Comparable endemic coronavirus nucleoprotein-specific antibodies in mild and severe Covid-19 patients

Received: 5 March 2021 | Revised: 31 March 2021 | Accepted: 9 April 2021

DOI: 10.1002/jmv.27038

SHORT COMMUNICATION

JOURNAL OF MEDICAL VIROLOGY WILEY

Comparable endemic coronavirus nucleoprotein-specific antibodies in mild and severe Covid-19 patients

Susannah Leach[1,2] | Ali M. Harandi[1,3] | Tomas Bergström[4] | Lars-Magnus Andersson[4,5] | Staffan Nilsson[6,7] | Lia van der Hoek[8] | Magnus Gisslén[4,5]

[1]Department of Microbiology and Immunology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

[2]Department of Clinical Pharmacology, Sahlgrenska University Hospital, Gothenburg, Sweden

[3]Vaccine Evaluation Center, BC Children's Hospital Research Institute, The University of British Columbia, Vancouver, Canada

[4]Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

[5]Department of Infectious Diseases, Sahlgrenska University Hospital, Gothenburg, Sweden

[6]Department of Mathematical Sciences, Chalmers University of Technology, Gothenburg, Sweden

[7]Department of Laboratory Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

[8]Laboratory of Experimental Virology, Department of Medical Microbiology and Infection Prevention, Amsterdam Infection & Immunity Institute, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

Correspondence
Susannah Leach, Department of Microbiology and Immunology, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Box 435, 40530 Gothenburg, Sweden.
Email: susannah.leach@microbio.gu.se

Funding information
Science for Life Laboratory, Grant/Award Number: V-2020- 0250; VINNOVA, Grant/Award Number: 2020-02205; Swedish State Support for Clinical Research, Grant/Award Numbers: ALFGBG-679621, ALFGBG-717531

Abstract

The severity of disease of Covid-19 is highly variable, ranging from asymptomatic to critical respiratory disease and death. Potential cross-reactive immune responses between SARS-CoV-2 and endemic coronavirus (eCoV) may hypothetically contribute to this variability. We herein studied if eCoV nucleoprotein (N)-specific antibodies in the sera of patients with mild or severe Covid-19 are associated with Covid-19 severity. There were comparable levels of eCoV N-specific antibodies early and during the first month of infection in Covid-19 patients with mild and severe symptoms, and healthy SARS-CoV-2-negative subjects. These results warrant further studies to investigate the potential role of eCoV-specific antibodies in immunity to SARS-CoV-2 infection.

KEYWORDS
COVID-19, disease severity, endemic coronaviruses, nucleoprotein-specific antibodies

1 | INTRODUCTION

Among the large family of coronaviruses (subfamily Orthocoronavirinae in the family of Coronaviridae of the order Nidovirales), seven are known to infect humans. Four endemic coronaviruses (eCoVs), the species HCoV-NL63 and HCoV-229E within the genus *Alphacoronavirus* and the species HCoV-OC43 and HCoV-HKU1 within the genus *Betacoronavirus*, spread seasonally and cause the common cold.[1] Three additional betacoronaviruses, namely SARS-CoV, MERS-CoV, and SARS-CoV-2, can cause severe respiratory

syndromes in humans.[2] A hallmark of Covid-19, the disease caused by SARS-CoV-2 infection, is the great variability in the severity of disease, ranging from asymptomatic to critical respiratory disease and death. Potential cross-reactive immune responses between SARS-CoV-2 and eCoVs may hypothetically contribute to this variability, either by affording cross-protection or by being detrimental via antibody-dependent enhancement.

During Covid-19, antibody responses mainly target the spike (S) glycoprotein and the nucleoprotein (N).[3] Antibodies against the receptor-binding domain (RBD) of the S protein possess potent virus-neutralizing capacity, whereas antibodies against the internal structure N protein do not. Nevertheless, N-specific antibodies are indicative of antiviral immunity, exemplified in a recent epidemiological study, which demonstrated that healthcare workers with N-antibodies had a much lower risk of testing polymerase chain reaction (PCR)-positive for SARS-CoV-2 than those without, and that the incidence of PCR-positive results fell with increasing N-antibody titers.[4] Several studies have demonstrated the presence of T-cell and antibody cross-reactivity between several epitopes of SARS-CoV-2 and the eCoVs.[5–7] Although eCoV S-specific antibody responses in Covid-19 patients have been studied,[8] there is a dearth of information on eCoV N-specific antibody responses in Covid-19 patients with varying severity of the disease. Importantly, a recent report that studied medical records found that though recent eCoV infections were not associated with lower rates of SARS-CoV-2 infection, they were associated with reduced severity of Covid-19.[9]

Herein, we explored eCoV N-specific antibodies in the sera of Covid-19 patients with mild or severe symptoms and healthy SARS-CoV-2-negative individuals by an assessment of specific antibodies to N-protein of the four eCoVs and SARS-CoV-2. Our results showed that comparable levels of eCoV N-specific antibodies were observed in patients with mild and severe Covid-19. These results enhance our understanding of the impact of human antibody responses to eCoVs on the severity of Covid-19.

2 | MATERIALS AND METHODS

2.1 | Patients and sample collection

A cohort of 43 patients (70% female; age range, 23–84 years) with PCR-confirmed SARS-CoV-2 infection with mild ($n = 21$) or severe/critical ($n = 22$) symptoms of Covid-19 were recruited between February 25 and November 23, 2020, at the Department of Infectious Diseases, Sahlgrenska University Hospital, Gothenburg, Sweden. The study protocol was approved by the Swedish Ethical Review Authority (Registration Number: 2020–01771) and patients were included in the study after written informed consent. Severe/critical disease was defined as the requirement of intensive care with mechanical ventilation, and mild disease as not requiring supplementary oxygen or hospitalization. Medical conditions, defined by the Centers for Disease Control and Prevention, USA, as having a significant association with risk of severe Covid-19 illness (e.g., cancer, cerebrovascular disease, chronic kidney disease, chronic obstructive pulmonary disease, heart conditions, immunocompromised state, obesity, pregnancy, and diabetes mellitus type 1 and 2[10]), were collected from the subjects' medical records. Information on the smoking status of the study participants was incomplete and therefore not included. Serum samples retrieved from healthy volunteers who tested negative for SARS-CoV-2 with PCR ($n = 27$; 56% female; age range, 20–79 years) between January 29 and September 23, 2020, were included as controls. In the study that compared antibody levels between the mild and severe Covid-19 patient groups, acute serum samples collected within 10 days after debut of symptoms (mean four days post symptom onset for patients with mild disease and eight days for patients with severe disease) were used. For the analysis of longitudinal antibody development, subjects were included for which two or more samples from the first 30 days after symptom debut were available ($n = 23$).

2.2 | Antibody detection

Antibodies specific for HCoV-NL63, HCoV-229E, HCoV-HKU1, and HCoV-OC43 were analyzed using the C-terminal part of the N-protein as antigen in enzyme-linked immunosorbent antibodies (ELISAs) as described elsewhere.[1] SARS-CoV-2-specific total antibody was analyzed using the SARS-CoV-2 N-protein double recognition ELISA INgezim test as previously described.[11] This assay is based on the use of the same protein (in this case, the N protein) as the target antigen and detection molecule, using the principle that antibodies possess multiple antigen-binding regions (2 for IgG, 4 for IgA, and 10 for IgM), allowing their simultaneous binding to both the target and detection antigen. Double recognition tests have the advantage that they detect all antigen-specific antibodies, regardless of their class (IgA, IgG, or IgM).

2.3 | Statistical analysis

Differences between the groups were analyzed using analysis of variance with Tukey's multiple comparisons test for eCoV antibodies, and Fisher's exact test for SARS-CoV-2 antibodies for which there is a cut-off for positivity. Correlations were analyzed using Pearson. $p < 0.05$ was considered statistically significant.

3 | RESULTS AND DISCUSSION

Due to the ubiquitous nature of the eCoV, all adults are considered to be exposed to eCoVs, though eCoV-specific antibody levels have been shown to rapidly wane and reinfections are common.[1] SARS-CoV-2-specific antibodies are detectable a median of 11 days (interquartile range, 7–14 days) post symptom onset,[3] although with a considerable interpatient variability and differences in results depending on the assay used.

First, we investigated if magnitudes of antibodies against eCoVs in the early serum samples (collected maximum 10 days post symptom onset) in SARS-CoV-2-infected patients with mild or severe Covid-19 are correlated with the severity of Covid-19 developed, using N-specific antibody ELISAs. No statistically significant differences in the levels of N-specific antibodies against any of the four eCoVs were observed in Covid-19 patients with mild disease, severe disease, or healthy controls (Figure 1A). Although SARS-CoV-2 N-specific antibodies were higher in patients with severe compared to mild symptoms, this difference is likely partly due to the longer duration of disease in the patients with severe symptoms. At least one pre-existing comorbidity known to increase the risk of severe Covid-19 was present in a higher proportion of patients with severe disease (27%) compared with mild disease (14%), which could bias these results. However, when all subjects with comorbidities ($n = 9$) were excluded, conclusions based on statistical significance levels remain unchanged.

There were no statistically significant correlations between the magnitudes of SARS-CoV-2 N-specific antibodies and any of the eCoV N-specific antibodies (data not shown). This confirms that SARS-CoV-2 N-specific antibodies have no substantial cross-reactivity with eCoV epitopes in the C-terminal part of the N-protein used as antigen in the present study. Our finding on the lack of inverse correlation between eCoV N-specific antibodies and disease severity is in line with, and extends, a previous report on the lack of cross-reactive neutralizing activity against SARS-CoV-2 in the pre-pandemic sera of individuals with prior PCR-confirmed eCoV infection.[12]

As the samples in patients with mild disease were collected mean 4 days earlier than in patients with severe disease, the antibody levels in patients with mild disease could be misleadingly low. To examine this, longitudinal samples retrieved from the same patients during the first month of infection were analyzed. As expected, SARS-CoV-2 antibody levels increased significantly over time ($r = 0.4$; $p = 0.003$; Figure 1B). In contrast, N-specific antibody levels to

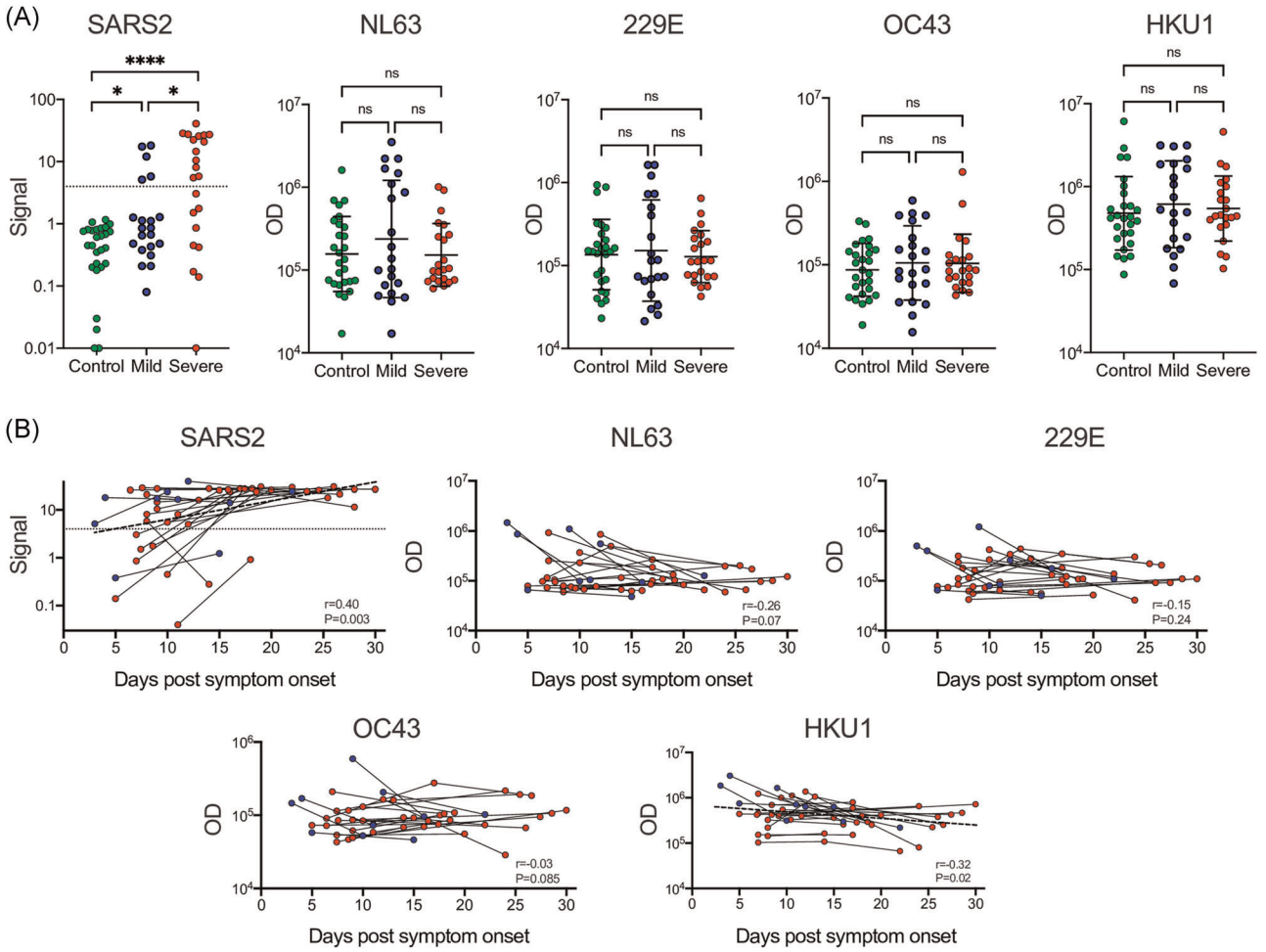

FIGURE 1 (A) Magnitudes (mean ± *SD* where relevant) of N-specific antibodies against SARS-CoV-2 and the endemic coronaviruses. Samples collected within 10 days of symptom onset in Covid-19 patients with mild symptoms ($n = 21$, blue) or severe/critical symptoms ($n = 22$, red), and SARS-CoV-2-negative controls ($n = 27$, green). (B) Serial samples collected during the first 30 days since symptom onset in Covid-19 patients with mild symptoms ($n = 5$, blue) or severe/critical symptoms ($n = 18$, red). Dotted line in (A) indicates assay cut-off for positivity for SARS-CoV-2 antibodies. ****$p < 0.0001$, *$p < 0.05$, ns: not significant

HCoV-NL63, HCoV-229E, and HCoV-OC43 did not increase over time, and HCoV-HKU1 antibodies even showed a trend toward decreasing over time ($r = -0.32$; $p = 0.02$). Thus, the lack of difference between eCoV-specific antibodies in those patients that are severely ill and those that show only mild symptoms is unlikely influenced by the 4-day difference in sampling timing.

The eCoV N-specific antibody levels detected in the samples probably reflect pre-existing serum antibodies, which do not appear to have been boosted by the SARS-CoV-2 infection, supported by the fact that these serum antibody levels remain stable or even slightly decrease during the first month and do not increase in parallel to the SARS-CoV-2 antibodies (Figure 1B). Notwithstanding, the possibility that memory responses to eCoV N-protein elicited by prior eCoV infections were very rapidly boosted by SARS-CoV-2 infection cannot be entirely ruled out. Additionally, it is conceivable that memory B-cells specific for other eCoV proteins, such as the S2 subunit,[6,7] with more cross-reactivity to SARS-CoV-2 counterparts, could be induced by SARS-CoV-2 infection.

In conclusion, we report comparable levels of eCoV N-specific antibodies early and during the first month after the onset of symptoms in Covid-19 patients with mild and severe symptoms. These results warrant further studies to investigate the potential role of eCoV-specific antibodies in immunity to SARS-CoV-2 infection.

ACKNOWLEDGMENTS

This study was supported by the Swedish State Support for Clinical Research (https://www.alfvastragotaland.se, ALFGBG-717531 [Magnus Gisslén] and 679621 [Susannah Leach]), SciLifeLab/KAW national COVID-19 research program (https://www.scilifelab.se/covid-19#nationalprogram, V-2020- 0250 [Magnus Gisslén]) and Sweden's Innovation Agency (Vinnova) project ID 2020-02205 (Ali M. Harandi). The funders had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

AUTHOR CONTRIBUTIONS

Study conception and design: Susannah Leach, Ali M. Harandi, Lars-Magnus Andersson, Lia van der Hoek, and Magnus Gisslén. *Data collection*: Susannah Leach, Lars-Magnus Andersson, Lia van der Hoek, and Magnus Gisslén. *Analysis and interpretation of results*: Susannah Leach, Ali M. Harandi, Tomas Bergström, Lars-Magnus Andersson, Staffan Nilsson, Lia van der Hoek, and Magnus Gisslén. *Draft manuscript preparation*: Susannah Leach and Magnus Gisslén. All authors reviewed the results and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

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

ORCID

Susannah Leach https://orcid.org/0000-0002-9614-2753

REFERENCES

1. Edridge AWD, Kaczorowska J, Hoste ACR, et al. Seasonal coronavirus protective immunity is short-lasting. *Nat Med*. 2020;26(11):1691-1693.
2. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med*. 2020;382(8):727-733.
3. Huang AT, Garcia-Carreras B, Hitchings MDT, et al. A systematic review of antibody mediated immunity to coronaviruses: kinetics, correlates of protection, and association with severity. *Nat Commun*. 2020;11(1):1-16.
4. 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(384):533-540.
5. Mateus J, Grifoni A, Tarke A, et al. Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans. *Science*. 2020;370(6512):89-94.
6. Ng KW, Faulkner N, Cornish GH, et al. Pre-existing and de novo humoral immunity to SARS-CoV-2 in humans. *Science*. 2020;370(6522):1339-1343.
7. Shrock E, Fujimura E, Kula T, et al. Viral epitope profiling of COVID-19 patients reveals cross-reactivity and correlates of severity. *Science*. 2020;370(6520):eabd4250.
8. Loos C, Atyeo C, Fischinger S, et al. Evolution of early SARS-CoV-2 and cross-coronavirus immunity. *mSphere*. 2020;5(5):163.
9. Sagar M, Reifler K, Rossi M, et al. Recent endemic coronavirus infection is associated with less-severe COVID-19. *J Clin Invest*. 2021;131(1):270.
10. Underlying Medical Conditions Associated with High Risk for Severe COVID-19: Information for Healthcare Providers. https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-care/underlyingconditions.html. Accessed March 31, 2021.
11. Hoste ACR, Venteo A, Fresco-Taboada A, et al. Two serological approaches for detection of antibodies to SARS-CoV-2 in different scenarios: a screening tool and a point-of-care test. *Diagn Microbiol Infect Dis*. 2020;98(4):115167.
12. Poston D, Weisblum Y, Wise H, et al. Absence of severe acute respiratory syndrome coronavirus 2 neutralizing activity in prepandemic sera from individuals with recent seasonal coronavirus infection. *Clin Infect Dis*. 2020;24:490.

How to cite this article: Leach S, Harandi AM, Bergström T, et al. Comparable endemic coronavirus nucleoprotein-specific antibodies in mild and severe Covid-19 patients. *J Med Virol*. 2021;1-4. https://doi.org/10.1002/jmv.27038