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1 # Omicron neutralizing antibody response following booster vaccination compared
2 # with breakthrough infection

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21 **ABSTRACT**

22 The rapid spread of the vaccine-resistant Omicron variant of SARS-CoV-2 presents a renewed
23 threat to both unvaccinated and fully vaccinated individuals, and accelerated booster
24 vaccination campaigns are underway to mitigate the ongoing wave of Omicron cases. The
25 degree of immunity provided by standard vaccine regimens, boosted regimens, and immune
26 responses elicited by the combination of vaccination and natural infection remain incompletely
27 understood. The relative magnitude, quality and durability of serological responses, and the
28 likelihood of neutralizing protection against future SARS-CoV-2 variants following these modes
29 of exposure are unknown but are critical to the future trajectory of the COVID-19 pandemic. In
30 this study of 99 vaccinated adults, we find that compared with responses after two doses of an
31 mRNA regimen, the immune responses three months after a third vaccine dose and one month
32 after breakthrough infection due to prior variants show dramatic increases in magnitude,
33 potency, and breadth, including increased antibody dependent cellular phagocytosis and robust
34 neutralization of the recently circulating Omicron variant. These results suggest that as the
35 number of Omicron cases rise and as global vaccination and booster campaigns continue, an
36 increasing proportion of the world's population will acquire potent immune responses that may
37 be protective against future SARS-CoV-2 variants.

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38 **INTRODUCTION**

39 Since 2020, the global COVID-19 pandemic has been punctuated by episodic waves of
40 increased incidence associated with the emergence of new SARS-CoV-2 variants with
41 progressively greater transmissibility and resistance to immune responses elicited by currently
42 approved vaccines. The most epidemiologically important variants have been classified as
43 variants of concern (VOC) by the World Health Organization, and include Alpha, Beta, Gamma,
44 Delta, and Omicron. Presently, the Omicron variant (including sub-linages BA.1 and BA.2) is the
45 globally dominant circulating strain, notable for its high transmissibility and resistance to
46 neutralization by vaccine-induced antibodies (1,2). The spike protein of the Omicron variant
47 contains 39 amino acid changes (30 substitutions, 6 insertions, and 3 deletions), nearly half of
48 which fall within the receptor binding domain (RBD) that is responsible for binding to the human
49 receptor angiotensin converting enzyme 2 (ACE2) (3). All known neutralizing antibodies bind to
50 the spike protein, with the vast majority targeting the RBD (4–6). Mutations within this region
51 have caused a dramatic loss of neutralizing ability among several of the approved monoclonal
52 antibody therapeutics, and has resulted in restriction of their use in cases of infection with
53 Omicron (7).
54
55 It is known that the additional antigenic exposure from boosters and breakthrough infections
56 bolster serological immunity, and third-dose vaccine booster campaigns are underway
57 worldwide to mitigate the ongoing wave of omicron cases (8,9). Vaccine breakthrough infections
58 can directly train the immune system against variant spike proteins, but come with medical risks
59 including prolonged illness (long COVID) and death (10,11). Conversely, booster vaccination is
60 generally safe, and has been shown to effectively increase the neutralizing response against
61 Omicron (12–14). The durability of responses due to boosting and breakthrough infection are
62 unknown, but antibody levels have been shown to decrease over time following primary
63 vaccination, suggesting that waning of the augmented immunity following additional exposure is
64 likely (15,16). It is also unknown whether recovery from breakthrough infection or booster
65 vaccination provide greater protection from reinfection with Omicron and future variants, which
66 will likely affect the future trajectory of the pandemic. To address these knowledge gaps, we
67 examined serological immune responses and antibody-dependent cell-mediated phagocytosis
68 in individuals who had received either two doses of a standard vaccine regimen, a standard
69 regimen followed by a booster, or breakthrough infection following vaccination.
70
71

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72 **METHODS**

73 **Cohort selection and serum collection** – Two- and three-dose group participants were
74 selected from a larger cohort of vaccinated health care workers at Oregon Health & Science
75 University recruited at the time of their first vaccine dose. Participants were asked to return after
76 either their second or third vaccine dose to provide whole blood samples. Breakthrough group
77 participants were recruited and enrolled at Oregon Health & Science University from among fully
78 vaccinated health care workers receiving positive results during PCR-based diagnostic testing
79 for SARS-CoV-2 infection, at which time participants provided information on symptoms of
80 illness by direct interview. Whole blood (4-6 mL) was collected with a BD Vacutainer® Plus
81 Plastic Serum Tube and centrifuged for 10 minutes at 1000xg, then stored at -20°C. Two- and
82 three-dose group participants confirmed no history of COVID-19 by direct interview and
83 validated by nonreactivity in a SARS-CoV-2 N protein ELISA. All vaccines used in this study
84 were BNT162b2 (Pfizer) and only individuals with no reported immunocompromising conditions
85 were included.

86

87 **Enzyme-linked immunosorbent assays (ELISA)** – ELISAs were performed as previously
88 described.[1] In 96-well plates (Corning Incorporated, EIA/RIA High binding, Ref #359096). Plates
89 were coated with SARS-CoV-2 RBD (produced in Expi293F cells and purified using Ni-NTA
90 chromatography), N (SARS-CoV-2 Nucleocapsid-His, insect cell-expressed, SinoBio Cat:
91 40588-V08B, Item #NR-53797, lot #MF14DE1611) at 100 µL/well at 1 µg/mL in PBS and
92 incubated overnight at 4°C with rocking. Plates were washed three times with .05% Tween-20 in
93 PBS (wash buffer) and blocked with 150 uL/well with 5% nonfat dry milk powder and .05%
94 Tween 20 in PBS (blocking buffer) at room temperature (RT) for 1 hour with rocking.
95 Breakthrough and control sera were aliquoted and frozen in dilution plates then resuspended in
96 blocking buffer; sera were diluted and added to ELISA plates 100 µL/well (6 x 4-fold dilutions
97 from 1:50 to 1:51,200, except for IgM (6 x 4-fold dilutions from 1:25 to 25,600). Sera was
98 incubated in coated plates for 1 hour at RT, then washed three times with wash buffer. Plates
99 were incubated with anti-human IgA-HRP at 1:3,000 (BioLegend, Ref #411002), Mouse anti-
100 human IgG-HRP Clone G18-145 at 1:3,000 (BD Biosciences, Ref #555788), or Goat anti-
101 human IgM-HRP at 1:3,000 (Bethyl Laboratories, Ref #A80-100P) at RT for 1 hour with rocking,
102 then washed three times with wash buffer prior to developing with o-phenylenediamine
103 dihydrochloride (OPD, Thermo Scientific #34005) according to manufacturer instructions. The
104 reaction was stopped after 25 minutes using an equivalent volume of 1 M HCl; optical density
105 was measured at 492 nm using a CLARIOstar plate reader.

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106

107 **Antibody dependent cellular phagocytosis (ADCP)** – ADCP was assessed as described
108 previously.[2] Biotinylated SARS-CoV-2 RBD protein was incubated with neutravidin beads
109 (Invitrogen, F8775) for 2 hours at room temperature then washed with PBS with 1% BSA
110 (dilution buffer) two times. 10 µL of 1:100 diluted RBD beads were incubated with an equal
111 volume of diluted serum for 2 hours at 37°C. Bead-serum mixtures were then incubated with
112 20,000 THP-1 cells in a final volume of 100 uL overnight in a tissue culture incubator. 100 µL of
113 PBS with 4% formaldehyde was then used to fix each well for 30 minutes prior to flow
114 cytometry. Triplicate, samples were flowed on a CytoFLEX flow cytometer. 2500 events were
115 recorded per replicate. Phagocytosis scores were calculated as the product of percent bead-
116 positive cells and mean fluorescence intensity of bead-positive cells, then divided by 10^6.
117
118 **SARS-CoV-2 growth and titration** – SARS-CoV-2 isolates USA-WA1/2020 [lineage A] (NR-
119 52281), hCoV-19/USA/PHC658/2021 [lineage B.1.617.2 – Delta] (NR-55611), and hCoV-
120 19/USA/MD-HP20874/2021 [lineage B.1.1.529 – Omicron] (NR-56461) were obtained from BEI
121 Resources. Viral stocks were propagated as previously described.[3] Sub-confluent Vero E6 cells
122 (CRL-1586) grown in Dulbecco's Modified Eagle Medium (DMEM), 10% fetal bovine serum
123 (FBS), 1% nonessential amino acids, 1% penicillin-streptomycin (complete media) were infected
124 at an MOI of 0.05 in a minimal volume (0.01 mL/cm^2) of Opti-MEM + 2% FBS (dilution media)
125 for 1 hour at TCC then 0.1 mL/cm^2 additional complete media was added and incubated until at
126 least 20% cytopathic effect (CPE) was observed, typically 72-96 hours. Culture supernatant was
127 centrifuged for 10 min at 1000xg and frozen at -80°C. Titration was performed by focus forming
128 assay on sub-confluent Vero E6 cells. 10-fold dilutions were prepared in dilution media and
129 incubated for 1 hour, then covered with Opti-MEM, 2% FBS, 1% methylcellulose (overlay
130 media) and incubated for 24 hours (48 hours for Omicron). Plates were then fixed in 4%
131 formaldehyde in phosphate buffered saline (PBS) for 1 hour then removed from BSL3 following
132 institutional guidelines. Cells were permeabilized in 0.1% bovine serum albumin (BSA), 0.1%
133 saponin in PBS (perm buffer) for 30 minutes, then with polyclonal anti-SARS-CoV-2 alpaca
134 serum (Capralogics Inc.) (1:5000 in perm buffer, or 1:2000 for Omicron) overnight at 4°C. Plates
135 were washed three times with 0.01% Tween 20 in PBS (wash buffer), then incubated for 2
136 hours at RT with 1:20,000 anti-alpaca-HRP (Novus #NB7242), or 1:5000 for Omicron. Plates
137 were washed three times with wash buffer, then incubated with TrueBlue (Sera Care #5510-
138 0030) for 30 minutes or until sufficiently developed for imaging. Foci images were captures with
139 a CTL Immunospot Analyzer and counted with Viridot (1.0) in R (3.6.3).[4] Viral stock titers in

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140 focus forming units (FFU) were calculated based on the dilution factor and volume used for
141 infection.
142
143 **Focus reduction neutralization test (FRNT)** – FRNT assays were carried out as previously
144 described.[3] We prepared 5x4.7-fold (1:10-1:4879) serial dilutions in duplicate for each serum
145 sample. An equal volume of viral stock was added to each well (final dilutions of sera, 1:20 –
146 1:9760) such that approximately 50 FFU were added to each well. Virus-serum mixtures were
147 incubated for 1 h before being used to infect sub-confluent Vero E6 cells in 96-well plates for 1
148 hour, then covering with 150 µL/well overlay media. Each 5-point serum dilution series was
149 accompanied by a virus only control well. Fixation, development, and counting of FRNT plates
150 was carried out as described in SARS-CoV-2 growth and titration. Percent neutralization values
151 were calculated for each well as focus count divided by the average of virus-only wells from the
152 same plate.
153
154 **Statistical analysis** – $FRNT_{50}$ and EC_{50} values were calculated by fitting to a dose-response
155 curve as previously described.[3] Final $FRNT_{50}$ values below the limit of detection (1:20) were set
156 to 1:19. Final EC_{50} values below the limit of detection of 1:25 for N, full-length Spike, Spike
157 RBD, IgG, IgA were set to 1:24 and 1:12.5 for IgM was set to 1:12. Aggregated EC_{50} and
158 $FRNT_{50}$ values were analyzed in Graphpad Prism (9.3.1). Significance was determined using
159 Kruskal-Wallis tests with Dunn's multiple comparison correction, P-values were two-tailed.
160 Correlations were calculated with log-transformed EC_{50} and/or $FRNT_{50}$ values with the
161 Spearman method, with corresponding two-tailed P values. Best fit lines were calculated via
162 simple linear regression.
163
164 **Study approval** – This study was conducted with approval of the Oregon Health and Sciences
165 University Institutional review board (IRB# 00022511). All participants were enrolled following
166 written informed consent.
167
168 **RESULTS**
169 **Cohort**: A total of 97 individuals were studied (Table 1). Participants from the two-dose group
170 provided serum samples a median of 24 days after the second dose. The three-dose group
171 received a third vaccine dose a median of 253 days after the second, and then provided serum
172 samples a median of 86 days after the third dose. Both two- and three-dose groups reported no
173 history of SARS-CoV-2 infection and displayed a lack of Nucleocapsid antibodies (Fig. 1).

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174 Breakthrough group participants received positive PCR-based COVID-19 test results a median
175 of 159.5 days after their final vaccine dose and provided serum samples a median of 29 days
176 after the date of PCR testing (Table 1 and Figure 2A).
177
178 **Approach**: In each sample, we analyzed the spike receptor-binding domain-specific IgG, IgA,
179 and IgM antibody levels by enzyme-linked immunosorbent assay (ELISA). We also measured
180 each serum's ability to neutralize authentic wild-type SARS-CoV-2 (WA1, Wuhan strain) and
181 clinical isolates of the Delta (B.1.617.2) and Omicron (B.1.1.529) variants with focus reduction
182 neutralization tests (FRNT). Finally, we examined the ability of serum in each group to trigger
183 antibody-depended cell-mediated phagocytosis (ADCP) of spike protein-coated beads.
184
185 **Binding antibody responses:** Compared to two-dose vaccination, the geometric mean of
186 serum dilutions with half-maximal binding in ELISA (EC_{50}) to full-length SARS-CoV-2 spike
187 protein was 223% higher in the three-dose group and 278% higher in the breakthrough group;
188 the three-dose and breakthrough groups were not significantly different from each other (Fig.
189 2B). Spike RBD specific antibodies did not significantly increase in the three-dose group but did
190 in the breakthrough group, which was 259% higher than in the two-dose group (Fig. 2C). Spike-
191 specific IgG and IgA levels showed similar increases relative to the two-dose group, with 224%
192 and 261% higher IgG levels and 221% and 279% higher IgA in the three-dose and
193 breakthrough groups, respectively (Fig. 2D and 2E). IgM levels were not significantly different
194 between any of the groups (Fig. 2F).
195
196 **Antibody-dependent cell-mediated phagocytosis:** Similarly to neutralizing antibody
197 responses, ADCP also increased in the three-dose (202%), and breakthrough (210%) groups,
198 compared to two-dose vaccination; here as well, the three-dose and breakthrough groups were
199 not significantly different from each other (Fig. 2G).
200
201 **Neutralizing antibody responses:** Consistent with previous reports, neutralization of live
202 SARS-CoV-2 improved to a greater degree than the observed rise in binding antibody levels
203 (9,17). The geometric mean titers (GMT) showing 50% neutralization of the original SARS-CoV-
204 2 virus (WA1, Wuhan strain) in FRNT assays were 463% and 732% higher for the three-dose
205 and breakthrough groups, respectively, compared to two-dose vaccination, but were not
206 significantly different from each other (Fig. 3A). Among the breakthrough infections, 10 of the 30
207 participants were infected with the Delta variant. The GMT of the breakthrough group to

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208 neutralize the Delta variant increased 948% while the three-dose group increased only 424%,
209 compared to two-dose vaccination. However, the difference between the 3-dose and
210 breakthrough groups did not rise to the level of statistical significance (Fig. 3B). Against
211 Omicron, 25 of 42 (59%) sera in the two-dose group fell below the limit of detection for
212 neutralization, while all three-dose and breakthrough participants showed detectable
213 neutralization, with 1156% and 1810% higher neutralizing GMTs, respectively, which were not
214 significantly different from each other (Fig. 3C).
215
216 **Antibody response quality:** The relationship between spike binding antibody level and
217 neutralizing titer gives an indication of the quality of the antibody response by controlling for the
218 total quantity of antibodies present. In all three groups, neutralizing titer correlates strongly with
219 neutralization of WA1 and Delta. However, the correlation is much weaker for the two-dose
220 group for Omicron, largely due to the high proportion of samples below the detection limit (Fig.
221 4A-4C). We explored this association further by calculating the neutralizing potency index (NPI),
222 as the ratio of live-virus neutralization to spike-specific antibody EC_{50} for WA1, Delta, and
223 Omicron. For WA1, the median NPI was 0.60 for two-dose, 1.10 for three-dose, and 1.91 for
224 breakthrough, showing an increase in the ratio of neutralizing activity to spike binding EC_{50} (Fig.
225 4D). The median Delta NPI were 0.30, 0.52, and 0.93 and the median Omicron NPI were 0.03,
226 0.15, and 0.20 for two-dose, three-dose, and breakthrough groups, respectively (Fig. 4E and
227 4F). For all three viruses, the three-dose and breakthrough groups were found to be significantly
228 increased from the two-dose group, but not significantly different from each other. A similar
229 trend was seen when calculating the NPI using RBD-specific antibody levels instead of those for
230 full-length spike (Fig. 5).
231
232 **Relative loss of strain-specific neutralizing capacity:** To measure the relative loss of
233 neutralizing activity against the Delta and Omicron variants compared to WA1, we calculated
234 the ratio of neutralization for each variant to WA1 neutralization in each participant. For Delta,
235 the median ratio was 0.42 for two-dose, 0.43 for three-dose, and 0.55 for breakthrough, none of
236 which were significantly different (Fig. 3D). Against Omicron, however, the median ratio was
237 0.06 for two-dose, 0.12 for three-dose, 0.13 for breakthrough, which were significantly higher
238 than two-dose for both the three-dose and breakthrough groups (Fig. 3E). Comparing the
239 neutralization of Delta and Omicron with that of WA1 clearly showed a greater extent of
240 resistance by Omicron, with some individuals displaying nearly a 100-fold reduction in

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241 neutralization of Omicron compared to WA1, where grey lines indicate successive 10-fold
242 differences (Fig. 3F and 3G).
243
244 **Antibody response vs age and gender**: Previous studies have established a negative
245 correlation between antibody response and age among vaccinated individuals (18). Among
246 study participants, we observed a negative correlation between age and WA1 neutralizing titer
247 for the two-dose and also the three-dose groups, but not the breakthrough group (Fig. 4G-3I).
248 We found no difference in neutralizing titer based on gender.
249

250 **DISCUSSION**

251 Our data confirm the extent to which Omicron resists neutralization by vaccinated sera,
252 consistent with recent reports (2,3). Importantly, however, we find that both booster vaccination
253 and breakthrough infection enhance neutralizing titers to a similar degree when comparing
254 timepoints approximately 3 months after boosting with 1 month after breakthrough. Despite the
255 reliance of the vaccine on the original SARS-CoV-2 spike protein sequence, we see robust
256 boosting of the Omicron neutralizing response.
257
258 While boosting with updated vaccine inserts may ultimately enhance immune responses to new
259 variants such as Omicron, three-dose vaccine regimens and hybrid immune exposures
260 consisting of vaccination and breakthrough infection nevertheless improve the breadth of the
261 humoral response, as seen by improved Delta neutralization and an increased ratio of variant to
262 WA1 neutralizing titers. Further, we see an improvement in the amount of neutralizing activity
263 for a given amount of spike-binding antibody, indicating an improvement in antibody quality.
264 Thus, while two-doses of the currently available mRNA vaccines provide strong protection
265 against symptomatic infection due to the original SARS-CoV-2 and early variants, serological
266 immunity against the Omicron variant is substantially reduced (19), but is restored by booster
267 vaccination or breakthrough infection. A previous study also indicates that hybrid immunity from
268 SARS-CoV-2 infection followed by one or two mRNA vaccine doses provides similar antibody
269 responses to breakthrough infection (9). The similarity seen between immune responses to
270 three-dose regimens and breakthrough infection suggest that the original vaccines continue to
271 provide neutralizing antibody responses that are relevant to the current antigenic landscape,
272 dominated by the Omicron variant. As future variants will emerge from currently circulating
273 strains, the development of vaccines with inserts based on more contemporary variants may
274 further improve the protective efficacy of vaccine-induced immune responses.

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275 Critically, current two-dose vaccine regimens establish a foundation of immunity that is further
276 enhanced through boosting or breakthrough infections, resulting in substantial protection
277 against reinfection, even due to immune-resistant variants such as Omicron. Interestingly, the
278 negative correlation between age and antibody levels seen in vaccines is no longer apparent in
279 hybrid immunity. The enhanced immunity resulting from augmented vaccine regimens and
280 hybrid exposures studied here will apply to an ever-increasing proportion of the world's
281 population as individuals continue to be vaccinated and exposed to natural infection.
282
283

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284 **AUTHOR CONTRIBUTIONS**

285 Conceptualization: MEC, FGT, TAB. Recruitment and sample collection: MEC, DS, SDC.
286 Experimental design: MEC, FGT, TAB. Laboratory Analysis: FGT, TAB, GG, SKM. Statistical
287 analysis: TAB. Supervision: MEC, FGT. Manuscript drafting: MEC, TAB. Manuscript review and
288 editing: MEC, TAB, DS, SDC, FGT.

289

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372 **FIGURES AND TABLES**

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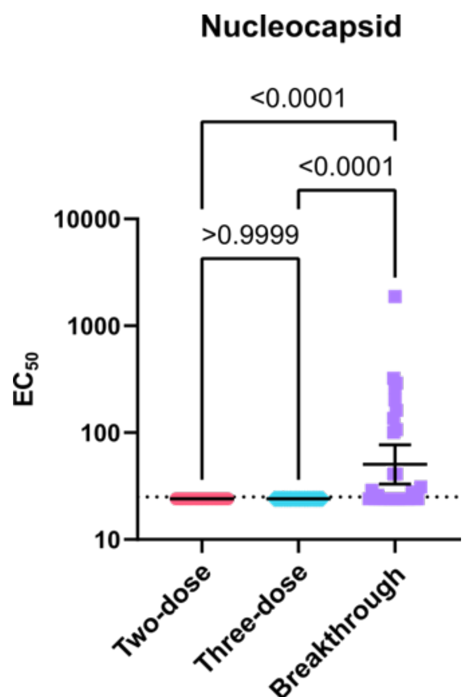

375

376 **Figure 1**: Nucleocapsid ELISA. Serum dilutions with half-maximal binding (EC_{50}) of IgG/A/M
377 antibodies to SARS-CoV-2 Nucleocapsid protein. Error bars indicate the geometric mean and
378 95% confidence intervals. P-values show the results of a two-tailed Kruskal-Wallis test with
379 Dunn's multiple comparison correction.

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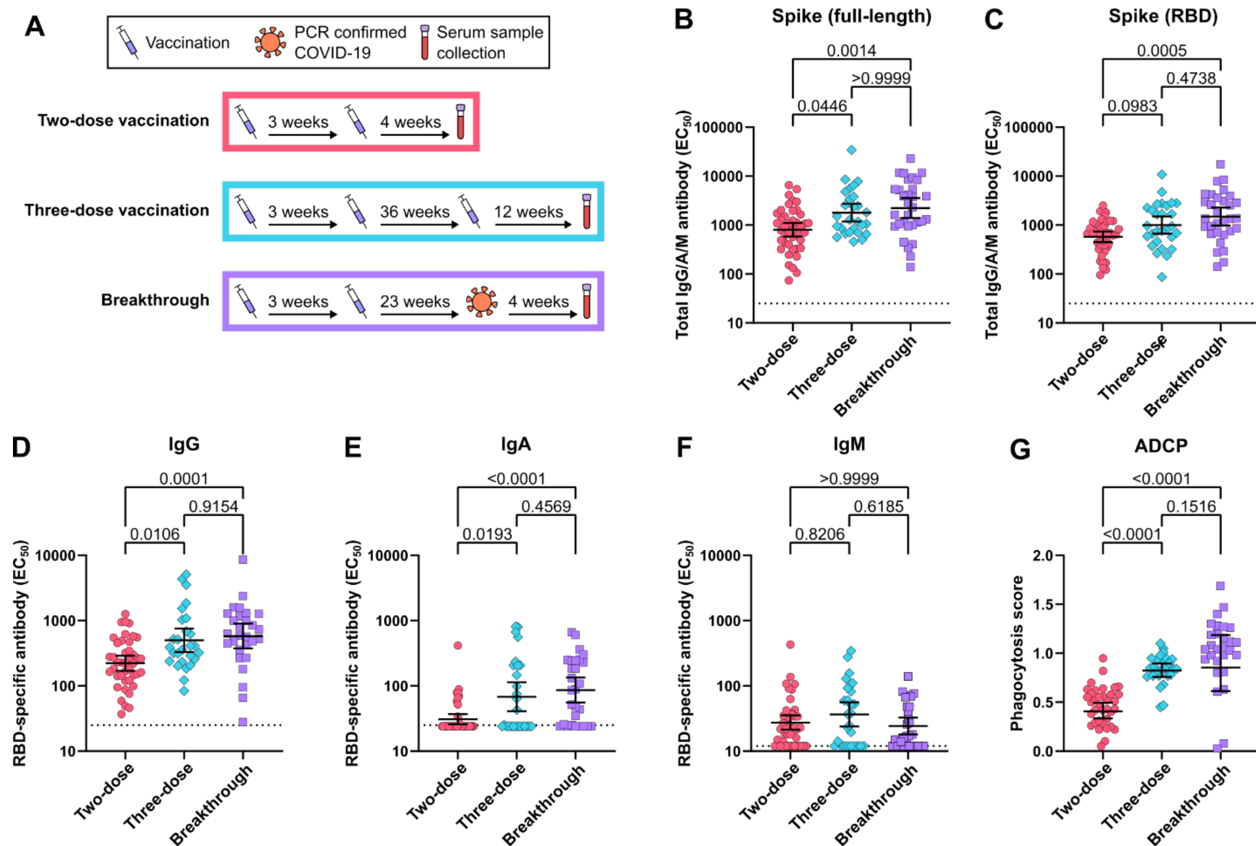

381

382 **Figure. 2:** Antibody response to two-dose vaccination, three-dose vaccination and breakthrough
383 infection. (A) Schematic describing median cohort vaccine dose, PCR-confirmed natural
384 infection, and sample collection timing. (B) Serum dilutions with half-maximal binding (EC_{50}) of
385 IgG/A/M antibodies to full-length SARS-CoV-2 spike protein. (C) Serum IgG/A/M antibody EC_{50}
386 to spike receptor binding domain (RBD). Serum (D) IgG-specific, (E) IgA-specific, (F) IgM-
387 specific antibody EC50's to RBD. (G) Antibody dependent cellular phagocytosis scores indicate
388 the increase in uptake of RBD-coated beads caused by sera. Error bars in B-G indicate the
389 geometric mean and 95% confidence intervals. P-values in B-G show the results of two-tailed
390 Kruskal-Wallis tests with Dunn's multiple comparison correction.

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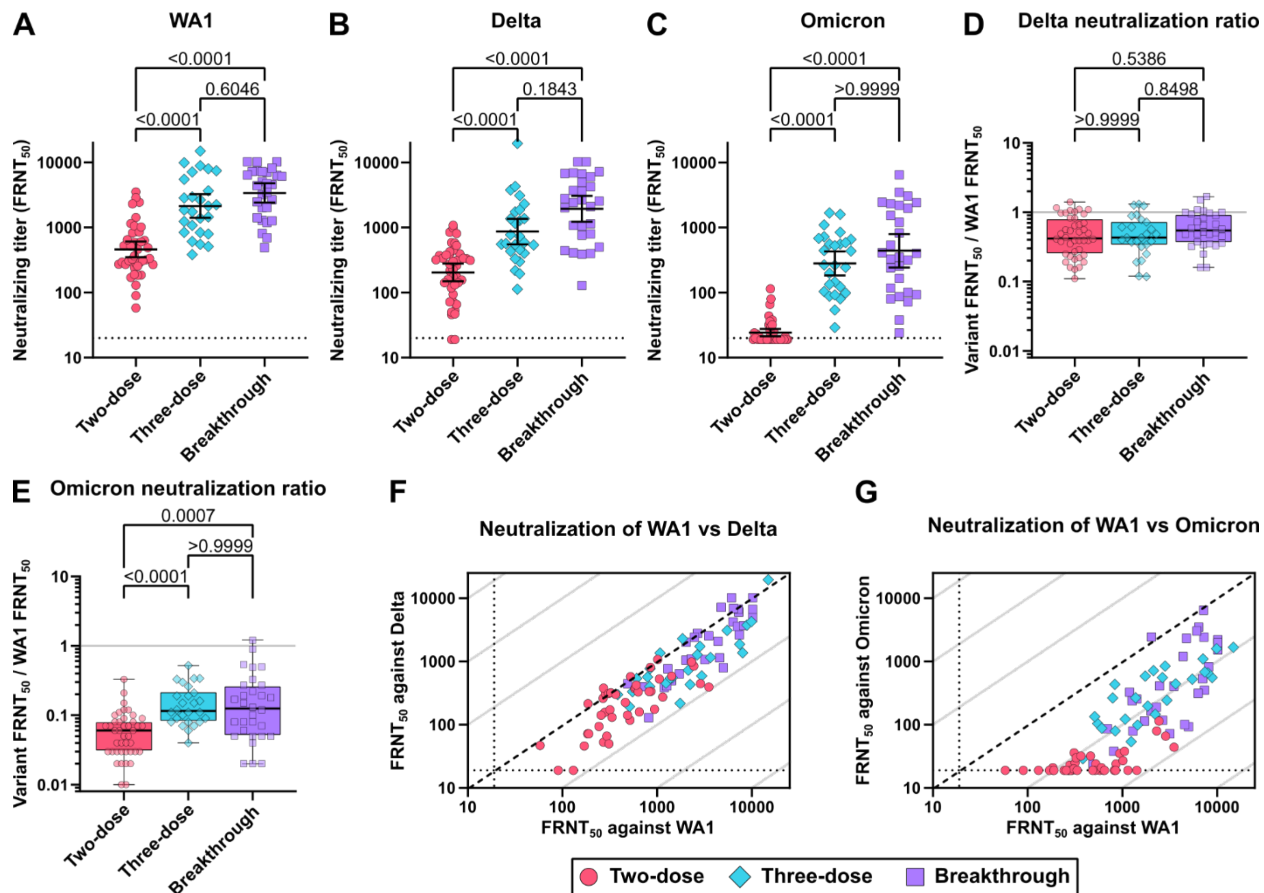

392

393 **Figure. 3:** Live SARS-CoV-2 neutralization by two-dose vaccination, three-dose vaccination and
394 breakthrough infection cohorts. (A) Original Wuhan strain WA1, (B) Delta variant, and (C)
395 Omicron variant neutralizing activity determined by 50% focus reduction neutralization test
396 ($FRNT_{50}$). Ratio of (D) Delta and (E) Omicron variant $FRNT_{50}$ over WA1 $FRNT_{50}$. The solid grey
397 lines indicate equal neutralization of variant and WA1. Scatter plots depicting (F) Delta and (G)
398 Omicron variant $FRNT_{50}$ versus WA1 $FRNT_{50}$. The broken lines indicate equal neutralization of
399 variants and WA1, while grey lines signify 10-fold differences. Error bars in A-C indicate the
400 geometric mean and 95% confidence intervals. Box plots in D and E show the median,
401 interquartile range, and full range. P-values in A-E show the results of two-tailed Kruskal-Wallis
402 tests with Dunn's multiple comparison correction.

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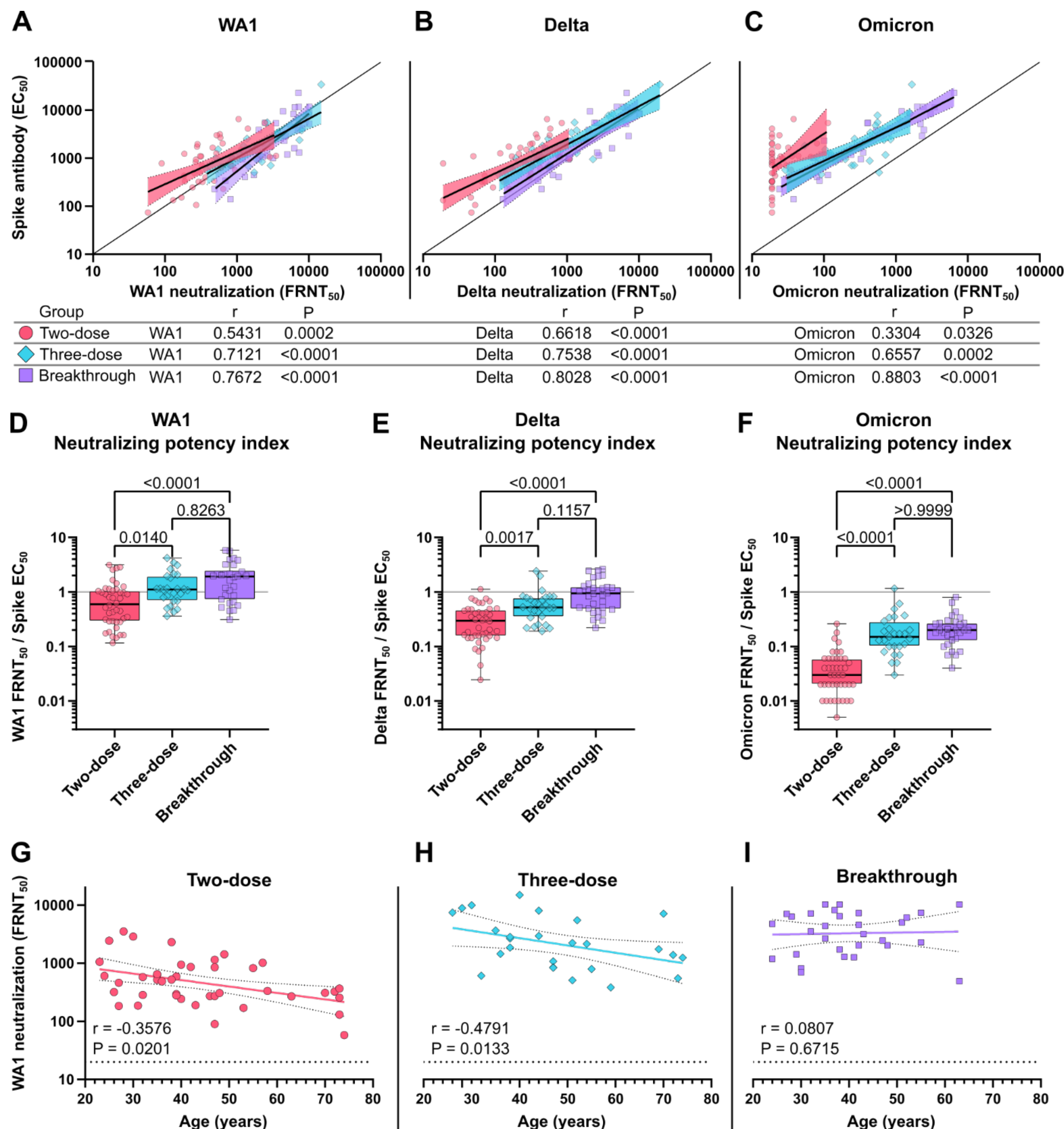

403

404 **Figure. 4:** Quality of the neutralizing antibody response to two-dose vaccination, three-dose
405 vaccination and breakthrough infection. Correlation of serum full-length spike-binding antibody
406 EC50 with (A) WA1 $FRNT_{50}$, (B) Delta $FRNT_{50}$, and (C) Omicron $FRNT_{50}$. The solid line
407 indicates equal EC_{50} and $FRNT_{50}$ values. Neutralizing potency indices indicate the ratio of (C)
408 WA1 $FRNT_{50}$, (D) Delta $FRNT_{50}$, and (E) Omicron $FRNT_{50}$ over full-length spike EC_{50}. The solid
409 line indicates equal EC_{50} and $FRNT_{50}$ values. Correlation of (G) WA1 $FRNT_{50}$, (H) Delta $FRNT_{50}$,
410 and (D) Omicron $FRNT_{50}$ with age at the time of study enrollment. r in A-C and G-H indicate the
411 Spearman correlation coefficients with corresponding two-tailed P values. Linear best fit-lines

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412 with 95% confidence bands were determined by simple linear regression of log transformed
413 EC_{50}, $FRNT_{50}$, and non-transformed Age values. Box plots in D-F show the median, interquartile
414 range, and full range. P-values in D-F show the results of two-tailed Kruskal-Wallis tests with
415 Dunn's multiple comparison correction.
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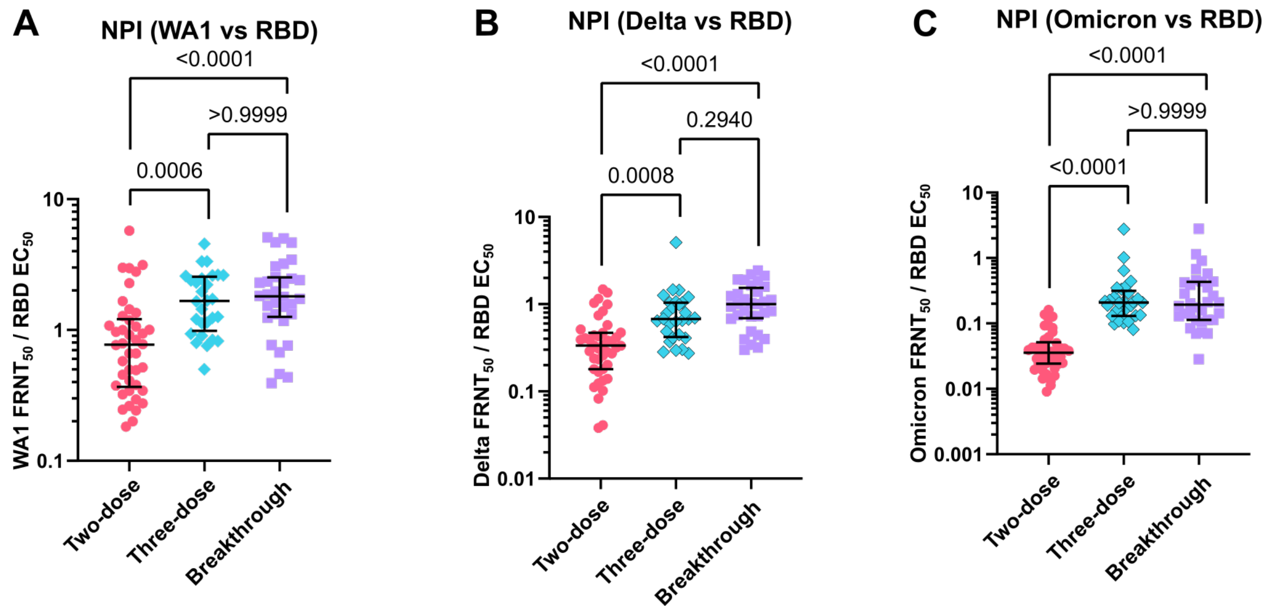

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419 **Figure 5**: Neutralizing potency indices (NPI) for receptor-binding domain antibodies. NPI
420 indicate the ratio of WA1 50% focus reduction neutralization test ($FRNT_{50}$) (panel A), Delta
421 $FRNT_{50}$, (panel B) and Omicron $FRNT_{50}$ (panel C) to spike receptor binding domain (RBD) EC_{50}.
422 Error bars indicate the median and interquartile range. P-values show the results of a two-tailed
423 Kruskal-Wallis test with Dunn's multiple comparison correction.
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425 **Table. 1:** Cohort demographics and clinical data

Characteristic	Two-dose	Three-dose	Breakthrough
Cohort characteristics			
N	42	27	30
Female - N (%)	35 (83.3)	19 (70.4)	23 (76.7)
Male - N (%)	7 (16.7)	8 (29.6)	7 (23.3)
Age (yr)	40 [23-74]	47 [26-74]	38 [24-63]
Time intervals (days)			
Last vaccine to blood draw	24 [17.25-35.75]	86 [79.5-93.5]	N/A
PCR positivity to blood draw	N/A	N/A	29 [23-47]
2nd vaccine to positive PCR	N/A	N/A	159.5 [81.25-202.25]
Time between 1st and 2nd vaccines	21 [21-22]	21 [21-22]	21 [21-23]
Time between 2nd and 3rd vaccines	N/A	253 [249-263.5]	N/A
Vaccine type			
BNT162b2 (Pfizer)	42 (100)	27 (100)	28 (93.3)
mRNA-1237 (Moderna)	0 (0)	0 (0)	1 (3.3)
Ad26.COV2.S (Janssen)	0 (0)	0 (0)	1 (3.3)
Breakthrough infection strain			
Alpha (B.1.1.7)	N/A	N/A	4 (16.7)
Beta (B.1.351)	N/A	N/A	1 (3.3)
Gamma (P.1)	N/A	N/A	3 (10)
Delta (B.1.617.2)	N/A	N/A	10 (33.3)
Unknown	N/A	N/A	11 (36.7)

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