1   **Title:** A Third Dose of SARS-CoV-2 Vaccine Increases Neutralizing Antibodies Against

2   Variants of Concern in Solid Organ Transplant Recipients

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31 **Abbreviations:**

32 Anti-N: Anti-nucleocapsid antibody

33 Anti-RBD: Anti-Receptor Binding Domain antibody

34 Anti-S: Anti-Spike antibody

35 AU: Arbitrary Unit

36 AUC: Area Under the Curve

37 CFR: Case Fatality Rate

38 CM: Complete Media

39 ELISA: enzyme-linked immunosorbent assay

40 HC: Healthy Control

41 IM: Infection Media

42 MSD: Meso Scale Diagnostics

43 nAb: Neutralizing antibody

44 NT50: 50% Neutralization Titer

45 OD: Optical Density

46 SOTR: Solid Organ Transplant Recipient

47 TCID50: 50% Tissue Culture Infectious Dose

48 VOC: Variant of Concern

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50 **Word Count:** 2,898

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ABSTRACT:

Vaccine-induced SARS-CoV-2 antibody responses are attenuated in solid organ

transplant recipients (SOTRs) and breakthrough infections are more common.

Additional SARS-CoV-2 vaccine doses increase anti-spike IgG in some SOTRs, but it is

uncertain whether neutralization of variants of concern (VOCs) is enhanced. We tested

47 SOTRs for clinical and research anti-spike IgG, pseudoneutralization (ACE2

blocking), and live-virus neutralization (nAb) against VOCs before and after a third

SARS-CoV-2 vaccine dose (70% mRNA, 30% Ad26.COV2.S) with comparison to 15

healthy controls after two mRNA vaccine doses. We used correlation analysis to

compare anti-spike IgG assays and focused on thresholds associated with neutralizing

activity. A third SARS-CoV-2 vaccine dose increased median anti-spike (1.6-fold) and

receptor-binding domain (1.5-fold) IgG, as well as pseudoneutralization against VOCs

(2.5-fold versus Delta). However, IgG and neutralization activity were significantly lower

than healthy controls (p<0.001); 32% of SOTRs had zero detectable nAb against Delta

after third vaccination. Correlation with nAb was seen at anti-spike IgG >4 AU on the

clinical assay and $>10^4$ AU on the research assay. These findings highlight benefits of

a third vaccine dose for some SOTRs and the need for alternative strategies to improve

protection in a significant subset of this population.

3

1. INTRODUCTION:

77

78   Solid organ transplant recipients (SOTRs) are at increased risk for severe

79 COVID-19[1]. For example, while the case fatality rate (CFR) for the general population in

80 the United States is 1-2%[2], CFRs range from 10-30% in SOTRs, due to a combination

81 of chronic disease and immunosuppressive medications[3]. Therefore, effective and

82 optimized vaccines that prevent COVID-19 disease in this group are critical.

83 Unfortunately, these patients were excluded from the phase III trials of the mRNA based

84 COVID-19 vaccines,[4,5] and recent publications suggest that breakthrough disease is

85 more common among fully-vaccinated SOTRs than the general population[6,7].

86 Furthermore, it has been demonstrated that many SOTRs develop weak SARS-CoV-2

87 antibody responses after the recommended two doses of an mRNA-based vaccine[8–11].

88 This led to the hypothesis that a third dose of mRNA-based SARS-CoV-2 vaccine may

89 improve the immune response to SARS-CoV-2 and protection from COVID-19. Although

90 third doses have been authorized for immunocompromised persons in several

91 countries, including the United States (US), published data on neutralizing capacity of

92 SOTR plasma after additional vaccine doses are limited[12–14]. In particular, it is unknown

93 if augmented immune responses to a third vaccine dose would result in protection

94 against more transmissible variants of concern (VOCs) that exhibit immune escape,

95 including the Delta variant which currently comprises >99% of new cases in the US[15]. In

96 an effort to assess whether a third dose of SARS-CoV-2 vaccine in SOTRs would

97 improve the SARS-CoV-2 specific neutralizing response, we measured total SARS-

98 CoV-2 specific IgG and neutralizing activity against the vaccine strain and four VOCs

99 before and after a third dose of SARS-CoV-2 vaccine and compared this to IgG levels

100   and neutralizing capacity of healthy controls who received a standard two-dose mRNA-

101   based vaccine series.

102

103   ## 2. MATERIALS AND METHODS:

104   *2.1 Cohorts:*

105   SOTR participants were enrolled in a national prospective, observational cohort:

106   COVID-19 Antibody Testing of Recipients of Solid Organ Transplants and Patients with

107   Chronic Diseases, Johns Hopkins IRB00248540, as previously described[9,16]. Healthy

108   control participants were enrolled under Johns Hopkins IRB00027183[17]. SOTRs

109   submitted blood samples to the investigators 0-4 weeks before and 2 weeks after third

110   vaccine doses, which were independently obtained in the community. Blood was

111   collected in Acid Citrate Dextrose tubes and plasma was isolated by Ficoll centrifugation

112   and stored at -80°C.

113   *2.2 IgG Measurement:*

114   Plasma was tested using the clinically available EUROIMMUN anti-SARS-CoV-2

115   IgG enzyme-linked immunosorbent assay (ELISA) versus the S1 domain of spike

116   protein, performed per the manufacturers' protocols. Optical density (OD) of the sample

117   was divided by calibrator provided arbitrary unit (AU) ratio, for which ≥ 1.1 was

118   considered positive and ≥ 0.8–1.1 were considered indeterminate. Plasma was thawed

119   and anti-N, anti-RBD, and anti-S IgG was measured using the multiplex

120   chemiluminescent Meso Scale Diagnostics (MSD) V-PLEX COVID-19 Respiratory

121   Panel 3 Kit according to the manufactures' protocol at a dilution of 1:5000.

122

123   *2.3 Pseudoneutralization/ACE2 Inhibition Measurement:*

124   Plasma from study participants was thawed and ACE2 blocking was measured

125   using the ACE2 MSD V-PLEX SARS-CoV-2 Panel 6 and Panel 14 kits according to the

126   manufacturers' protocol at a dilution of 1:100.

127   *2.4 Viruses and cells.*

128   VeroE6-TMPRSS2 cells[18] were cultured in complete media (CM) consisting of

129   DMEM containing 10% FBS (Gibco, Thermo Fisher Scientific), 1 mM glutamine

130   (Invitrogen, Thermo Fisher Scientific), 1 mM sodium pyruvate (Invitrogen, Thermo

131   Fisher Scientific), 100 U/mL penicillin (Invitrogen, Thermo Fisher Scientific), and 100

132   µg/mL streptomycin (Invitrogen, Thermo Fisher Scientific). Cells were incubated in a 5%

133   CO_2 humidified incubator at 37°C.

134   The SARS-CoV-2/USA-WA1/2020 virus was obtained from BEI Resources. The

135   delta variant of SARS-CoV-2 (hCoV19/USA/MD-HP05660/2021, EPI_ISL_2331507)

136   was isolated on Vero-E6-TMPRSS2 cells plated in 24-well dishes and grown to 75%

137   confluence. The CM was removed and replaced with 150 µL of infection medium (IM),

138   which is identical to CM but with the fetal bovine serum reduced to 2.5%, and 150 ul of

139   the viral transport media containing a swab from a patient confirmed to be SARS-CoV-2

140   positive was added to the culture. The cultures were incubated at 37°C for 2 hours, the

141   inoculum was aspirated and replaced with 0.5 mL of IM and the cells cultured at 37°C

142   for up to 5 days. When a cytopathic effect was visible in most of the cells, the IM was

143   harvested and stored at –70°C. The presence of SARS-CoV-2 was verified by

144   extracting RNA from the harvested supernatant using the Qiagen Viral RNA extraction

145   kit (Qiagen), and viral RNA detected using quantitative RT-PCR.[19] The consensus

6

146   sequence of the virus isolate did not differ from the sequence derived from the clinical

147   specimen.

148   The infectious virus titer was determined on VeroE6-TMPRSS2 cells using a

149   50% tissue culture infectious dose (TCID50) assay as previously described for SARS-

150   CoV.[20,21] Serial 10-fold dilutions of the virus stock were made in IM, and then 100 µL of

151   each dilution was added to the cells in a 96-well plate in sextuplicate. The cells were

152   incubated at 37°C for 4 days, visualized by staining with naphthol blue-black, and

153   scored visually for cytopathic effect.

154   *2.5 Neutralization assay.*

155   The neutralizing antibody (nAb) levels were determined as described for SARS-

156   CoV.[22] Two-fold dilutions of plasma (starting at a 1:20 dilution) were made in IM.

157   Infectious virus was added to the plasma dilutions at a final concentration of 1×10^4

158   TCID50/mL (100 TCID50 per 100 µL). The samples were incubated for 1 hour at room

159   temperature, and then 100 µL of each dilution was added to 1 well of a 96-well plate of

160   VeroE6-TMPRSS2 cells in sextuplet for 6 hours at 37˚C. The inocula were removed,

161   fresh IM was added, and the plates were incubated at 37˚C for 2 days or until complete

162   cytopathic effect was visible in wells exposed to virus without plasma. The cells were

163   fixed by the addition of 100 µL of 4% formaldehyde per well, incubated for at least 4

164   hours at room temperature, and then stained with Napthol Blue Black (MilliporeSigma).

165   The nAb titer was calculated as the highest serum dilution that eliminated the cytopathic

166   effect in 50% of the wells and area under the curve (AUC) was calculated using

167   GraphPad Prism.

168

7

169   *2.6 Statistical analysis:*

170   Only SOTRs with available demographic and immunological data on pre and

171   post third dose of SARS-CoV-2 vaccine were included in the analysis. Wilcoxon signed

172   rank test was used to compare the median of SARS-CoV-2 anti-Spike and anti-RBD

173   IgG level and percent ACE2 inhibition before and after third dose of vaccine among

174   SOTRs. The median of IgG level and ACE2 inhibition between SOTRs and HCs were

175   compared using Wilcoxon rank sum test. Pearson correlation was used to evaluate the

176   linear association between Spike IgG and percent ACE2 inhibition among SOTRs. A

177   spline knot was added at 4 $\log_{10}$(AU) MSD IgG. Bonferroni correction was conducted to

178   control multiple comparison when analyzing variants ($p < 0.01$ was considered

179   statistically significant). The analysis was also stratified by type of third dose vaccine,

180   age, sex, and graft transplanted to evaluate effect measure modification. Missing values

181   were treated using available case strategy in subgroup analysis.

182

183   **3.   RESULTS:**

184   *3.1* Pre- and post-third dose samples were available for 47 SOTRs followed in

185   our ongoing longitudinal observational cohort studying immunogenicity and safety of

186   SARS-CoV-2 vaccination. Most of these participants had previously undergone anti-

187   spike antibody testing using two clinically available assays[16]. The median age was 63

188   (interquartile range 49-70) years and 55% were female. Most SOTRs were kidney

189   transplant recipients (64%) and all initially received two doses of an mRNA-based

190   vaccine (23 Moderna mRNA-1273, 24 Pfizer BNT162b2). Most were taking a

191   calcineurin inhibitor-based maintenance immunosuppression regimen (77%) and 30%

192 were on "triple immunosuppression" with a calcineurin inhibitor, an antimetabolite, and

193 corticosteroids. 70% of SOTRs received a third mRNA vaccine dose and 30% received

194 the Janssen Ad26.COV2.S vaccine. None reported a known history of COVID-19.

195 Among mRNA-vaccinated healthy controls (HC, N=15), none had known medical

196 conditions, and all received two doses of BNT162b2. See **Table 1** for full demographic

197 and clinical data.

198 Anti-S1-Receptor binding domain (RBD), anti-Spike (S), and anti-Nucleocapsid

199 (N) total IgG were measured in plasma using a research assay (MSD) with FDA-verified

200 sero-positivity cutoffs before and after a third dose of SARS-CoV-2 vaccine in SOTRs

201 and after two doses of an mRNA-based vaccine in HCs. No participants had a positive

202 anti-N response at before or after a third dose of vaccine (**Supplemental Figure 1**).

203 Prior to a third dose of vaccine, 17 (36%) and 11 (23%) SOTRs were sero-positive for

204 anti-RBD and anti-S, respectively (**Figure 1A**). After the third dose, these numbers

205 increased to 36 (77%) and 34 (72%), respectively, and there was a significant increase

206 in the median total anti-S (1.6 fold change) and anti-RBD (1.5 fold change) IgG levels

207 compared to matched pre-third dose samples (**Figure 1A**). The median anti-RBD and

208 anti-S IgG values of SOTRs receiving a third dose remained significantly lower than the

209 median responses in fully vaccinated HCs after the two-dose series (**Figure 1B**). In

210 comparison to all other transplant recipients, kidney transplant recipients had

211 significantly lower anti-S IgG (**Figure 1C**). Eight (57%) of those receiving Ad26.COV2.S

212 as a third dose and 26 (79%) who received an mRNA-based vaccine as a third dose

213 became sero-positive. When stratifying by type of third dose received (mRNA versus

214 Ad.COV2.S), however, we did not observe a significant difference in median anti-S IgG

215   value (**Supplemental Figure 2C**). In exploratory analysis, median IgG levels did not

216   differ by other key clinical or demographic parameters such as age or sex, though

217   subgroup sizes were small (**Supplemental Figures 2A and 2B**). Notably, seven female

218   kidney recipients had the lowest post third dose IgG levels of all SOTRs in the study. All

219   were taking anti-metabolite maintenance immunosuppression, but they did not

220   otherwise share clinical or demographic factors.

221   *3.2* Next, we investigated the neutralizing potential of SOTR plasma versus major

222   SARS-CoV-2 VOCs after three vaccine doses with comparison to that of healthy

223   individuals after two vaccine doses. This was assessed initially via an assay measuring

224   capacity of plasma to inhibit S protein binding to the ACE2 receptor, termed

225   pseudoneutralization. There was a significant increase in the median

226   pseudoneutralization of all variants after a third vaccine dose among SOTRs: fold

227   changes 2.5, 2.2, 1.6, 1.5, and 2.5 for vaccine, Alpha, Beta, Gamma, Delta variants,

228   respectively (**Figure 2A**). However, pseudoneutralization of all variants was significantly

229   lower than that of healthy controls after two doses of an mRNA-based vaccine (**Figure**

230   **2B**). For example, only two (6%) SOTRs had pseudoneutralization values for the Delta

231   variant above the first quartile of the healthy control pseudoneutralization values; the

232   majority were below 20% inhibition for all variants. When stratified by type of organ

233   received, kidney transplant recipients had significantly lower ACE2 inhibition versus the

234   vaccine strain and Alpha variant compared to all other organs (**Figure 2C**). Stratification

235   by age, sex, or vaccine platform, did not identify any significant differences in

236   pseudoneutralization (**Supplemental Figure 3**).

237   We also examined the correlation between anti-S IgG and pseudoneutralization

238   for all the variants. We found a strong correlation between anti-S IgG and

239   pseudoneutralization, but the relationship only became linear around 4 $\log_{10}$(arbitrary

240   unit, AU) IgG, suggesting that values below this may not correlate with neutralizing

241   response (**Figure 2D**).

242   *3.3* Finally, we used live-virus neutralization (nAb) to assess 50% neutralization

243   titer (NT50) and area under the curve (AUC) against the vaccine strain and the Delta

244   variant before and after a third vaccine dose in SOTRs and in two-dose vaccinated

245   HCs. For SOTRs, median (IQR) NT50s were 40 (10-120) versus vaccine strain and 20

246   (10-40) versus Delta (**Figure 3A**), with median (IQR) AUC of 50 (2-145) and 9 (3-50),

247   respectively (**Figure 3B**) after a third vaccine dose. This corresponded to a fold change

248   in NT50 of 1.6 and 1.3 and a fold change in AUC 50.2 and 8.4 versus the vaccine strain

249   and Delta variant, respectively. Compared to HCs, NT50s and AUC versus vaccine and

250   Delta variant strains were significantly lower among SOTRs (**Figure 3D**). Fully 32% of

251   SOTRs had nAb NT50s at or below the limit of detection versus the Delta variant after a

252   third vaccine dose (as compared to 0% of HCs). There were two female liver transplant

253   recipients with very high neutralizing titers, even beyond those of the HCs.

254   *3.4* We assessed inter-assay correlation for both the vaccine strain (**Figure 4A**)

255   and Delta variants (**Figure 4B**) among clinical (EUROIMMUN) and research (MSD) anti-

256   spike IgG assays, as well as pseudoneutralization and nAb AUC for SOTRs and HCs.

257   For the vaccine strain, EUROIMMUN and MSD IgG showed excellent positive

258   correlation, particularly above the clinical manufacturer threshold for seropositivity (1.1

259   AU). Correlation of pseudoneutralization with both IgG assays was strong above a

260   threshold of 20% ACE2 blocking. Below this, there was marked variation in

261   corresponding IgG levels among SOTRs (e.g., EUROIMMUN IgG ranged 0-7.5 AU)

262   (**Supplemental Figure 4**). Correlation of both IgG assays and nAb AUC was moderate,

263   though markedly improved when restricting to higher IgG cutoffs (4 AU on the

264   EUROIMMUN assay and 4 log10(AU) on the MSD assay). Overall correlation of

265   pseudoneutralization and nAb was stronger, particularly when restricting each to one

266   patient group (SOTR or HC). These cross-correlation patterns were similar when

267   considering the Delta variant, although pseudoneutralization and nAb AUC correlation

268   was overall stronger as compared to the vaccine strain, reflecting reduction in ACE2

269   blocking for HCs.

270

271   **4. DISCUSSION**

272   Here, we provide evidence that a third dose of COVID-19 vaccine increases

273   plasma neutralization against VOCs for some SOTRs, including versus the highly

274   transmissible and now dominant Delta variant. This was robustly characterized using a

275   combination of clinical and research IgG assays, pseudoneutralization, and gold-

276   standard live-virus neutralization. Although median plasma neutralizing capacity did

277   increase for SOTRs, levels were generally far below that of HCs after the two-dose

278   mRNA series and 32% showed no nAb against the Delta variant using the live-virus

279   assay.

280   Other key findings include lower neutralization among kidney transplant

281   recipients versus other transplant recipients, potentially reflecting heavier maintenance

282   immunosuppression. Other factors previously associated with improved sero-response

283 such as younger age or third dose vaccine platform (i.e., mRNA) were not clearly

284 associated with response. Importantly, although there was significant variability in IgG

285 responses in SOTRs, we found evidence through correlation analysis that certain IgG

286 cutoffs were associated with clear increases in ACE2 blocking, as well as in nAb. This is

287 an early step toward establishing thresholds for high-throughput assays that may

288 indicate protection from COVID-19, including the Delta variant, though this will need to

289 be tested by assessing risk of infection in real-world cohort and clinical trial settings.

290   The observed variable humoral response to additional vaccine doses in this high-

291 risk group indicates that alternative strategies, such as immunosuppressive modulation

292 or using emerging vaccine platforms, may be necessary to induce a protective response

293 to vaccination. While some SOTRs clearly produce an antibody response on par with

294 HCs, they were a small minority in this study. Though we identified kidney

295 transplantation as a risk factor for decreased responsiveness, the mechanism

296 underlying this association is unknown. This is evident in the seven female kidney

297 recipients who all had very low IgG and neutralizing responses, yet had no clear pattern

298 in demographic features, age, or vaccine platform received as a third dose. Additional

299 investigations and deeper immunological analyses are warranted to understand in a

300 personalized fashion why some SOTRs respond to additional antigen exposure, while

301 others do not.

302   This study was limited by its observational nature and small number of

303 participants with demographic and immunosuppressive heterogeneity. Additionally, HC

304 comparators were younger than the SOTR group, which may contribute to observed

305 differences in humoral response. Although patient survey and anti-N IgG were used to

13

306   rule out prior COVID-19, it is possible that subclinical infections occurred in some

307   patients before or after vaccination. Furthermore, mucosal immune responses and

308   cellular immune responses were not characterized in this study.

309   In summary, a third dose of a SARS-CoV-2 vaccine increases anti-spike and

310   anti-RBD IgG levels and plasma neutralizing capability, including against the Delta

311   variant, in some SOTRs. Yet, a significant portion of SOTRs have limited or no

312   neutralizing activity against the dominant VOC indicating that a third dose of vaccine

313   may not be a fully effective strategy for a large portion of immunocompromised patients.

314   These data also inform how research and clinical anti-spike IgG measurements might

315   be used to estimate neutralizing ability and potential sero-protection thresholds. This is

316   novel and timely information regarding the potential improvement of immune protection

317   against SARS-CoV-2 variants in a highly vulnerable population amidst ongoing

318   community surges.

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AUTHOR CONTRIBUTIONS:

AHK and WAW conceived of the study and design. OA, JER, and YE processed the

samples and prepared them for the assays. AHK, KHW, and AGR performed the MSD

assays and collected the antibody data. AP and IS performed the live virus

neutralization. ATA, JLA, JNB, DW, and BJB assisted with participant enrollment and

collection of clinical data. XZ and TL performed the analysis. EAT assisted with sample

curation and data interpretation. AHK and WAW wrote the original manuscript. JMG,

ALC, JNB, SLK, AP, JRB, DLS, AART, and WAW supervised the studies, provided

material support, and contributed to the interpretation of results. All authors aided in

editing the manuscript.

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DLS has the following financial disclosures: consulting and speaking honoraria from

Sanofi, Novartis, CSL Behring, Jazz Pharmaceuticals, Veloxis, Mallinckrodt, Thermo

Fisher Scientific. None of the other authors have any relevant competing interests.

DATA AVAILABILITY:

Reasonable requests for deidentified data to the corresponding author will be granted.

352   **FIGURE LEGENDS**

353   **Figure 1. Changes in SARS-CoV-2 Specific IgG After a Third Dose of SARS-CoV-2**

354   **Vaccine**

355

356   **A.** Total SARS-CoV-2 S1 RBD (left) and Spike (right) specific IgG in SOTRs before

357   and after a third dose of vaccine. The dashed line represents the assay

358   manufacturer's cut-off for seropositivity based on convalescent samples.

359   **B.** Total SARS-CoV-2 S1 RBD (left) and Spike (right) specific IgG in fully mRNA

360   vaccinated healthy controls (HCs) (n = 15) and SOTRs after a third dose of

361   vaccine (n = 47).

362   **C.** Total SARS-CoV-2 Spike specific IgG in SOTRs who received kidney (n = 30)

363   and non-kidney (n = 17) transplant.

364
365   The boxplots represent the IQR. The median is represented by a horizontal line in

366   the box. The lower and upper whiskers represent 1.5x the IQR beyond the quartiles.

367   Each dot represents an individual sample. Statistical differences between groups

368   were determined by Wilcoxon signed rank test for panel A, and Wilcoxon rank sum

369   test for panel B and C. P-values of <0.05 were considered significant.

370

371 **Figure 2. SARS-CoV-2 Pseudoneutralization After a Third Dose of COVID-19**

372 **Vaccine in SOTRs**

373

374 **A.** Pseudoneutralization of full-length SARS-CoV-2 Spike variants (indicated in top

375   header of each panel) before and after a third dose of vaccine among SOTRs.

376 **B.** Pseudoneutralization of full-length SARS-CoV-2 Spike variants (indicated in top

377   header of each panel) in SOTRs (n = 47) after a third dose of vaccine compared

378   to fully vaccinated healthy controls (n = 15).

379 **C.** Comparison of pseudoneutralization of full-length SARS-CoV-2 Spike variants

380   (indicated in top header of each panel) in SOTRs who received kidney (n = 30)

381   and non-kidney (n = 17) transplant.

382 **D.** Correlation between total SARS-CoV-2 Spike IgG and pseudoneutralization of

383   full-length SARS-CoV-2 Spike variants among SOTRs receiving a third dose of

384   vaccine.

385 In panel A-C, the boxplots represent the IQR. The median is represented by a

386 horizontal line in the box. The lower and upper whiskers represent 1.5x the IQR

387 beyond the quartiles. Each dot represents an individual sample. Statistical

388 differences between groups were determined by Wilcoxon signed rank test for panel

389 A, and Wilcoxon rank sum test for panel B and C. Pearson correlation coefficient

390 were generated for panel D. P-values of <0.01 were considered significant after

391 Bonferroni correction.

392

393   **Figure 3. Neutralizing antibody (nAb) versus SARS-CoV-2 vaccine strain and**
394   **Delta variant**
395
396   A. nAb NT50 versus SARS-CoV-2 vaccine strain and Delta variant before and after

397   a third dose SARS-CoV-2 vaccine among SOTRs.

398   B. nAb area under curve (AUC) versus SARS-CoV-2 vaccine strain and Delta

399   variant before and after a third dose SARS-CoV-2 vaccine among SOTRs.

400   C. Comparison of nAb reciprocal NT50 versus SARS-CoV-2 vaccine strain and

401   Delta variant between SOTRs after a third dose of SARS-CoV-2 vaccine and

402   HCs after two mRNA vaccine doses

403   D. Comparison of nAb AUC of SARS-CoV-2 vaccine strain and Delta variant

404   between SOTRs after a third dose of SARS-CoV-2 vaccine and HCs after two

405   mRNA vaccine doses.

406
407   In panel A-D, the boxplots represent the IQR. The median is represented by a

408   horizontal line in the box. The lower and upper whiskers represent 1.5x the IQR

409   beyond the quartiles. Each dot represents an individual sample. Statistical

410   differences between groups were determined by Wilcoxon signed rank test for panel

411   A and B, and Wilcoxon rank sum test for panel C and D.

412
413
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415

18

416 **Figure 4. Correlations between neutralizing antibody (nAb), percent ACE2**
417 **inhibition, MSD anti-spike IgG and EUROIMMUN anti-spike IgG of SARS-CoV-2**
418 **among SOTRs and HCs**

419

420   A. Correlations between neutralizing and IgG assays versus the SARS-CoV-2

421   vaccine strain among SOTRs after a third dose of vaccine and HCs after two

422   doses.

423   B. Correlations between neutralizing and IgG assays versus the Delta variant

424   among SOTRs after a third dose of vaccine and HCs after two doses.

425 Each point on the scatter plots represents an individual sample. Pearson correlation

426 coefficients between assays are presented in the upper panels. "Corr" represents

427 the correlation across all samples. "HCs" (in red) represents the correlation among

428 only HCs. "SOTR" (in blue) represents the correlation among only SOTRs. * $p <$

429 0.05; ** $p < 0.01$; *** $p < 0.001$. Density plots of SOTRs and HCs are shown in

430 diagonal panels. Unit of analysis: nAb AUC, $\log_{10}$AUC; ACE2: percent ACE2

431 inhibition; MSD IgG, $\log_{10}$IgG AU/mL; EUROIMMUN IgG, AU/mL.

432

Table 1. Clinical and Demographic Characteristics of SOTRs and Healthy

Controls.

	Overall n = 62	SOTR n = 47	Healthy controls, n = 15
Age, years			
20-39	10 (16)	3 (6)	7 (47)
40-59	26 (42)	18 (38)	8 (53)
60-79	26 (42)	26 (55)	0 (0)
Sex			
Female	31 (50)	26 (55)	5 (33)
Male	31 (50)	21 (45)	10 (67)
Race			
White	57 (92)	46 (98)	11 (73)
Asian	4 (6)	1 (2)	3 (20)
African American	1 (2)	0 (0)	1 (7)
Graft transplanted			
Kidney*	-	30 (64)	-
Liver	-	10 (21)	-
Heart	-	4 (9)	-
Lung	-	2 (4)	-
Pancreas	-	1 (2)	-
Anti-rejection medication†			
Prednisone	-	22 (47)	-
Calcineurin Inhibitors	-	36 (77)	-
mTOR inhibitors	-	7 (15)	-
anti-metabolites	-	30 (64)	-
Type of the third dose vaccine			
mRNA	-	33 (70)‡	-
Ad26.COV2.S	-	14 (30)	-
Days between second dose and third dose vaccine	-	102 (70 - 124)	-
Days between transplant and third dose vaccine	-	1778 (930 - 4419)	-
Days post second dose vaccine	-	-	8 (7 – 10)

Note: all study participants received mRNA vaccine for the first two doses. Categorical variables were presented in n (%), and continuous variables were presented in median (interquartile range). *1 person had both kidney and pancreas transplanted and has been grouped into kidney category. †Anti-rejection medication use was not mutually exclusive. ‡10 (30%) of the 33 participants received a third mRNA vaccine that differed from their initial two-dose series.

447   **Supplemental Figure 1.**

448   Total SARS-CoV-2 Nucleocapsid specific IgG in SOTRs before and after a third dose of

449   vaccine. The dashed line represents the assay manufacturer's cut-off for positivity

450   based on convalescent samples. P-values were calculated using Wilcoxon rank sum

451   test.

452

453   **Supplemental Figure 2.**
454
455   Total SARS-CoV-2 Spike specific IgG in SOTRs who received a third dose of COVID-19

456   vaccine stratified by age (<60 n = 21 and ≥60 years n = 26), sex (female n = 26 and

457   male n = 21), and type of third dose vaccine received (mRNA n = 33 and Ad26.COV2.S

458   n = 14). P-values were calculated using Wilcoxon rank sum test and should be

459   considered exploratory given the small subgroups.

460

461   **Supplemental Figure 3.**

462   Pseudoneutralization of full-length SARS-CoV-2 Spike variants in SOTRs who received

463   a third dose of COVID-19 vaccine stratified by age (<60 n = 21 and ≥60 years n = 26),

464   sex (Female n = 26 and male n = 21), and type of third dose vaccine received (mRNA n

465   = 33 and Ad26.COV2.S n = 14). P-values were calculated using Wilcoxon rank sum test

466   and should be considered exploratory given the small subgroups.

467

468

469

470 **Supplemental Figure 4. Correlation between percent ACE2 inhibition and**

471 **neutralizing antibody (nAb) and IgG of SARS-CoV-2 among SOTRs after a third**

472 **dose of COVID-19 vaccine, stratified by ACE2 inhibition level**

473 A. Pearson correlation between vaccine strain percent ACE2 inhibition and vaccine

474 strain neutralizing antibody (nAb), MSD IgG and EUROIMMUN IgG of SARS-

475 CoV-2 among SOTRs after a third dose of COVID-19 vaccine.

476 B. Pearson correlation between Delta variant percent ACE2 inhibition and Delta

477 variant neutralizing antibody (nAb), MSD IgG and EUROIMMUN IgG of SARS-

478 CoV-2 among SOTRs after a third dose of COVID-19 vaccine.

479 Units of analysis: nAb AUC, $\log_{10}$AUC; ACE2: percent ACE2 inhibition; MSD IgG,
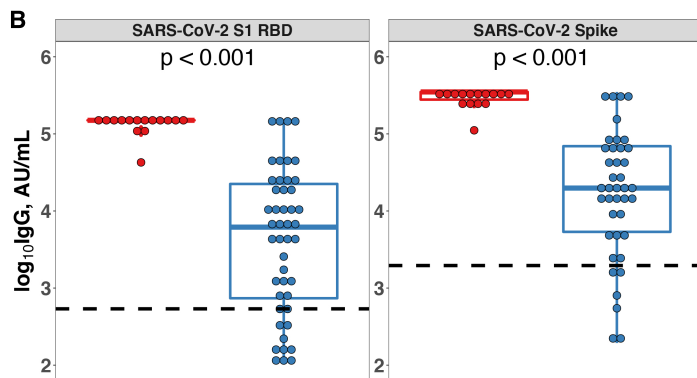
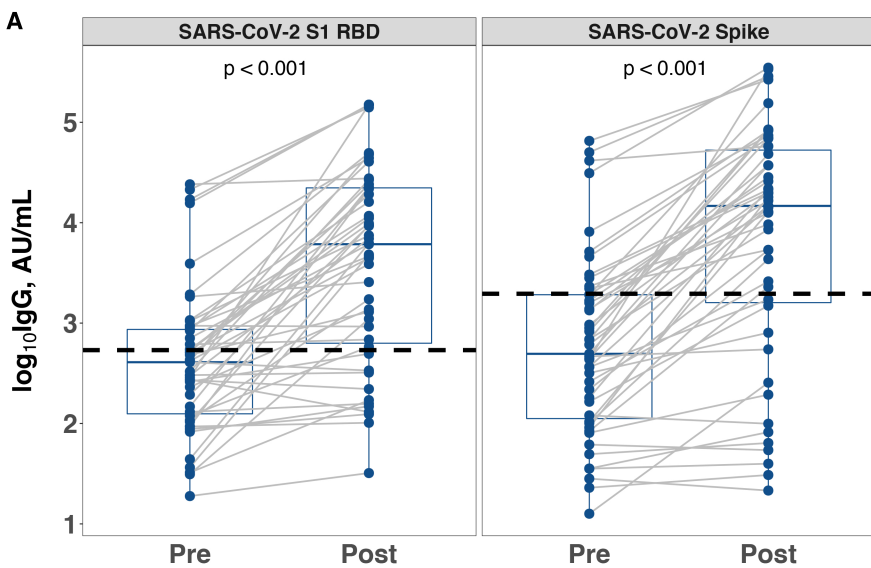480 $\log_{10}$IgG AU/mL; EUROIMMUN IgG, AU/mL.

481

482

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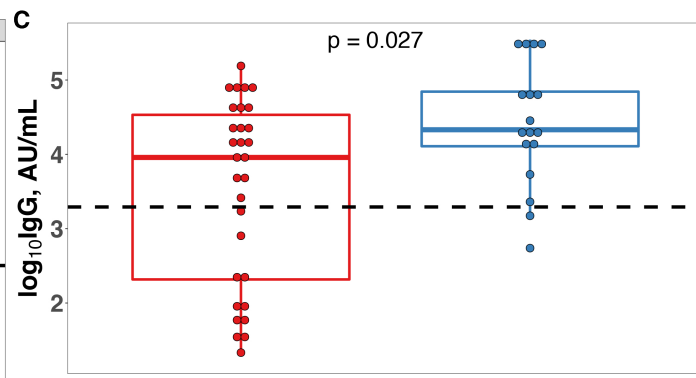
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552

A

| Vaccine nAb AUC | Vaccine ACE2 | MSD IgG | EUROIMMUN IgG |

Corr: 0.730***
HCs: 0.761***
SOTR: 0.734***

Corr: 0.895
HCs: 0.803***
SOTR: 0.891***

Corr: 0.882
HCs: 0.805***
SOTR: 0.877***

Corr: 0.770***
HCs: 0.917***
SOTR: 0.681***

Corr: 0.858***
HCs: 0.732**
SOTR: 0.822***

Corr: 0.926***
HCs: 0.828***
SOTR: 0.892***

B

| Delta nAb AUC | Delta ACE2 | MSD IgG | EUROIMMUN IgG |

Corr: 0.790***
HCs: 0.840***
SOTR: 0.722***

Corr: 0.865***
HCs: 0.709**
SOTR: 0.825***

Corr: 0.867***
HCs: 0.545*
SOTR: 0.835***

Corr: 0.719***
HCs: 0.822***
SOTR: 0.617***

Corr: 0.797***
HCs: 0.652*
SOTR: 0.744***