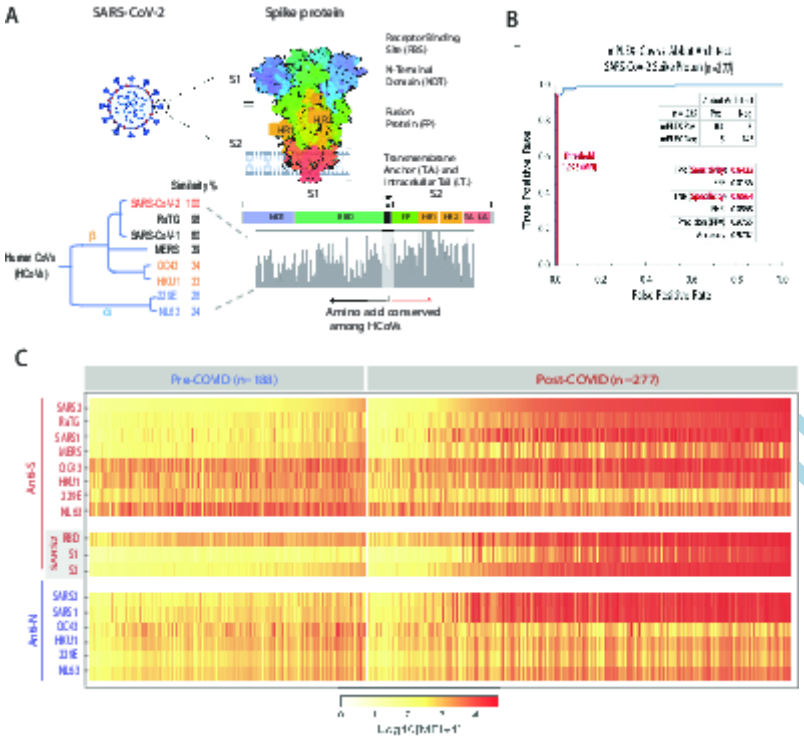

Figure 2

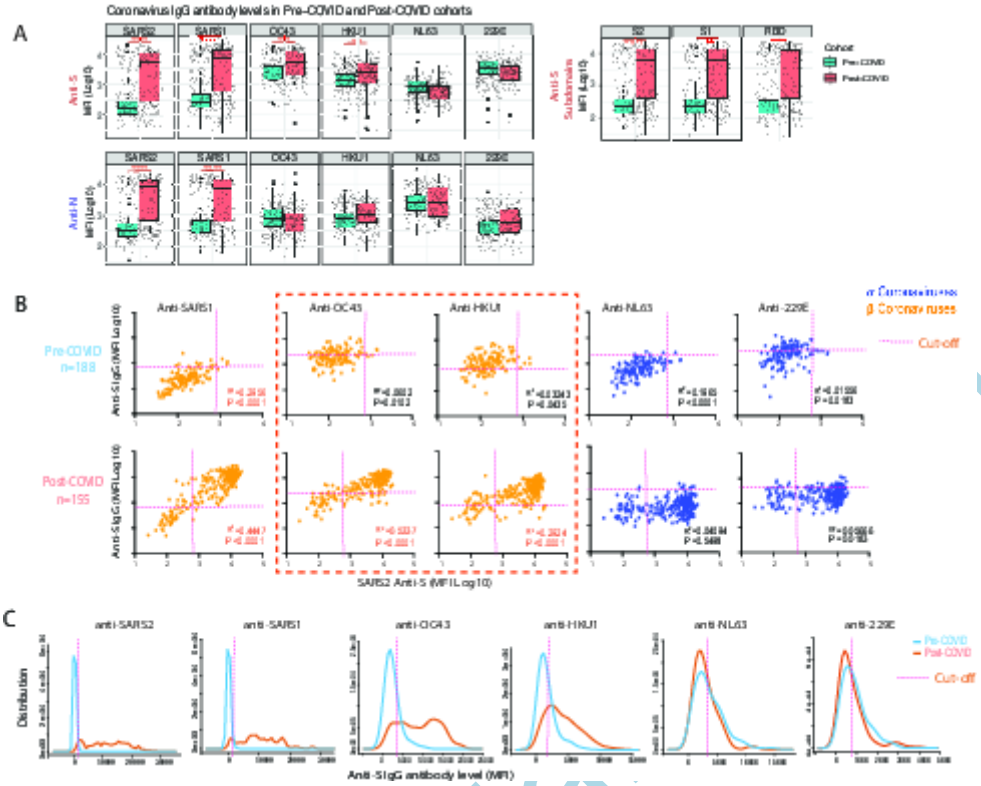

Figure 3

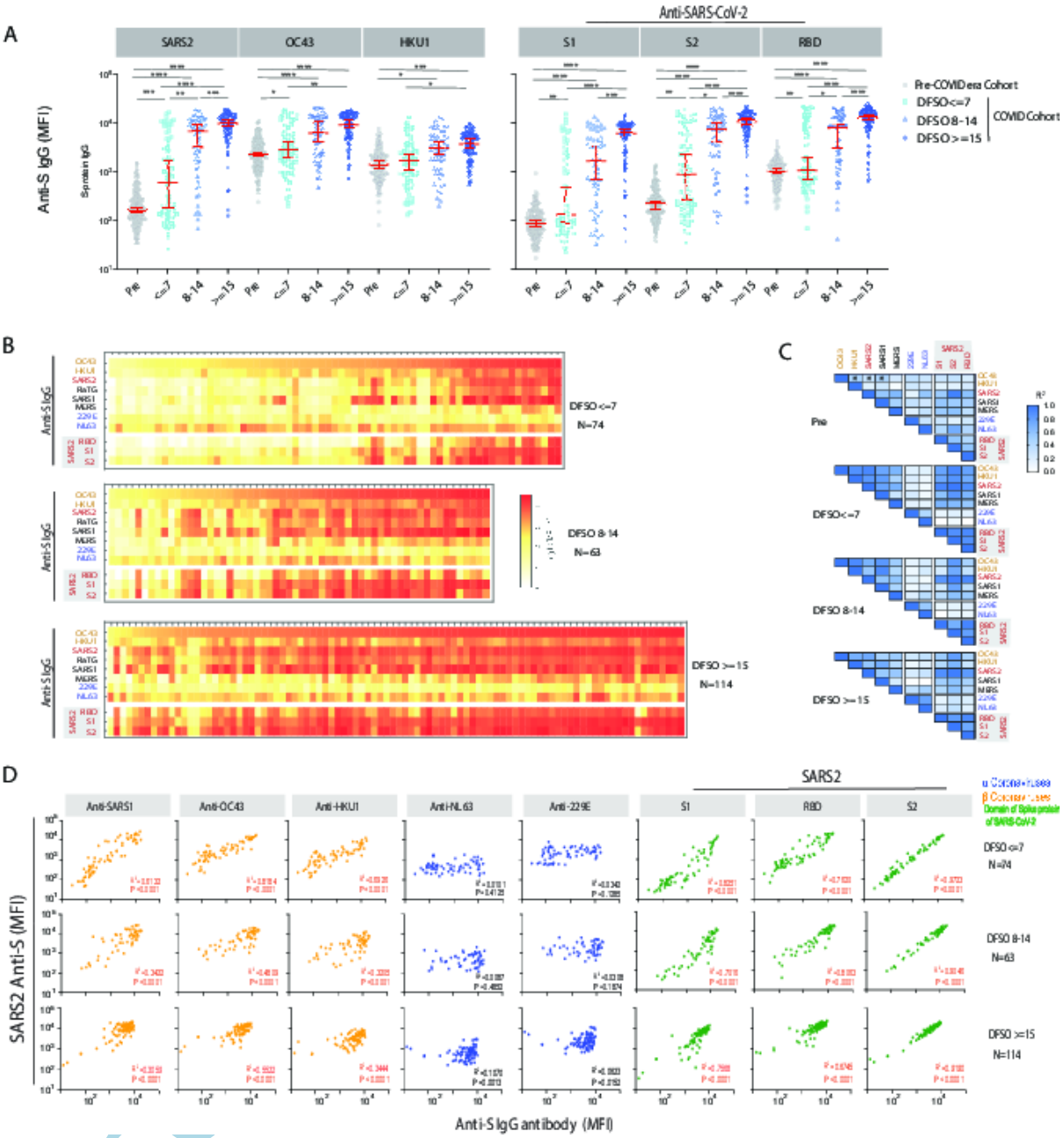
A
SARS2
OC43
HKU1
Anti-SARS-CoV-2
S1
S2
RBD
Anti-S IgG (MFI)
Spike IgG
Pre
<=7
8-14
>=15
Pre-COVID era Cohort
DFSO<=7
DFSO 8-14
DFSO >=15
COVID Cohort
B
Anti-S IgG
DFSO <=7
N=74
DFSO 8-14
N=63
DFSO >=15
N=114
C
Pre
DFSO<=7
DFSO 8-14
DFSO >=15
D
SARS2
Anti-SARS1
Anti-OC43
Anti-HKU1
Anti-NL63
Anti-229E
S1
RBD
S2
SARS2 Anti-S (MFI)
Anti-S IgG antibody (MFI)
DFSO <=7
N=74
DFSO 8-14
N=63
DFSO >=15
N=114

Figure 4