Vaccination after SARS-CoV-2 infection increased antibody avidity against the Omicron variant compared to vaccination alone

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Running title: SARS-CoV-2 Omicron neutralization

Keywords: SARS-CoV-2; COVID-19; Antibody response; Vaccine

Funding statement

Funding for this work was through internal institutional funds to T.B. from Children's Mercy Research Institute and Children's Mercy Kansas City. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of interest statement

The authors have declared no competing interests.

Abstract

The SARS-CoV-2 Omicron variant has caused infections among individuals vaccinated or with prior COVID-19, suggesting immune escape. Here, we showed a decrease in binding and surrogate neutralizing antibody responses to the Omicron variant after two doses of the Pfizer COVID-19 mRNA vaccine. Individuals recovered from infection before vaccination had higher antibody levels and avidity to the Omicron variant compared to individuals vaccinated without infection. This suggested that COVID-19 infection before vaccination elicited a higher magnitude and affinity antibody response to the Omicron variant, and repeated exposure through infection or vaccine may be required to improve immunity to emerging SARS-CoV-2 variants.

Background (Main 1664 words)

During the course of the COVID-19 pandemic, several variants of the original 2019 strain of SARS-CoV-2 have emerged. Two recent variants of concern (VOC), Delta and Omicron have quickly become dominant in the most wide-spread waves of infection globally [1]. The amino acid alterations in the Alpha, Beta, Gamma and Delta VOCs were associated with increased viral transmissibility and only modest escape from vaccine-induced immunity or therapeutic monoclonal antibodies [2]. The most recent Omicron VOC (classified by WHO on November 26th 2021) has 26-32 mutations in the spike protein, including 15 mutations in the receptor-binding domain (RBD) [3]. These regions are often targeted by neutralizing antibodies elicited by both vaccination and SARS-CoV-2 infection, and observations that the Omicron variant is more likely to cause reinfection suggests the possibility of immune escape [4-7]. Thus, there is an urgent need to determine whether the mutations in the Omicron VOC impact humoral immunity in vaccinated individuals. Previously, we have shown that individuals with prior SARS-CoV-2 infection have increased magnitude and longevity of antibody responses after vaccination compared with infection-naïve vaccinated individuals [8-10]. Here, we determined the levels of antibody activity and avidity to the original 2019 strain, and the Omicron variant of SARS-CoV-2, after two doses of the BNT162b2 Pfizer COVID-19 mRNA vaccine. We compared individuals with no history of prior SARS-CoV-2 infection and those who had recently recovered from infection before vaccination.

Methods

Plasma samples collected four weeks after the second dose of the Pfizer BNT162b2 COVID-19 mRNA vaccine from individuals who had SARS-CoV-2 infection 30-60 days before initiating vaccination (Convalescent-Vaccinated, n=43) and individuals who did not have previous SARS-CoV-2 infection (Vaccinated, n=128) were compared (**Table S1**). Samples were obtained in January-March 2021, prior to the emergence of the Omicron variant, and thus, natural SARS-CoV-2 infection was not with the Omicron variant. Informed consent was obtained from all participants and the use of

biospecimens was reviewed and approved by the Children's Mercy Institutional Review Board (STUDY00001670). We utilized a surrogate neutralizing antibody assay that allows indirect detection of potential SARS-CoV-2 neutralizing antibodies in the blood through determination of plasma antibody blocking of the binding of the SARS-CoV-2 RBD to the human host receptor angiotensin converting enzyme 2 (ACE2) (SARS-CoV-2 Surrogate Virus Neutralizing Test Kit, Genscript; **Supplemental Appendix**). This assay has been demonstrated to correlate significantly with SARS-CoV-2 primary or pseudovirus neutralization assays, but has a shorter assay time and is more accessible to labs due to reduced biosafety concerns [11, 12]. We used the RBDs derived from both the wild-type strain (2019-nCoV) and the Omicron variant of SARS-CoV-2. A single 1:10 dilution of plasma was utilized for this assay. A threshold of 30% blocking compared to control wells, with no plasma antibodies, indicated positive detection of surrogate neutralizing antibodies as recommended by the manufacturer.

Total IgG antibody binding was determined using enzyme-linked immunosorbent assay (ELISA), where microtiter plates were coated with recombinant RBD proteins (Genscript) from the 2019-nCoV strain or the Omicron variant. Plasma was serially diluted (1:3) seven times beginning at a 1:30 dilution. Anti-human IgG secondary antibody was used, and absorbance was measured at optical density 450nm.

To measure antibody avidity, the plasma dilution that resulted in binding optical densities of around 2.5 was used, which corresponded to a 1:21870 dilution. Plates were coated with recombinant RBD proteins (Genscript) from the 2019-nCoV strain or the Omicron variant. Plasma dilutions were incubated for 1 hour. The plates were then washed either with phosphate buffered saline (PBS) or 8M urea in PBS for 5 minutes before being washed with PBS with 0.1% Tween 20. Wells were then incubated with Goat anti-human IgG secondary antibody. SureBlue Reserve Microwell Substrate (95059-294, VWR, Radnor, PA, USA) was added and incubated in the dark for 15 minutes. Absorbance was measured at 450 nm immediately after 0.33 N HCl Acid Stop solution was added to the plate. The

avidity index was calculated for each sample as the optical density ratio of the urea-washed to PBS-washed wells (**Supplemental Appendix**).

Descriptive statistics and group differences were determined at each timepoint using a nonparametric paired Wilcoxon-Mann-Whitney test that was corrected for multiple comparisons (False Discovery Rate (FDR)). GraphPad prism (v9) was used to generate graphs and perform statistical tests.

Results

2019-nCoV RBD-ACE2 blocking antibodies were detected in all individuals after two doses of the BNT162b2 COVID-19 mRNA vaccine in both the vaccinated (128/128 positive) and convalescent-vaccinated groups (43/43 positive; **Figure 1A**). There was a significant decrease in blocking antibody levels against the SARS-CoV-2 Omicron variant RBD in both individuals without prior SARS-CoV-2 infection (7/128 positive) and in individuals recovered from SARS-CoV-2 infection prior to the two-dose vaccine regimen (32/43 positive; **Figure 1A**). Individuals who were convalescent before vaccination had significantly higher levels of RBD-ACE2 blocking antibodies against the Omicron variant (median: 40.36%; 32.88-59.36%, 95% CI) compared to vaccinated individuals with no history of SARS-CoV-2 infection (median: 0.51%; 0.000-3.997%, 95% CI). This occurred despite the impossibility of infection with the Omicron variant due to the time of sampling.

We detected binding of immunoglobulin G (IgG) antibodies to both the 2019-nCoV and the Omicron RBDs in a subset of convalescent-vaccinated (n=32) and vaccinated individuals (n=32; **Figure 1B**). There was a significant decrease ($P \leq 0.0001$; Wilcoxon-Mann-Whitney), although still detectable, in the levels of binding antibodies to the Omicron RBD compared to the 2019-nCoV RBD for both vaccine groups. In the vaccinated group, the median area under the curve (AUC) was 10.05 for the 2019-nCoV RBD and 8.46 for the Omicron RBD. In the convalescent-vaccinated group the median AUC was 10.77 for the 2019-nCoV RBD compared to 9.06 for the Omicron RBD (**Figure 1B**). However, there was no significant difference in binding to either the 2019-nCoV or the Omicron RBDs between convalescent-vaccinated and vaccinated individuals (**Figure 1B**). Thus, after two doses of the

BNT162b2 COVID-19 vaccine there was a significant reduction in surrogate neutralizing antibodies and overall binding antibodies against the Omicron RBD. Individuals with prior SARS-CoV-2 infection had significantly higher levels of surrogate neutralizing antibodies against the Omicron variant compared to those without SARS-CoV-2 infection, but no differences in the overall levels of binding antibodies were observed between the two vaccine groups. This suggested that differences existed in the quality of the neutralizing antibody response in individuals with prior SARS-CoV-2 infection before vaccination.

Antibody avidity is a measure of the overall strength of the antibody-antigen interaction and is often a reflection of antibody maturation and affinity. We measured the avidity of the plasma anti-RBD IgG against both the 2019-nCoV strain and the Omicron variant (**Figure 2A**). Convalescent-vaccinated individuals had significantly ($P \leq 0.0001$, Wilcoxon-Mann-Whitney) higher antibody avidity to the 2019-nCoV RBD compared to the vaccinated individuals (median 60.97 avidity index and median 27.84 avidity index, respectively). Both groups showed a significant ($P \leq 0.0001$, Wilcoxon-Mann-Whitney) decrease in avidity to the Omicron RBD, but convalescent-vaccinated individuals had significantly ($P \leq 0.0001$, Wilcoxon-Mann-Whitney) higher antibody avidity to the Omicron RBD than the vaccinated individuals (**Figure 2A**). Specifically, the median avidity index to the Omicron RBD for the convalescent-vaccinated group was 36.12 compared to 18.94 for the vaccinated group. Antibody avidity to the Omicron RBD significantly correlated ($r=0.62$, $P=0.0002$; Pearson) with the surrogate neutralizing antibody levels for the convalescent-vaccinated individuals but not for the vaccinated group (**Figure 2B**). However, antibody avidity for the 2019-nCoV RBD did correlate ($r=0.41$, $P=0.02$; Pearson) with surrogate neutralizing antibodies for the vaccinated group (**Figure 2B**). These data suggested that prior SARS-CoV-2 infection, coupled with vaccination, elicited increased antibody avidity to both the 2019-nCoV and the Omicron RBDs. Moreover, the increased avidity correlated with improved antibody surrogate neutralization measures.

Discussion

We found that individuals who had recovered from COVID-19 prior to SARS-CoV-2 vaccination with the Pfizer mRNA vaccine had increased levels of surrogate neutralizing antibodies and increased antibody avidity when compared to individuals with no prior SARS-CoV-2 infection before vaccination. The antibody levels were increased against both the vaccine-matched 2019-nCoV strain and the Omicron variant that emerged after sample collection. Moreover, we found that antibody avidity correlated with the levels of surrogate neutralizing antibodies. This suggested that individuals with prior SARS-CoV-2 infection before vaccination could have increased neutralizing antibodies with higher avidity against emerging variants of SARS-CoV-2.

Our results are consistent with recent reports showing significant humoral immune escape of the Omicron variant from both vaccinated and convalescent serum antibodies [5, 7, 13]. Moreover, another study has demonstrated escape of the Omicron variant from many of the neutralizing monoclonal antibodies that were being utilized for therapy [4]. These studies indicated that two doses of the BNT162b2 mRNA vaccine may not be sufficient to protect against infection with the Omicron variant, despite prior SARS-CoV-2 infection. Here, we found significantly higher antibody avidity to both the wild-type and Omicron RBDs for individuals with prior SARS-CoV-2 infection. This suggested that SARS-CoV-2 infection before vaccination elicited a different quality of antibody response that resulted in increased antibody immunity to SARS-CoV-2 variants. It remains unclear if natural SARS-CoV-2 infection was required for this increased avidity or if this could be achieved through repetitive vaccination. Two studies have recently demonstrated that a third vaccine dose increased antibody titers to the Omicron variant [14, 15]. Therefore, repeated vaccination may lead to a broader antibody response to SARS-CoV-2 that could protect from infection with future variants. It will be critical to determine whether additional vaccine doses without infection could also boost antibody avidity. This study stresses the urgent need to make vaccines available globally and to increase the uptake of vaccines by the public in order to prevent generation of future variants that can escape humoral immunity. Future

studies examining the antibody breadth and longevity against SARS-CoV-2 VOC will be required to determine if repetitive boosting is the optimal strategy to protect against future variants.

While neutralizing antibodies are a critical layer of defense for immunity against SARS-CoV-2, cross-reactive T cell and innate immune responses could have an impact on the severity of COVID-19, reducing the hospitalization and mortality from infection. Multiple arms of the immune system may need to be engaged in order to generate broad immunity to SARS-CoV-2 variants.

Acknowledgements

We thank all the health care workers that participated in this study. Special thanks to Occupational Health and Children's Mercy Research Institute for their support of this study. Daniel Louiselle, Nick Nolte, Rebecca Biswell, assisted with peripheral blood sample processing and storage. Elin Grundberg, Tomi. Pastinen, Bradley Beldon, Dithi Banerjee, Angela Myers, and Jennifer Schuster were involved in overseeing study design and clinical implementation with the mRNA vaccine. Funding for this work was through internal institutional funds to T.B. from Children's Mercy Research Institute and Children's Mercy Kansas City. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors have declared no competing interests.

Figure legends

Figure 1. Binding and surrogate neutralizing antibodies are reduced against SARS-CoV-2 Omicron variant. (A) Neutralization antibody proxy assay that determines the level of antibodies that block binding of the spike protein receptor-binding domain to the human host receptor angiotensin-converting enzyme 2 (ACE2), expressed as the percentage of binding that was blocked relative to a control with no plasma (representing maximum binding). The assay threshold for positivity was 30% indicating the presence of surrogate neutralizing antibodies. RBD from the original 2019-nCoV or RBD from the SARS-CoV-2 VOC Omicron were utilized. Plasma samples were obtained after 2-doses of the Pfizer COVID-19 mRNA vaccine from individuals with no history of infection before vaccination (Vaccinated, blue; n=128) or recent history of SARS-CoV-2 infection before vaccination (Convalescent-Vaccinated, red; n=43). Number of individuals above positive threshold displayed above graph. **(B)** IgG binding to the original 2019-nCoV or Omicron RBD measured by ELISA from plasma samples were obtained after 2-doses of the Pfizer COVID-19 mRNA vaccine from individuals with no history of infection before vaccination (Vaccinated, blue; n=32) or recent history of SARS-CoV-2 infection before vaccination (Convalescent-Vaccinated, red; n=32). Binding shown as area under the curve (AUC). P values determined using Wilcoxon matched-pairs signed rank test. Group median values displayed above graph.

Figure 2. Prior infection before vaccination increased antibody avidity to SARS-CoV-2 RBD. (A) IgG antibody avidity to the original 2019-nCoV or Omicron RBD measured by ELISA from plasma samples were obtained after 2-doses of the Pfizer COVID-19 mRNA vaccine from individuals with no history of infection before vaccination (Vaccinated, blue; n=32) or recent history of SARS-CoV-2 infection before vaccination (Convalescent-Vaccinated, red; n=32). Avidity index is the ratio of optical density in wells with Urea-PBS and PBS. P values determined using Wilcoxon matched-pairs signed rank test for paired analysis and Wilcoxon-Mann-Whitney for intergroup analysis. Group median values displayed above graph. **(B)** Graphs comparing antibody avidity to surrogate neutralizing antibody assay levels. Pearson correlations were performed with Pearson r and p values (two-tailed) displayed on graphs.

References

1. Organization WH. Tracking SARS-CoV-2 Variants. Available at: http://www.who.int/en/activities/tracking-SARS-CoV-2-variants/.

2. Planas D, Veyer D, Baidaliuk A, et al. Reduced sensitivity of SARS-CoV-2 variant Delta to antibody neutralization. Nature **2021**; 596:276-80.

3. Organization WH. Classification of Omicron (B.1.1.529): SARS-CoV-2 variant of concern. Available at: https://www.who.int/news/item/26-11-2021-classification-of-Omicron-(b.1.1.529)-sars-cov-2-variant-of-concern.

4. Cao Y, Wang J, Jian F, et al. Omicron escapes the majority of existing SARS-CoV-2 neutralizing antibodies. Nature **2021**.

5. Cheng SMS, Mok CKP, Leung YWY, et al. Neutralizing antibodies against the SARS-CoV-2 Omicron variant following homologous and heterologous CoronaVac or BNT162b2 vaccination. Nat Med **2022**.

6. Perez-Then E, Lucas C, Monteiro VS, et al. Neutralizing antibodies against the SARS-CoV-2 Delta and Omicron variants following heterologous CoronaVac plus BNT162b2 booster vaccination. Nat Med **2022**.

7. Rossler A, Riepler L, Bante D, von Laer D, Kimpel J. SARS-CoV-2 Omicron Variant Neutralization in Serum from Vaccinated and Convalescent Persons. N Engl J Med **2022**.

8. Bradley T, Grundberg E, Selvarangan R, et al. Antibody Responses after a Single Dose of SARS-CoV-2 mRNA Vaccine. N Engl J Med **2021**; 384:1959-61.

9. Fraley E, LeMaster C, Geanes E, et al. Humoral immune responses during SARS-CoV-2 mRNA vaccine administration in seropositive and seronegative individuals. BMC Med **2021**; 19:169.

10. Fraley E, LeMaster C, Khanal S, et al. Prior infection and age impacts antibody persistence after SARS-CoV-2 mRNA vaccine. Clin Infect Dis **2021**.

11. Chan KH, Leung KY, Zhang RR, et al. Performance of a Surrogate SARS-CoV-2-Neutralizing Antibody Assay in Natural Infection and Vaccination Samples. Diagnostics (Basel) **2021**; 11.

12. 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**; 38:1073-8.

13. Muik A, Lui BG, Wallisch AK, et al. Neutralization of SARS-CoV-2 Omicron by BNT162b2 mRNA vaccine-elicited human sera. Science **2022**:eabn7591.

14. Garcia-Beltran WF, St Denis KJ, Hoelzemer A, et al. mRNA-based COVID-19 vaccine boosters induce neutralizing immunity against SARS-CoV-2 Omicron variant. Cell **2022**.

15. Gruell H, Vanshylla K, Tober-Lau P, et al. mRNA booster immunization elicits potent neutralizing serum activity against the SARS-CoV-2 Omicron variant. Nat Med **2022**.

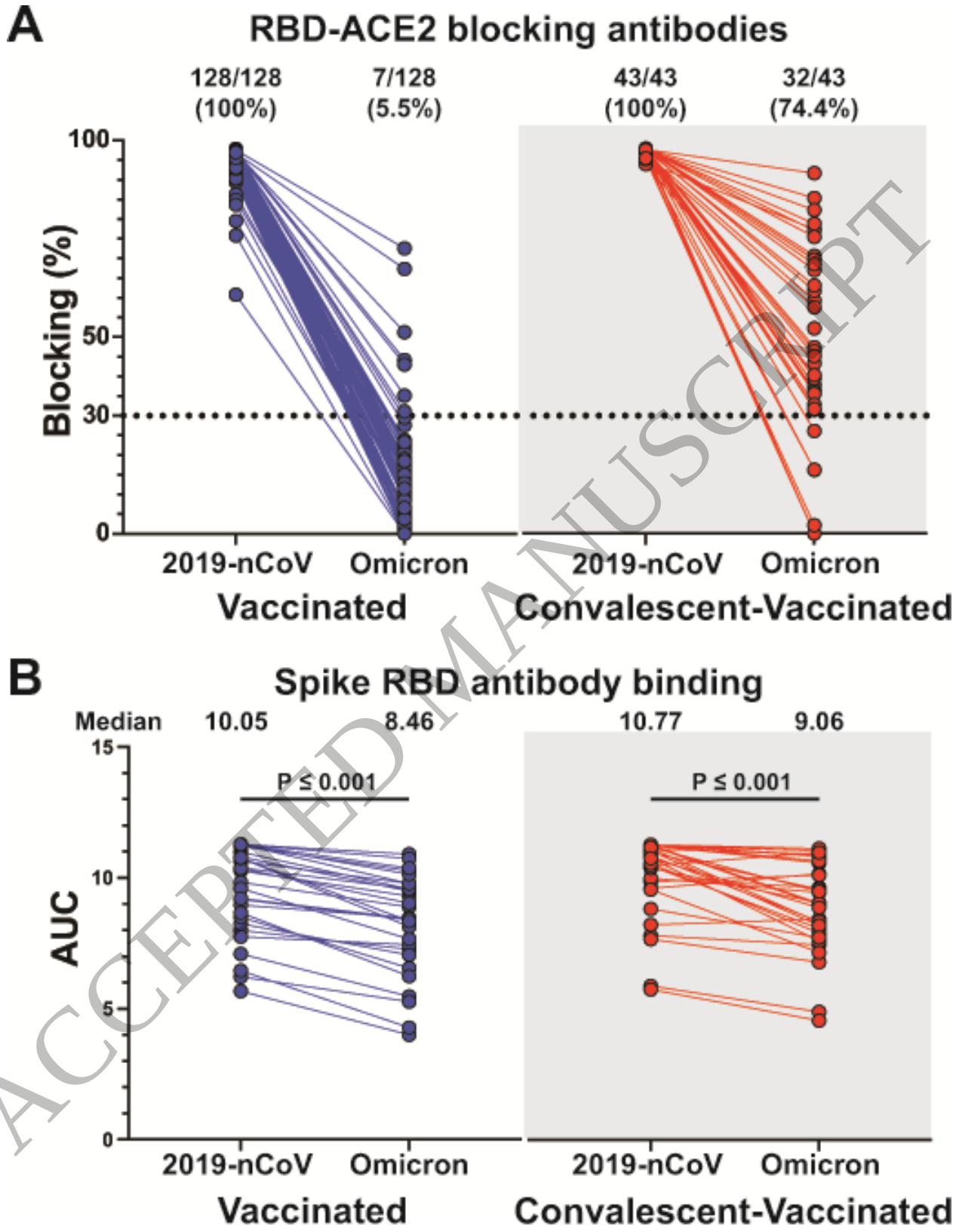

Figure 1
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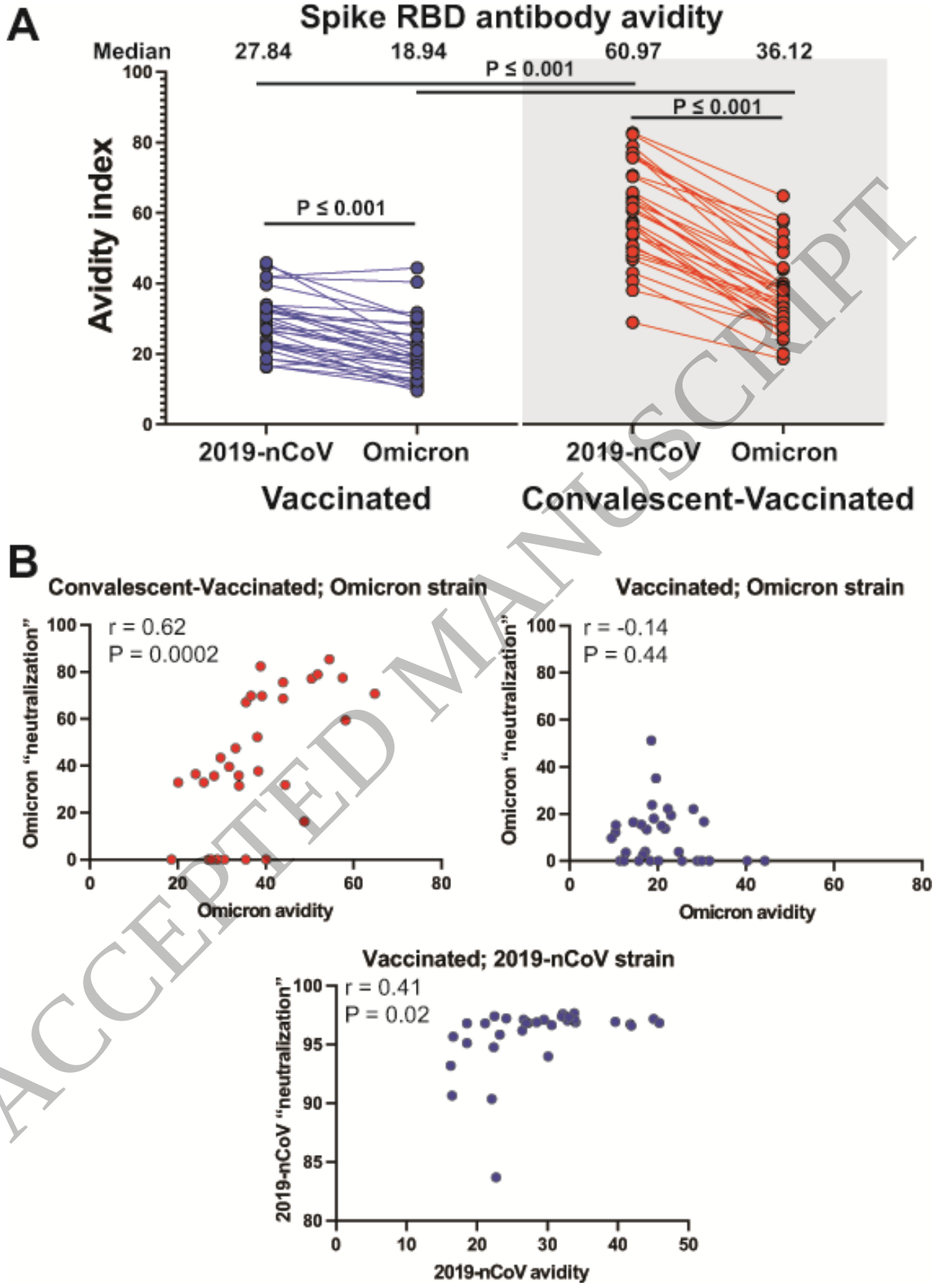

Figure 2
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