Impact of Seasonal Coronavirus Antibodies on SARS-CoV-2 Vaccine Responses in Solid Organ Transplant Recipients

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Running Title: Seasonal Coronavirus Antibodies in SOTR

Abstract:

Antibody responses to SARS-CoV-2 vaccination are reduced in solid organ transplant recipients (SOTRs). We report that increased levels of pre-existing antibodies to seasonal coronaviruses are associated with decreased antibody response to SARS-CoV-2 vaccination in SOTRs, supporting that antigenic imprinting modulates vaccine responses in this immunosuppressed population.

Keywords: SARS-CoV-2, COVID-19, Vaccines, Immunocompromised Hosts

Introduction:

Solid organ transplant recipients (SOTRs) have decreased antibody responses to SARS-CoV-2 vaccines and are at increased risk for severe COVID-19 as compared to the general population [1–3]. Although age, use of antimetabolite immunosuppression, time since transplantation, and transplanted organ type are known to affect vaccine response in SOTRs, their impact is not uniform, and precise mechanisms governing sero-response remain unknown [1,3–5]. Seasonal coronaviruses (sCoVs) are ubiquitous causes of the common cold and the vast majority of the adult population has been infected (and often reinfected) throughout life [6]. SARS-CoV-2, the etiological agent of COVID-19, is a betacoronavirus, as are two of the sCoVs, OC43 and HKU1, which share some sequence homology. The other two sCoVs, 229E and NL63, are alphacoronaviruses and more distantly related (though NL63 also utilizes ACE2 for cell entry) [6]. Pre-existing antibodies to these sCoVs can impair the antibody response to SARS-CoV-2 infection in the general population, but a significant impact on vaccine response has not been observed [7,8]. This phenomenon, known as antigenic imprinting, takes place when the original adaptive immune response to an antigen influences the subsequent response to closely related antigens. Antigenic imprinting has been studied in influenza, but is only recently being appreciated in the context of SARS-CoV-2 [9]. Whether pre-existing antibodies to sCoVs independently impact the response to SARS-CoV-2 vaccination in immunosuppressed SOTRs has not been studied. To investigate this, we measured SARS-CoV-2 spike-specific antibodies pre- and post-two-dose mRNA vaccine in an observational cohort of SOTRs and tested the association of baseline sCoVs antibodies with humoral vaccine response.

Materials and Methods:

Study Participants

Participants were recruited and consented virtually to enroll in a national prospective, observational cohort approved by the Johns Hopkins IRB (00248540), as previously described [1,3]. Participants were included if they did not report a diagnosis of COVID-19 prior to vaccination and their pre-vaccine SARS-CoV-2 anti-spike and anti-RBD antibodies were below the manufacturer's cutoffs for positivity.

Antibody Detection

Plasma antibodies that bound the spike (S) proteins from 229E, NL63, OC43, HKU1, SARS-CoV-1, and SARS-CoV-2, as well the respiratory syncytial virus (RSV) pre-fusion F protein, and SARS-CoV-2 nucleocapsid (N) were measured using the Meso Scale Diagnostics (MSD, Rockville, MD) Respiratory Panel 3 IgG kit according to the manufacturer's protocol.

Statistical Analysis

Two-sided Wilcoxon signed-rank tests were applied to test the difference in antibody levels pre- and post-vaccine. Spearman correlation was used to measure the correlation between pre-existing sCoVs antibodies and SARS-CoV-2 antibody response (i.e., difference between pre- and post-vaccine SARS-CoV-2 spike antibody levels as well as absolute levels). Association between clinical factors and SARS-CoV-2 vaccine response were assessed via univariable linear regression. Multivariable linear regression was applied to assess the independent association of pre-vaccine sCoV antibody levels individually with (i) change in SARS-CoV-2 anti-spike antibody and (ii) absolute level of SARS-CoV-2 anti-spike antibody after vaccination, with adjustment for potential confounders (age at vaccination, months since transplant, use of antimetabolite immunosuppression, and receipt of liver transplant) chosen via an a priori explanatory model for vaccine response in SOTRs [1,4]. Results were considered significant for $p < 0.05$.

Additional methods details can be found in the supplemental appendix.

Results:

Demographic and clinical data were available for fifty-one participants, (**Supplemental Table 1)**. The median (IQR) age was 59 (40 – 67). The cohort was majority white (94%), female (51%), and kidney recipients (71%). Most participants were vaccinated more than three years post-transplant with two-dose mRNA-based vaccines.

Antibodies that bind SARS-CoV-2 S significantly increased after vaccination (fold change (fc) = 75.3, absolute post-vaccine median and IQR ($_{Q1}Med_{Q3}$) = $_{666}6362_{54671}$ AU/mL). As specified, none of the participants reported a COVID-19 diagnosis or had pre-vaccine anti-SARS-CoV-2 S antibody titers above the threshold for positivity. Thirty-one (61%) participants developed a positive anti-S antibody response after vaccination, consistent with previous reports after two doses of vaccine in this population [1,3]. Antibodies against seasonal betacoronaviruses, HKU1 and OC43, also increased significantly, albeit to a lesser degree (1.1, $_{7157}13135_{270}$ AU/mL and 1.2, $_{20455}30117_{247181}$ AU/mL). In contrast, antibodies against alphacoronaviruses (229E and NL63 (1.0, $_{10350}17796_{26323}$ AU/mL and 1.0, $_{1682}3403_{5085}$ AU/mL)), RSV pre-fusion F, and SARS-CoV-2 N did not increase (**Figure 1a and Supplemental Figure 1a**). Although pre-vaccine anti-SARS-CoV-2 antibody levels were qualitatively negative in all participants, low-level signals were detected suggesting, as previously reported, probable cross-reactivity of pre-existing sCoV antibodies with SARS-CoV-2 spike on this sensitive assay [10]. We noted pre-vaccine antibodies against OC43 and NL63 were significantly correlated with pre-vaccine anti-SARS-CoV-2 S antibodies (**Supplemental Figure 1b**). Moreover, we observed similar levels of cross-reactivity with SARS-CoV-1 antibodies and sCoV antibodies prior to vaccination, demonstrating that the assay is able to detect low-level antibody to related coronaviruses (**Supplemental Figure 1c**). We examined the relationship between pre-vaccine sCoV antibodies and both the change in SARS-CoV-2 anti-spike antibodies as well as the absolute value after vaccination. Pre-vaccine sCoV antibodies negatively correlated with changes in SARS-CoV-2 S antibodies post-vaccine, though this did not reach statistical

significance for NL63 (**Figure 1b**). Notably, only pre-vaccine levels of the beta sCoVs HKU1 and OC43 were significantly negatively correlated with the absolute level of SARS-CoV-2 spike antibodies post-vaccine (**Supplemental Figure 2**). In contrast, there was no observed relationship between pre-vaccine antibodies against RSV and anti-SARS-CoV-2 spike antibodies after vaccination.

We explored univariable associations of clinical and transplant factors with SARS-CoV-2 vaccine response based on published literature [1,3,4]. Receipt of liver transplant and time from transplant were significantly associated with a greater response, while antimetabolite immunosuppression (mycophenolate or azathioprine) was significantly associated with a decreased response (**Supplemental Figure 3**). There was no significant association between age at vaccination and response, but there was a significant negative correlation between older age at transplant and response (**Supplemental Figures 3 and 4**). No significant association was observed between sex or vaccine manufacturer and response (**Supplemental Figure 4**). When using multivariable linear regression to adjust for factors known to associate with vaccine response in SOTRs, pre-vaccine levels of antibodies against all sCoV (each individually adjusted for potential confounders) remained negatively correlated with change in anti-SARS-CoV-2 spike antibodies, yet only OC43 and 229E were statistically significant (**Figure 1c; Supplemental Table 2**). When using absolute anti-SARS-CoV-2 spike antibody level as the dependent variable, a similar negative correlation was observed, but did not reach statistical significance (OC43 β = -0.9, p=0.052) (**Supplemental Figure 5; Supplemental Table 3**).

Discussion:

We found a negative correlation between pre-existing anti-sCoV (HKU1, OC43, and 229E) antibodies and change in anti-SARS-CoV-2 S antibodies post vaccination among SOTRs. This association persisted after controlling for key factors, with antibodies against OC43, which shares an immunogenic epitope with SARS-CoV-2 near the S2 cleavage site [11].

This independent negative association of preexisting sCoV antibodies was equivalent to that of antimetabolite immunosuppression, widely recognized as a major deleterious factor influencing SARS-CoV-2 vaccine response [12].

Significant back-boosting of antibodies against betacoronaviruses OC43 and HKU1 after SARS-CoV-2 vaccination was observed. These findings suggest that antigenic imprinting may be influencing the anti-S antibody response to vaccination in this immunosuppressed population. This previously unexplored factor may explain some of the marked variability in SARS-CoV-2 antibody responses among SOTRs [2,5,13]. In contrast to immunocompetent persons who develop high-level antibody responses to SARS-CoV-2 vaccines [3], the weaker antibody responses in SOTRs might be more impacted by immune memory specific for sCoVs, which was established during pre-transplant infection(s) when immune responses were generated in the absence of immunosuppressive medications. Similar observations with cytomegalovirus (CMV) have been reported, where SOTRs who are CMV seronegative prior to transplant and receive an organ from a CMV positive donor (D+/R-), are at greater risk for CMV disease post-transplant than recipients who are CMV seropositive prior to transplant (D+/R+)[14,15].

This investigation was limited by a relatively small observational convenience sample that included a heterogenous mix of graft types and immunosuppressive agents. Lung transplant recipients were notably absent from our cohort. Given the focus on serological changes, we cannot comment on cellular responses in this cohort and caution against drawing definitive conclusions on vaccine induced protection against COVID-19 based on these data alone. Neutralizing activity of anti-spike antibody was not tested, though this correlates well with anti-spike IgG [3]. It is possible that some participants had subclinical or otherwise undiagnosed SARS-CoV-2 infection prior to study entry, which could affect antibody response to vaccination. That said, in this population with extensive health care experience, we used a combination of participant self-report as well as screening by both anti-spike and anti-RBD antibody to reduce

this potential bias. Despite these limitations, this is the first study to investigate the impact of preexisting antibodies against sCoV on SARS-CoV-2 humoral response in SOTRs, a group with known poor vaccine responses and at high risk for severe COVID-19.

These findings support the rationale for maximizing vaccination efforts before undergoing intense immunosuppression that may impair downstream response to SARS-CoV-2-specific antigens. Furthermore, mechanisms of antigenic imprinting suggested by this work raise concern regarding the potential for impaired responses to variant-specific booster vaccination among transplant recipients and should be a focus of future study.

NOTES

Acknowledgments:

We thank all participants who enrolled in this study and donated plasma.

Funding:

This work was supported by the Ben-Dov family; the National Cancer Institute [U54CA260491 to A.L.C. and S.L.K.]; the National Institute of Diabetes and Digestive and Kidney Diseases [T32DK007713 to J.L.A]; and the National Institute of Allergy and Infectious Diseases [K24AI144954 to D.L.S., K08AI156021 to A.H.K., K23AI157893 to W.A.W., U01AI138897 to D.L.S., and R01AI120938S1 to A.A.R.T.]. A.L.C, J.R.B, J.N.B, J.M.G.W, A.H.K, S.L.K, A.P, D.L.S, A.A.R.T, and W.A.W reports support from NIH (grants to institution).

Potential conflicts of interest:

D.L.S. has the following financial disclosures: consulting and speaking honoraria from Sanofi, Novartis, CSL Behring, Jazz Pharmaceuticals, Veloxis, Mallinckrodt, Regeneron, and AstraZeneca, Thermo Fisher Scientific. D.L.S. reports consulting fees from Sanofi, Novartis,

CSL Behring, Jazz Pharmaceuticals, Veloxis, Mallinckrodt, Thermo Fisher Scientific, Regeneron, and AstraZeneca, and speaking honoraria from Sanofi and Novartis (paid to author). A.L.C has received consulting fees from Janssen. A.H.K. has received consulting fees from Roche. None of the other authors have any relevant competing interests.

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Figure Legend:

Changes in anti-spike specific antibody levels before and after SARS-CoV-2 vaccination.

A. Pre-(grey) and post-(orange) vaccine antibodies against the indicated antigens measured in arbitrary units (AU/mL) after log-transformation. Differences were analyzed using a two-sided Wilcoxon signed-rank test. NS indicates $p > 0.05$, *** indicates $p \leq 0.001$. Dashed red line indicates manufacturer cutoff for SARS-CoV-2 S positivity.

B. Scatterplots of $\log_{10}$ change (AU/mL) in anti-SARS-CoV-2 spike antibodies on the y-axis and pre-vaccine antibodies against indicated antigens on the x-axis. The blue line is the least square regression line. Spearman's correlation coefficients were calculated and are displayed for each antigen at the top of each panel along with the corresponding p-value.

C. Dot and whisker plots of the coefficients (beta values) and 95% confidence intervals for the clinical factors and the pre-vaccine antibodies against indicated sCoV antigens in each of the multivariable linear regression models where change in anti-SARS-CoV-2 spike antibodies after vaccination is the dependent variable. A solid dot indicates $p \leq 0.05$ and a circle indicates $p > 0.05$. A sensitivity analysis substituting age at vaccination for age at transplant did not affect the sCoV coefficients (**Supplemental Tables 2 and 3**).

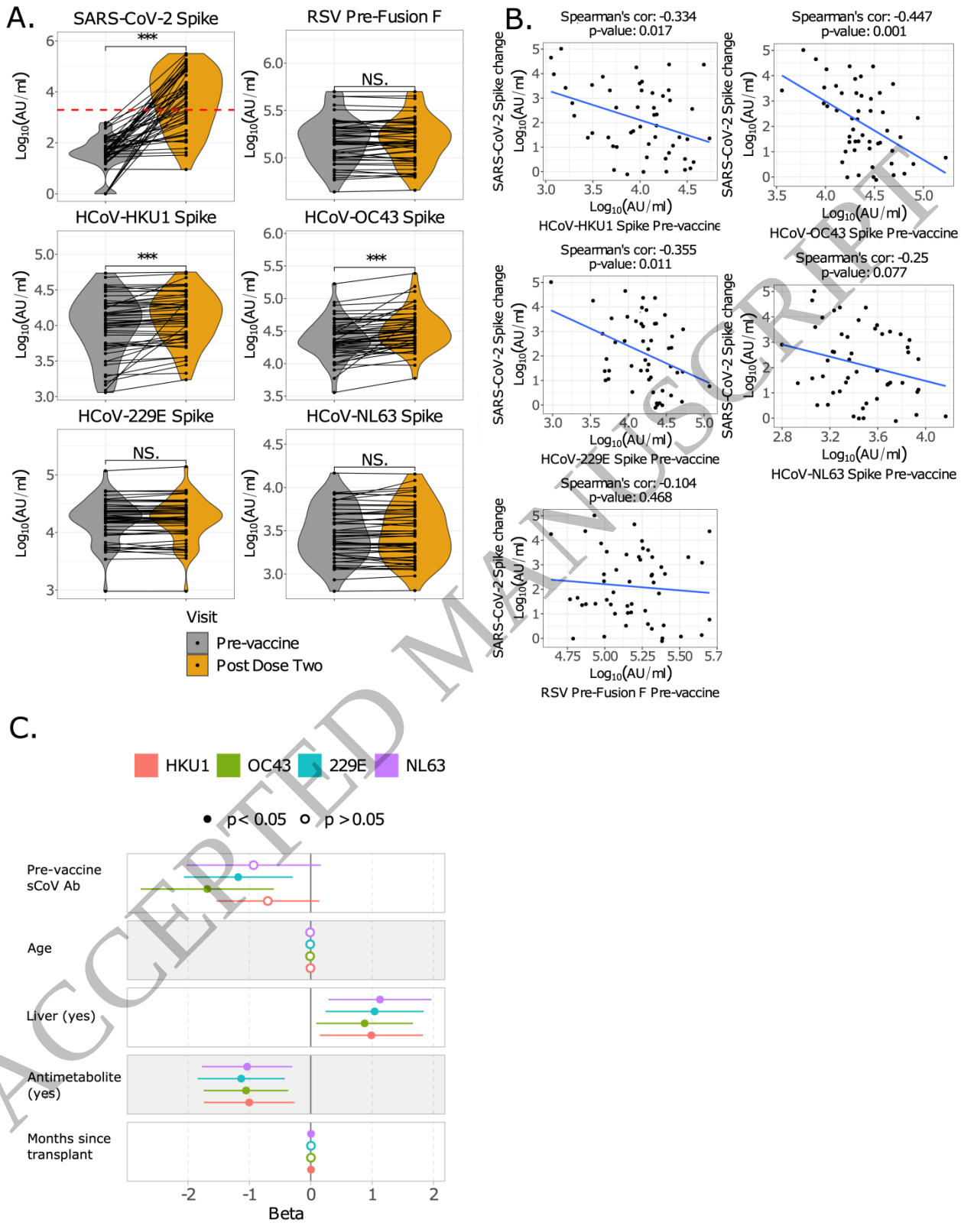

Figure 1
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