Longitudinal SARS-CoV-2 seroconversion and functional heterogeneity in a pediatric dialysis unit

To the editor: Health care settings have a high risk of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread and high seroconversion rates.[1,2] Dialysis units are at high risk for SARS-CoV-2 exposure because of frequent close patient contact, a highly mobile patient population, and open bay formats that limit social distancing.[3]

Little is known regarding longitudinal serologies in SARS-CoV-2 and factors impacting neutralization.[2] We have previously reported on 3 weeks of SARS-CoV-2 seroconversion in health care workers and patients in a pediatric dialysis unit.[2] This study describes 10 additional weeks of SARS-CoV-2 antibody measurements and neutralization potential.

Serum IgM and IgG levels were measured weekly on an expanded cohort of participants for 10 additional weeks, for 13 weeks total (Supplementary Table S1). Five different SARS-CoV-2 enzyme-linked immunosorbent assays and surrogate virus neutralization assays were performed (Supplementary Methods).

Fourteen patients on hemodialysis and 34 health care workers participated in the study. By week 13, 12 of 34 health care workers (35%) and 5 of 14 patients (38%) had SARS-CoV-2 antibodies. During the first 6 weeks of the study, 4 patients and 10 health care workers seroconverted. Over the remaining 4 weeks, 1 patient and 2 health care workers seroconverted (Figure 1). Among those seroconverting in the second portion of the study, all were asymptomatic except 1 nurse who developed anosmia after positive IgG test. The majority of patients (80%) and health care workers (83%) maintained seroconversion at week 13. Only 3 of 12 participants (25%) with polymerase chain reaction (PCR) test were positive (Supplementary Figure S1).

Conceptually, antibodies that disrupt the receptor binding domain (RBD) of the spike peptide binding to angiotensin II converting enzyme (ACE2) will be neutralizing.[4] Monoclonal antibodies to the N-terminal domain (NTD) of spike are also associated with neutralizing properties.[4] Therefore, we quantified NTD, RBD, and spike-specific antibodies.[5] Additionally, we evaluated antibody status in SARS-CoV-2 PCR-positive community volunteers. One-half (50%) of our health care worker and patient cohort developed spike antibodies, and 15% developed NTD antibodies. Of the PCR-positive participants from the local community, 90% developed spike antibodies and 47% developed NTD antibodies (Supplementary Figure S1).

In our surrogate viral neutralization assay, we found a variable range of neutralization rates from 0% to over 90%. We correlated spike protein and NTD IgG optical density (OD) values to quantitative neutralization (Supplementary Figure S2A–F). In health care workers and the PCR-positive participants from the local community, a strong correlation between NTD and neutralization ($R^2 = 0.879$) existed. A weaker correlation between spike and neutralization ($R^2 = 0.410$) existed (Supplementary Figure S3). In our dialysis population, we did not find the same correlation between NTD and neutralization ($R^2 = 0.055$) (Supplementary Figure S2G and H). We confirmed these findings with microneutralization studies in a subset of this cohort (Supplementary Figure S4).

This study found rapid initial seroconversion followed by slower ongoing seroconversion in individuals interacting in a pediatric dialysis unit. To the best of our knowledge, this represents the first report of variable NTD antibody development after SARS-CoV-2 infection and correlation with neutralization of RBD-ACE2 binding. The lack of correlation in patients on dialysis between neutralization and serologies offers insight into SARS-CoV-2 susceptibility and immune dysfunction in patients on hemodialysis.[6,7]

Our antibody profiling and neutralization studies separate our cohort into distinct groups. In the PCR-positive participants from the local community and health care workers, we demonstrate a strong correlation between NTD and RBD-ACE2 neutralization. It is unclear why some individuals make spike antibodies to spike but not antibodies to the NTD. Based on the *in vitro* assays, spike antibodies alone do not prevent complexing of RBD to ACE2 receptors.[4] Why only certain individuals make high-quality RBD and NTD antibodies that correlate to neutralization needs investigation. Our finding of NTD conferring neutralization by preventing RBD binding to ACE2 is novel. We postulate that NTD serves as a surrogate marker for other mechanisms. Our pilot microneutralization studies confirmed this finding. Elucidating factors that lead to neutralizing antibodies will be paramount for vaccine development and community immunity.

Our patients on dialysis made NTD, RBD, and spike antibodies, but they did not neutralize. Patients on dialysis have adaptive immune deficiency, despite normal antibody levels.[8] We hypothesize that the uremic milieu of end-stage renal disease leads to dysfunction much like uremic platelet dysfunction (Supplementary Figure S5).[9] This report offers potential insight into the poor outcomes of patients on dialysis who have coronavirus disease 2019.[3]

A decreased rate of seroconversion over time occurred in our participants despite children, some with documented SARS-CoV-2, being grouped together during hemodialysis sessions. A statewide "shelter in place" order and mandatory mask requirement were implemented during the first 3 weeks of this study and may be responsible for this decreased seroconversion rate.[2] We recognize limitations including that the clinical consequences of serological neutralizing capability are unknown. Seroconversion was generally sustained over

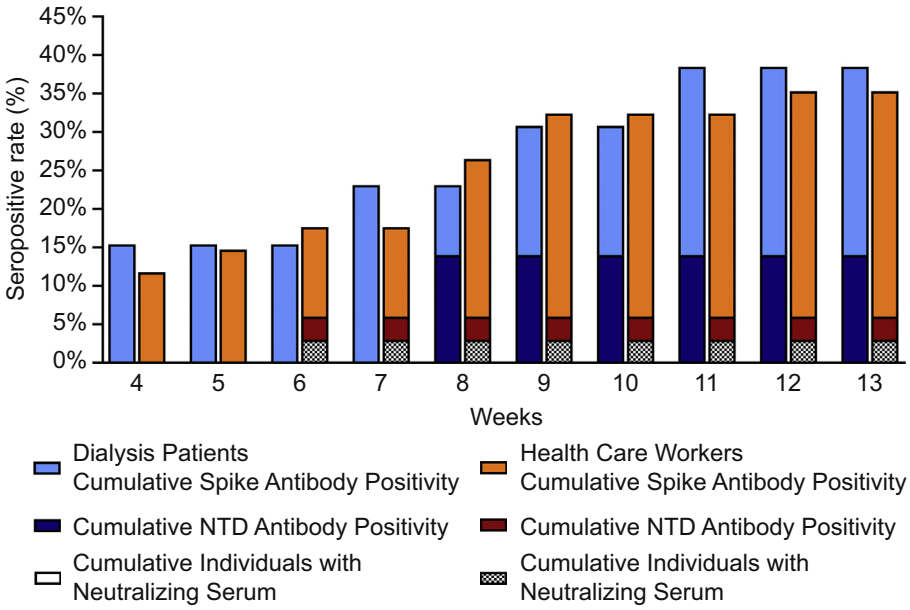

Figure 1 | Cumulative seroconversion (development of severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2] IgM or IgG antibodies) rates by week of study in patients receiving dialysis and health care workers. Individuals were considered seropositive based on the study week in which they were first found to be seropositive for IgM, IgG, or both. The percentage of seroconverted pediatric patients on dialysis ($n = 14$) and health care workers ($n = 34$) with cumulative N-terminal domain (NTD) antibody positivity and cumulative neutralizing antibodies are shown as a proportion of the total seropositive rate in each week of the study.

several months in contrast to past studies, indicating decline in seroconversion over time.[10] Additionally participants with no neutralization antibodies had mild symptoms or none at all. Asymptomatic seroconversion without neutralization capabilities might result from a lower viral load, preexisting resistance to disease conferred by T cells, or host-specific factors.[11]

DISCLOSURE

ALS has received grants from Eli Lilly Foundation and the National Institutes of Health. FK reported that a patent to The Icahn School of Medicine at Mount Sinai is in the process of licensing assays based on the assays described herein to commercial entities and that the school of medicine has filed for patent protection, pending and licensed. All other authors declared no competing interests.

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The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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AUTHOR CONTRIBUTIONS

ALS and DSH had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. MCS, ACW, ALS, and DSH conceived of and designed the study. JJC, MCS, JH, SA, ACW, AEC, FA, JF, AS, AC, ALS, and DSH acquired, analyzed, or interpreted (or a combination of these) the data. JJC, MCS, ACW, FA, and ALS drafted the manuscript. ACW, AEC, VS, FK, JF, AS, AC, JS, ALS, and DSH performed the critical revision for important intellectual content. JJC, MCS, FA, ALS, and DSH performed the statistical analysis. ALS obtained the funding. ACW, AEC, FK, ALS, and DSH provided administrative, technical, or material support (or a combination of these). AEC, ALS, and DSH supervised the study.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Figure S1. Study flow diagram. Flow diagram depicting symptoms, PCR testing results and laboratory testing for pediatric patients on dialysis ($n = 14$), community of SARS-CoV-2 PCR-positive control participants ($n = 17$), and health care workers ($n = 34$).

Figure S2. Neutralizing antibodies against SARS-CoV-2 in patients receiving dialysis, health care workers, and PCR-positive individuals from the local community. Scatter plot illustrating distribution of (**A**) spike IgG, (**B**) receptor-binding domain (RBD) IgG, and (**C**) N-terminal domain (NTD) IgG to neutralization percentage in the community of SARS-CoV-2 PCR-positive control participants (n = 17) and health care workers (n = 21). Graph illustrating the relationship of the optical density (OD) of (**D**) spike IgG, (**E**) RBD IgG, and (**F**) NTD IgG to percentage of neutralization in the community of SARS-CoV-2 PCR-positive control participants ($n = 17$) and health care workers ($n = 21$). NTD IgG was associated with higher neutralization effect in the PCR-positive

control participants from the local community and health care workers ($R^2 = 0.879$) than was spike IgG antibody ($R^2 = 0.410$). Open elements (diamonds, triangles, or circles) represent high titer samples, gray-shaded elements represent positive but not high titer, and filled black elements represent negative samples to correspond to **A** to **C**. Threshold for IgG positivity denoted with horizontal dashed line. Linear regression correlations were plotted with 95% confidence intervals (dotted curves). (**G**) Scatter plot illustrating distribution of spike IgG (diamonds), RBD IgG (triangles), and NTD IgG (circles) in pediatric patients on dialysis ($n = 10$). Open elements represent positive samples and closed represent negative samples. (**H**) Distribution of neutralization for spike IgG-, RBD IgG-, and NTD IgG-positive samples.

Figure S3. Neutralizing antibodies against SARS-CoV-2 in health care workers and PCR-positive individuals from the local community by IgG antibody status. Relationship of spike IgG, receptor-binding domain (RBD) IgG, and spike peptide N-terminal domain (NTD) IgG to percentage of neutralization. NTD IgG was associated with higher neutralizing effect in the community of PCR-positive control participants and health care workers with high OD ($R^2 = 0.888$) and spike IgG antibody ($R^2 = 0.834$). No association was seen in those with low OD. Threshold for IgG positivity denoted with horizontal dashed line. Linear regression correlations were plotted with 95% confidence intervals (dotted curves). Open elements (diamonds, triangles, or circles) represent positive samples and closed represent negative samples.

Figure S4. Microneutralization correlation to SARS-CoV-2 antibody subsets. The community of SARS-CoV-2 PCR-positive control participants ($n = 17$) illustrating the relationship among levels of spike IgG, receptor-binding domain (RBD) IgG, and spike peptide N-terminal domain (NTD) IgG to one-half maximal inhibitory concentration (IC50) against live SARS-CoV-2.

Figure S5. Surrogate viral neutralization assay in different clinical cohorts. SARS-CoV-2 binds to the ACE2 receptor for host cell internalization via the receptor-binding domain (RBD) of the spike protein. Signal is produced by the absence of antibodies in the subject's serum enabling the binding of RBD to the ACE2 receptor. Signal is absent when serum contains neutralization antibodies reflecting inhibition of binding. When serum contains robust spike IgG antibodies and no neutralization antibodies, an increase in signal is demonstrated potentially from spike IgG to RBD complexes binding to ACE2. During uremia, we do not see the effect of neutralization antibodies on RBD-ACE2 binding.

Table S1. Characteristics and cumulative SARS-CoV-2 seroconversion for patients receiving dialysis and health care workers.

1. Kliger AS, Silberzweig J. Mitigating risk of COVID-19 in dialysis facilities. *Clin J Am Soc Nephrol*. 2020;15:707–709.
2. Hains DS, Schwaderer AL, Carroll AE, et al. Asymptomatic seroconversion of immunoglobulins to SARS-CoV-2 in a pediatric dialysis unit. *JAMA*. 2020;323:2424–2425.
3. Valeri AM, Robbins-Juarez SY, Stevens JS, et al. Presentation and outcomes of patients with ESKD and COVID-19. *J Am Soc Nephrol*. 2020;31:1409–1415.
4. Tan CW, Chia WN, Qin X, et al. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2–spike protein–protein interaction. *Nat Biotechnol*. 2020;38:1073–1078.
5. Chi X, Yan R, Zhang J, et al. A neutralizing human antibody binds to the N-terminal domain of the Spike protein of SARS-CoV-2. *Science*. 2020;369:650–655.
6. Kunori T, Fehrman I, Ringdén O, Möller E. In vitro characterization of immunological responsiveness of uremic patients. *Nephron*. 1980;26:234–239.
7. Tsakolos ND, Theoharides TC, Hendler ED, et al. Immune defects in chronic renal impairment: evidence for defective regulation of lymphocyte response by macrophages from patients with chronic renal impairment on haemodialysis. *Clin Exp Immunol*. 1986;63:218–227.
8. Okasha K, Saxena A, el Bedowey MM, Shoker AS. Immunoglobulin G subclasses and susceptibility to allosensitization in humans. *Clin Nephrol*. 1997;48:165–172.
9. Kozek-Langenecker SA, Masaki T, Mohammad H, et al. Fibrinogen fragments and platelet dysfunction in uremia. *Kidney Int*. 1999;56:299–305.
10. Ibarrondo FJ, Fulcher JA, Goodman-Meza D, et al. Rapid decay of anti–SARS-CoV-2 antibodies in persons with mild Covid-19. *N Engl J Med*. 2020;383:1085–1087.
11. 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.

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Decline and loss of anti–SARS-CoV-2 antibodies in kidney transplant recipients in the 6 months following SARS-CoV-2 infection

To the editor: The dynamics of immune response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in kidney transplant recipients (KTRs) remains largely unknown. KTRs have been reported to develop serological responses to SARS-CoV-2.[1,2] However, information about the duration and significance of antibody response in this immunocompromised population is still critically lacking. We herein report anti–SARS-CoV-2 IgG trajectory in a cohort of KTRs followed at Necker Hospital (Paris, France) between 2 and 6 months after symptomatic coronavirus disease 2019 (COVID-19) infection.

Forty-two patients (22 men [52.4%]; median age of 57.7 years; interquartile range [IQR]: 47.2–67.0), who developed COVID-19 infection between March 14 and May 2, 2020, were included. COVID-19 was defined by typical clinical symptoms associated to a positive SARS-CoV-2 polymerase chain reaction test on nasopharyngeal swab. Sera were tested for the presence

Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.