

Correlates of Neutralizing/SARS-CoV-2-S1-binding Antibody Response with Adverse Effects and Immune Kinetics in BNT162b2-Vaccinated Individuals

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Running Title: Correlates of SARS-CoV-2-Neutralizing Antibody Response with Adverse Effects and Immune Kinetics in BNT162b2-Vaccinees

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1 **SUMMARY**

2 **Background** While mRNA vaccines against SARS-CoV-2 have been exceedingly effective in
3 preventing symptomatic viral infection, the features of immune response remain to be clarified.

4

5 **Methods** In the present prospective observational study, 225 healthy individuals in Kumamoto
6 General Hospital, Japan, who received two BNT162b2 doses in February 2021, were enrolled.
7 Correlates of BNT162b2-elicited SARS-CoV-2-neutralizing activity (50% neutralization titer: NT₅₀;
8 assessed using infectious virions and live target cells) with SARS-CoV-2-S1-binding-IgG and -IgM
9 levels, adverse effects (AEs), ages, and genders were examined. The average half-life of neutralizing
10 activity and the average time length for the loss of detectable neutralizing activity were determined
11 and the potency of serums against variants of concerns was also determined.

12

13 **Findings** Significant rise in NT₅₀s was seen in serums on day 28 post-1st dose. A moderate inverse
14 correlation was seen between NT₅₀s and ages, but no correlation was seen between NT₅₀s and AEs.
15 NT₅₀s and IgG levels on day 28 post-1st dose and pain scores following the 2nd shot were greater in
16 women than in men. The average half-life of neutralizing activity in the vaccinees was approximately
17 67.8 days and the average time length for their serums to lose the detectable neutralizing activity was
18 198.3 days. While serums from elite-responders (NT₅₀>1,500-fold: the top 4% among all participants'
19 NT₅₀s) potently to moderately blocked the infectivity of variants of concerns, some serums with
20 moderate NT₅₀s failed to block the infectivity of a beta strain.

21

22 **Interpretation** BNT162b2-elicited immune response has no significant association with AEs.
23 BNT162b2-efficacy is likely diminished to under detection limit by 6-7 months post-1st shot. High-
24 level neutralizing antibody-containing serums potently to moderately block the infection of SARS-
25 CoV-2 variants; however, a few moderate-level neutralizing antibody-containing serums failed to do
26 so. If BNT162b2-elicited immunity memory is short, an additional vaccine or other protective
27 measures would be needed.

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31 **Research in context**

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33 **Evidence before this study**

34 While mRNA vaccines against SARS-CoV-2 have been exceedingly effective in preventing
35 symptomatic viral infection, the salient features of immune response including the persistence of
36 protection remain to be clarified. There is a report that anti-SARS-CoV-2 antibodies persist through 6
37 months after the second dose of mRNA-1273 vaccine (Doria-Rose *et al. N Engl J Med.*
38 2021;384:2259-2261); however, more definite immune kinetics following mRNA-vaccine-elicited
39 protection have to be clarified. The mRNA-vaccine-elicited protection against SARS-CoV-2 variants
40 are also to be determined.

41

42 **Added value of this study**

43 In the present prospective study, 225 twice-BNT162b2-dose-receiving individuals in Japan were
44 enrolled. No significant correlation was seen between 50% neutralizing titers (NT_{50S}), determined by
45 using infectious SARS-CoV-2 virions and live target cells, and adverse effects. Largely, NT_{50S} and
46 IgG levels were greater in women than in men. Following 28 days post-2nd shot, significant reduction
47 was seen in NT_{50S}, IgG, and IgM levels. The average half-life of NT_{50S} was ~68 days and the average
48 time-length for participants' serums to lose the detectable activity was ~198 days. Although serums
49 from elite-responders potently to moderately blocked the infectivity of variants of concerns, some
50 serums with moderate NT_{50S} failed to block the infectivity of a beta strain.

51

52 **Implications of all the available evidence**

53 BNT162b2 efficacy is likely to be diminished to under detection limit by 6-7 months post-1st shot on
54 average. Individuals with moderate NT_{50S} may fail to block beta variants. If BNT162b2-elicited
55 immune memory is lost soon, additional vaccine(s) or other protective means would be needed.

56

57

58 **Introduction**

59 Since the emergence of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory
60 syndrome coronavirus 2 (SARS-CoV-2) in Wuhan, China, the disease quickly spread to the world. As
61 of June 29, 2021, more than 180 million SARS-CoV-2-infected individuals and almost 4 million death
62 cases have been reported in over 200 countries¹⁻⁴. Since the beginning of the pandemic, researchers
63 and pharmaceutical companies around the world have been working on developing vaccines⁵.
64 Currently, more than 10 vaccines have been authorized for public use worldwide. The development
65 of vaccines against SARS-CoV-2 was achieved time- and efficacy-wise beyond our expectations
66 within a single calendar year from the availability of the viral sequence to the initiation of
67 immunization of many people in several countries^{6,7}.

68 Among various vaccines, two RNA vaccines (BNT162b2 and mRNA-1273/TAK-919) have been
69 shown to be as much as 94-95% effective and safe⁸⁻¹⁰. In addition, inactivated vaccines or viral vector
70 vaccines have also been available in certain countries and areas^{5,7,9,10}. For example, the adenovirus-
71 vector-based vaccine (ChAdOx1 nCoV-19/AZD1222) has reportedly achieved 62% efficacy in initial
72 trials¹¹. The phase 3 reports of another adenovirus-based vaccine (Ad26.COV2.S) has indicated 85%
73 efficacy against severe disease or death^{12,13}.

74 However, the recent emergence of various SARS-CoV-2 variants with mutations in the spike
75 region is raising concerns about the efficacy of vaccines. The D614G and B.1.1.7 (alpha/N501Y)
76 variants appear to be without antigenic escape^{14,15}. However, the B.1.351 (beta) variant is reportedly
77 represents a neutralization escape variant to convalescent sera¹⁶. The phase 3 results of NVX-
78 CoV2373 (a nanoparticle, protein-based vaccine) from the United Kingdom indicated 89% efficacy
79 with over 50% of cases attributable to the more transmissible alpha variant¹⁷. However, a phase 2b
80 trial in South Africa showed 60% efficacy, in which approximately 90% of the endpoints occurred in
81 subjects infected with the beta variant^{18,19}, suggesting that the beta variant is less susceptible to
82 antibodies elicited with the original Wuhan strain antigens, which is in the composition of all the
83 vaccines currently being evaluated⁷. Another recent concern is the emergence of a B.1.617 (delta)
84 variant, which was first detected in India, is now spreading around the world. This variant of concern
85 (VOC) seems to have less susceptibility to vaccine-elicited protection and increased transmissibility
86 beyond alpha strains²⁰.

87 In the present study, we examined neutralizing activity and S1-binding-antibody response in
88 BNT162b2-vaccinated health care workers (n=225) in Japan. We also investigated the correlation
89 among neutralizing activity levels, S1-binding-IgG and -IgM levels, genders, and adverse events.
90 Decline of BNT162b2-elicited immune response and activity of the elite and moderate responders
91 against VOCs were also investigated.

92

93 **Methods**

94 **Participants and serum specimens.**

95 Serum samples were collected from 225 vaccinated health care workers at JCHO Kumamoto
96 General Hospital (Kumamoto, Japan). All the 225 participants were of Japanese citizen. Serum
97 samples were analyzed at the National Center for Global Health and Medicine (NCGM) in Tokyo.
98 The Ethics Committees from the Kumamoto General Hospital and NCGM approved this study
99 (Kumamoto General Hospital No. 180, and NCGM-G-004176-00, respectively). Each participant
100 provided a written informed consent, and this study abided by the Declaration of Helsinki principles.
101 The vaccination (on days 0 and 21) and serum collection (from day 7 through day 90 post-1st shot)
102 were conducted as shown in Table 1.

103

104 **Cells and viruses.**

105 VeroE6^{TMPRSS2} cells²¹ were obtained from Japanese Collection of Research Bioresources (JCRB)
106 Cell Bank (Osaka, Japan). VeroE6^{TMPRSS2} cells were maintained in DMEM supplemented with 10%
107 FCS, 100 µg/ml of penicillin, 100 µg/ml of streptomycin, and 1 mg/mL of G418. SARS-CoV-2
108 NCGM-05-2N strain (SARS-CoV-2_{05-2N}) was isolated from nasopharyngeal swabs of a patient with
109 COVID-19, who was admitted to the NCGM hospital^{22,23}. Five clinically isolated SARS-CoV-2
110 mutant strains were used in the current study: two B.1.1.7 (alpha) strains [hCoV-
111 19/Japan/QHN001/2020 (SARS-CoV-2_{QHN001}, GISAID Accession ID; EPI_ISL_804007) and hCoV-
112 19/Japan/QK002/2020 (SARS-CoV-2_{QK002}, GISAID Accession ID; EPI_ISL_768526)] and a B.1.351
113 (beta) strain [hCoV-19/Japan/TY8-612-P0/2021 (SARS-CoV-2_{TY8-612})] were obtained from National
114 Institute of Infectious Diseases, Tokyo, Japan. A B.1.617.1 (kappa) strain [TKYTK5356_2021
115 (SARS-CoV-2₅₃₅₆, DDBJ Accession ID; LC633761)] and a B.1.617.1 (beta) strain [hCoV-
116 19/Japan/TKYK01734/2021 (SARS-CoV-2₁₇₃₄, GISAID Accession ID; EPI_ISL_2080609)] were
117 provided from Tokyo Metropolitan Institute of public Health, Tokyo, Japan. Each variant was
118 confirmed to contain each VOC-specific amino acid substitutions before the assays conducted in the
119 present study (*vide infra*).
120

121

121 **Neutralization assay.**

122 The neutralizing activity of serums from vaccinated individuals was determined by quantifying
123 the serum-mediated suppression of the cytopathic effect (CPE) of each SARS-CoV-2 strain in
124 VeroE6^{TMPRSS2} cells as previously described with minor modifications²². In brief, each serum was 4-
125 fold serially diluted in culture medium. The diluted sera were incubated with 100 50% tissue culture
126 infectious dose (TCID₅₀) of viruses at 37°C for 20 min (final serum dilution range of 1:20 to 1:4000),
127 after which the serum-virus mixtures were inoculated to VeroE6^{TMPRSS2} cells (1.0 x 10⁴/well) in 96-
128 well plates. For SARS-CoV-2 strains used in this assay are as follows: a wild-type strain, SARS-CoV-
129 205-2N (PANGO lineage B)^{22,23}, two alpha variants (SARS-CoV-2_{QHN001} and SARS-CoV-2_{QK002}), a beta

130 variant SARS-CoV-2_{TY8-612}, a delta variant SARS-CoV-2₁₇₃₄, and a kappa variant SARS-CoV-2₅₃₃₆.
131 After culturing the cells for 3 days, the levels of CPE observed in SARS-CoV-2-exposed cells were
132 determined using the WST-8 assay employing Cell Counting Kit-8 (Dojindo, Kumamoto, Japan). The
133 serum dilution that gave 50% inhibition of CPE was defined as the 50% neutralization titer (NT₅₀).
134 Each serum was tested in duplicate.

135

136 **Measurement of anti-SARS-CoV-2 antibody titers.**

137 Measurement of 3 anti-SARS-CoV-2 antibody levels (anti-S1-IgG, anti-S1-IgM, and anti-N-IgG)
138 in each participant was performed using the chemiluminescence enzyme immunoassay (CLEIA)
139 platform (HISCL) manufactured by Sysmex Co. (Kobe, Japan) as previously reported²⁴.

140

141 **Statistical analyses.**

142 Out of the 225 participants, one participant, who had been infected by SARS-CoV-2 with PCR
143 positivity documented was primarily excluded. Demographic characteristics of the participants are
144 described in Table 1. Correlates of neutralizing activity levels with S1-binding-IgG and -IgM levels,
145 ages, genders, pain scores in the injection-site, and systemic fever up to 40°C were examined by
146 Spearman rank correlation coefficient. Also, neutralizing activity levels, S1-binding-IgG, and -IgM,
147 pain scores, systemic fever were compared between genders using the Wilcoxon rank sum test. As for
148 participants with normal fever, their temperature was treated as 36.89 degree, a normal body
149 temperature in Japanese²⁵. Percentage of the adverse events reported in writing following the 1st and
150 2nd dose administration were determined and assessed in regard to gender. The differences in
151 neutralization activity between each measurement time point were tested by the Wilcoxon rank sum
152 test, and were assessed among categorized age subgroups. Similarly, difference of S1-binding-IgG
153 and -IgM levels among time points were tested. Decline of neutralizing activity over 90 days post-1st
154 shot was assessed using the mixed-effects model including time as a main effect and intercept as a
155 random effect. Also, the prediction slope and its 80% prediction interval were generated by drawing
156 a sampling distribution for the fixed effects and then estimating the fitted value across that distribution.
157 The merTools package (version 0.5.2) in R software was used for prediction. The decline for S1-
158 binding-IgG and -IgM levels was assessed similarly. All the analyses were conducted with the use of
159 R, version 4.1.0 (R Foundation for Statistical Computing).

160

161 **Results**

162 **Demographic characteristics and immune response in the participants.**

163 We obtained blood samples for antibody testing from a total of 225, 220, 211, and 210 vaccine
164 recipients on days 7, 28, 60, and 90 post-1st shot, respectively (Table 1). Demographic characteristics
165 of the participants are shown in Table 1. As of the time of enrollment, the average age of the

166 participants was 41.8 years (range: 21 to 72 years), and 69.8% of the participants were female serving
167 as a physician, nurse, paramedical staff, or administrative staff. None of the participants was in the
168 immunodeficient state or was receiving immunosuppressants or steroids.

169 We first determined the neutralizing activity against SARS-CoV-2 in serum samples taken on day
170 7 post-1st shot from 225 participants; however, none of the samples showed detectable neutralization
171 activity ($NT_{50} < 20$ -fold). We then determined the neutralizing activity in samples taken on day 28
172 post-1st shot from 220 vaccinated participants. As shown in [Figure 1A](#), NT_{50} levels were substantially
173 diverse among the participants: the geometric mean of NT_{50} values was 375.2 (range 25.6-2680.0).
174 Very low or no correlation of the NT_{50} values with ages was identified ([Figure 1A](#): Spearman's $\rho = -0.22$;
175 95% CI -0.34 to -0.09). We also examined the levels of S1-binding-IgG and -IgM levels using
176 the HISCL system that enables quantitative and highly sensitive determination of S1-binding-IgG and
177 -IgM levels²⁴. The geometric mean of S1-binding-IgG values was 527.0 (range 44.6-3212.2), while
178 that of S1-binding-IgM was 85.1 (range 10.3-1406.5). There was a high positive correlation of the
179 NT_{50} values with S1-binding-IgG levels (Spearman's $\rho = 0.71$; 95% CI 0.63 to 0.77) as examined on
180 day 28 post-1st shot, while there was only a low positive correlation of the NT_{50} values with S1-
181 binding-IgM levels (Spearman's $\rho = 0.43$; 95% CI 0.31 to 0.53), suggesting that the neutralizing
182 activity largely resides in IgG fraction of the serum of vaccinated participants around on day 28 post-
183 1st shot ([Figures 1B and 1C](#)). However, when examined on day 60 post-1st shot, the correlations of
184 NT_{50} levels with both IgG and IgM levels became moderate or low (Spearman's $\rho = 0.56$ and 0.32,
185 respectively) ([Figure S1](#)).
186

187 **The occurrence of adverse effects has no association with the BNT162b2-elicited neutralizing 188 activity levels.**

189 Commonly observed adverse events reported following BNT162b2 vaccination include injection-
190 site pain, systemic fever, headache, and fatigue¹⁰. In the present study, the events were observed largely
191 more often following the 2nd shot ([Figure S2](#)) as previously reported by Polack *et al.*¹⁰ Pains in the
192 inject-site were reported by 67.6 and 61.6% of the participants and systemic fever ($\geq 37.1^{\circ}\text{C}$) was
193 reported by 3.6 and 46.4% of the participants following the 1st and 2nd shots, respectively. Since the
194 severity of pains can be relatively more quantitatively rated than that of other adverse effects such as
195 headache and fatigue, the possible correlate of the NT_{50} values with the severity of pains rated with
196 the short form McGill pain questionnaire²⁶ was first examined. No correlation was seen between the
197 NT_{50} values and the pain grades assessed following the 2nd shot (Spearman's $\rho = 0.14$; 95% CI 0.00 to
198 0.26). The correlation was also negligible between the NT_{50} values and the incidence of systemic fever
199 (Spearman's $\rho = 0.26$; 95% CI 0.13 to 0.38) ([Figures 2A and 2B](#)).
200

201 **The average half-life of neutralizing activity in the vaccinees is approximately 67.8 days and**
202 **the average time-length for their serums to lose the detectable activity is 198.3 days.**

203 Considering that recent multiple clinical studies strongly suggest that the presence of high-level
204 neutralizing antibodies is generally sufficient to confer protection against SARS-CoV-2 infection and
205 that the protection against COVID-19 development is largely explained by robust SARS-CoV-02-
206 neutralizing antibody response⁸⁻¹⁰. If so, the once-established neutralizing antibody levels will
207 decrease in time. We thus examined at what rate the levels of NT₅₀ and S1-bindng-IgG and -IgM levels
208 change by determining those levels from the data on day 28 (n=220), day 60 (n=211), and day 90
209 (n=210) post-1st shot (Figures 3A-C). The reduction of all NT₅₀, IgG, and IgM levels from day 28
210 through day 90 post-1st shot was found to occur virtually linearly. By computation, the predicted
211 average half-life of all the NT₅₀ values turned out to be 67.8 days and those of S1-binding-IgG and
212 IgM levels were 53.5 days and 43.6 days, respectively (Figure 3D). The half-life of the NT₅₀ values
213 and that of S1-binding-IgG were reasonably comparable, corroborating that the neutralizing activity
214 largely resides in the S1-binding IgG fraction. Based on the chronologically linear nature of the
215 reduction identified, we attempted to extrapolate from such half-life values and tried to predict the
216 average time-length for the serums of the participants having significant NT₅₀ values to lose the
217 activity down to under the undetectable level (UDL)(<20-fold)(Figure 3D). The predicted average
218 time-length for the serums to lose the activity was computated to be 198.3 days, while that of the top
219 10% participants to lose the activity was 204.3 days. The time-length of the middle 10% (between the
220 top 45% and 55%) participants to lose the activity was 187.6 days. For all participants, it was predicted
221 that day 160 after the 1st shot was when the 80% lower limit of predicted NT₅₀ levels drops under the
222 detection level (UDL), while day 237 after the 1st shot was when the 80% upper limit of predicted
223 NT₅₀ levels drops below UDL. Similarly, for the top 10% participants, the estimated days dropping
224 below UDL were 171 and 243 for lower and upper limits, respectively, while for the middle 10%, the
225 estimated days were 155 and 224, respectively. As for IgG, the predicted time-length for the serums
226 to get undetected was 346.8 days, and days reaching below UDL were 333 and 362 for lower and
227 upper limits, respectively. Likewise, the estimated day reaching below UDL for IgM was 183.6, and
228 the lower and upper limits were 170 and 198 days, respectively (Figure 3D). We also asked whether
229 the chronological decay rate of neutralization titers and S1-binding-IgG and -IgM differs among three
230 age subgroups: (i) 20-39 yo, (ii) 40-59 yo, and (iii) 60's and beyond. No significant difference was
231 identified among the three age subgroups in the levels of neutralizing titers, IgG, or IgM levels ($p=0.60$,
232 0.16, and 0.11, respectively: Figures S3A-C). The present data suggest that vaccinated individuals
233 with good neutralization response would lose BNT162b2's protection in 6 to 7 months without regard
234 to age subgroups unless such people achieve robust immune boost response upon the future exposure
235 to SARS-CoV-2. Otherwise, they should be protected by another booster vaccine shot or by other
236 protective means.

237

238 **Neutralization titers, S1-binding-IgG levels, and pain scores in the injection site were greater in
239 women than in men.**

240 We then asked whether there were differences between genders in neutralization activity levels,
241 S1-bindng-IgG and -IgM levels, injection-site pain scores, and systemic fever grades. Statistically
242 significant differences were identified in the levels of neutralization determined on 60 and 90 days
243 post-1st shot (p=0.002 and 0.002, respectively), S1-binding-IgG levels determined on 28, 60, and 90
244 days (p<0.001, p=0.001, and p=<0.001, respectively) post-1st-shot, and S1-binding-IgM levels on 60
245 and 90 days post-1st shot (p=0.025 and 0.044, respectively)(Figures S4A-C). The injection-site pain
246 score was greater in women (p<0.001) (Figure S4D). However, there was no difference in systemic
247 fever grades between genders (Figure S4E). However, no difference was seen in the decline rates of
248 neutralization activity, S1-binding-IgG and -IgM levels between men and women (Figure S5).

249

250 **Some serums retain potent activity against various VOCs, but others showed substantially less
251 potent or undetectable activity.**

252 We finally asked whether the neutralizing antibodies elicited by BNT162b2 vaccination blocked
253 the infectivity and replication of various variants of concerns (VOCs). To this end, we employed serum
254 samples from 6 elite responders (NT₅₀ values >1,500-fold: the top 4% of all participants' NT₅₀ values
255 as determined on 28 days post-1st dose) and serum samples from twelve randomly-selected moderate
256 responders (NT₅₀ values=200~1,500-fold) and tested them for their inhibition of the infectivity and
257 cytopathic effect of each variant in the VeroE6^{TMPRSS2} cell-based assay²¹. As shown in Figure 4, serums
258 from the elite responders (n=6) showed potent inhibition against SARS-CoV-2_{05-2N} (Wuhan strain,
259 PANGO lineage B), while they showed less activity against SARS-CoV-2_{QHN001} and SARS-CoV-
260 2_{QK002} (alpha), SARS-CoV-2₅₃₅₆ (kappa), SARS-CoV-2₁₇₃₄ (delta), and SARS-CoV-2_{TY8-612} (beta).
261 Serums from moderate responders (n=12) exerted less activity against the Wuhan strain than those
262 from the elite responders. Some serums from the moderate responders also showed substantially low
263 potency to all the VOCs tested. Notably, three serums from the moderate responders showed only
264 marginal activity against SARS-CoV-2_{TY8-612} (beta). Two of those three samples had no detectable
265 inhibitory activity against SARS-CoV-2_{TY8-612} (Figure 4).

266

267 **Discussion**

268 In this prospective observational study, 225 healthy individuals [physicians (n=36), nurses
269 (n=125), and other healthcare professionals (n=64)], who received two doses of 30 µg BNT162b2
270 (Pfizer–BioNTech) vaccine in February 2021, were enrolled, and the correlates of neutralization
271 activity represented by 50% neutralization titers (NT₅₀) determined by employing the target living
272 VeroE6^{TMPRSS2} cells and live SARS-CoV-2 with ages, adverse effects (AEs) that occur often such as

273 injection-site pain and systemic fever were examined. The kinetics of NT₅₀ values and S1-binding
274 antibody levels were also examined. There was a significant rise in the NT₅₀ values as determined on
275 day 28 post-1st shot (a week after post 2nd shot) compared to those on day 7 post-1st shot. Correlation
276 was negligible between NT₅₀ values and ages or systemic fever grades. In this regard, most adverse
277 effects that occur within 1-3 days following vaccine shots are thought to be caused by the release of
278 certain pyrogenic and inflammatory cytokines (*e.g.*, interleukin-1, interleukin-6, and tumor-necrosis
279 factor) from antigen-presenting cells (APCs) such as macrophages and dendritic cells when they ingest
280 and process the exogenous antigens (*i.e.*, SARS-CoV-2 spike antigens) and transmit the antigenic
281 information to relevant immune cells. Such early-phase defense events include response to antigenic
282 determinants irrelevant to neutralizing activity but those eliciting S1-binding antibodies. The release
283 of the pyrogenic and inflammatory cytokines mostly subsides within days following the vaccine shot.
284 The released cytokines activate the antigen-specific-antibody-producing B-cells, which respond to the
285 processed antigenic determinants presented by the APCs and start to produce antigen-specific
286 antibodies such as neutralizing antibodies as well as non-neutralizing but S1-binding antibodies. Such
287 antigen-specifically-activated and antibody-producing B-cells continue to produce antibodies. In the
288 case of BNT162b2 vaccination, it appears that it takes 10 to 12 days from the 1st vaccine shot for the
289 vaccinated individuals to achieve the amounts of neutralizing antibodies that are enough to block the
290 infection of substantial numbers of the virally-targeted cells and to inhibit further spread of the
291 infection^{9,10}. It is thought that the release of pyrogenic and inflammatory cytokines and the build-up
292 of the protective antibody levels are different events, occurring chronologically ~10-12 days apart.
293 These two different events appear to have resulted in the absence of significant correlates between
294 NT₅₀ levels and AEs examined in the present study.

295 In the present study, the NT₅₀ values had a substantial correlation with S1-binding-IgG levels but
296 had only moderate correlation with S1-binding IgM levels, suggesting that the major neutralizing
297 activity resides within the S1-binding IgG fraction. Interestingly, the approximate half-life of NT₅₀
298 values (67.8 days) and that of S1-binding-IgG levels (53.5 days) were reasonably close to each other,
299 corroborating the assumption of the presence of the major fraction of neutralizing antibodies within
300 IgG fraction. In human body, IgG has concentration-dependent half-life of approximately 21 days and
301 IgM around 5-6 days²⁷. By contrast, the half-lives of NT₅₀, S1-bindng-IgG, and -IgM levels determined
302 in the present study were much longer with 43.6-67.8 days. This discrepancy is perhaps attributed to
303 the persistence of continuously-antibody-producing B cells over weeks or months in the body of the
304 participants following the vaccination^{28,29}, thereby the half-lifes of neutralizing activity and S1-
305 binding-IgG and -IgM levels have been extended as compared to the physiological half-lifes of IgG
306 and IgM. However, the assumption of the half-lifes in the present work was based on the
307 chronologically linear nature of the reduction observed during the present study period (Figure 3D).
308 Also, the sensitivity and quantitativeness of S1-binding-IgG and -IgM levels determined with using

309 the chemiluminescence enzyme immunoassay (CLEIA) platform (HISCL)²⁴ were much greater (the
310 dynamic range is 0.1 to 2,000 SU/ml)²⁴ than that of neutralizing activity, whose dynamic range is 20
311 to 4,000²². Thus, the time length of S1-binding-IgG becoming under detection levels was calculated
312 to be longer than that of neutralizing activity, although it should be noted that the protective effect of
313 BNT162b2 judged by neutralizing activity is most likely associated with clinical outcomes. In
314 addition, recently, Doria-Rose et al. reported that anti-SARS-CoV-2 antibodies persist through 6
315 months after the second dose of mRNA-1273 administration³⁰. However, the study was of a relatively
316 small scale (n=33), and more definite data are needed for constructing more protective measures.
317 However, a caution should be used in assuming half-lives since we presently have no knowledge as to
318 how long neutralizing antibody- or S1-binding antibody-producing B-cells continue to produce
319 antibodies following the administration of two doses of BNT162b2. If such B-cells produce antibodies
320 for a shorter period of time than we assumed in the present study, the half-lives of neutralizing and S1-
321 binding antibodies could be shorter than we estimated in the present work.

322 There is a growing body of evidence that COVID-19 results in more severe symptoms and greater
323 mortality among men than among women^{31,32}. A cohort study of 17 million adults in England has
324 revealed a strong association between male sex and the risk of death from COVID-19 (hazard ratio
325 1.59, 95% confidence interval 1.53–1.65)³³. In the present data set, significantly greater levels of NT₅₀,
326 S1-binding-IgG and -IgM were documented in women than in men when examined on 28, 60, and 90
327 days post-1st shot, while there was no difference in either of NT₅₀, S1-binding-IgG or -IgM levels on
328 day 7 post-1st dose (Figures S4A-C). These results apparently relate to the findings by others reporting
329 that women, in general, have more robust ability to control infectious pathogens (*i.e.*, SARS-CoV-2)
330 than men^{33,34}. Indeed, there is increasing evidence indicating strong correlation between SARS-CoV-
331 2-neutralizing antibody titers and clinical efficacy, suggesting that a neutralizing antibody response
332 provides the primary contribution to protection against COVID-19³⁵ and that the presence of high
333 levels of neutralizing antibody is largely sufficient for protection against SARS-CoV-2 infection and
334 clinical onset upon exposure to the virus^{36,37}. In fact, Imai *et al.* have reported that the administration
335 of convalescent plasma from previously-SARS-CoV-2-infected hamsters completely protected newly
336 SARS-CoV-2-exposed hamsters from contracting viral pneumonitis³⁸. Thus, the greater neutralizing
337 activity in women than in men observed in the present study can contribute at least in part to the gender
338 differences in COVID-19 disease outcomes. Also, of note, the number of participants with ages greater
339 than 60 years was rather small (18 of 225), which might have made the statistical power insufficient
340 to find significant differences with other two age groups (20-39 yo and 40-59 yo groups)(Figures S3A-
341 C).

342 We also examined how the BNT162b2-elicited neutralizing antibodies blocked the infectivity and
343 cytopathic effect of five different variants of concerns in the cell-based assays using various infectious
344 variants (one Wuhan strain, two alpha strains, one strain each of beta, delta and kappa strains). Six

345 selected serums from elite responders showed quite potent activity to the alpha, kappa, and delta
346 variants, while they exerted relatively moderate activity against the beta strain (Figure 4). On the other
347 hand, one of the randomly-selected 12 serums from moderate responders showed a marginal activity
348 (NT_{50} value of 40-fold) and two of them failed to show detectable activity (NT_{50} values <20-fold)
349 against the beta strain (Figure 4). These data suggest that BNT162b2-receiving vaccinees who develop
350 high magnitudes of neutralizing antibody should probably be well protected against the infection by
351 most variants; however, those who develop only low levels of neutralizing antibody may be vulnerable
352 to the infection by certain variants such as beta strains. If so, to such low-responders to BNT162b2
353 even after the 2nd shot, an additional 3rd shot may be needed. If the 3rd dose of the same vaccine fails
354 to elicit good levels of neutralizing antibodies, new types of vaccines with different platform have to
355 be stratified.
356

357 **Contributors**

358 KM and HM had access to all data in this study and took and hold all responsibility for the integrity
359 of the data and the accuracy of the data analysis. KM and HM: Concept and design. KM, MA, KT,
360 TM, KN, YT, HG, KY, SO, TS, and TY: Acquisition, analysis, or interpretation of data. YU, YS, AF,
361 and HM: Statistical analysis. AF, YI, HN, MK, SM, AM, WS, and SS: Administrative, technical, or
362 material support. KM and HM: Original draft writing. All authors: Reading and writing manuscript.
363

364 **Declaration of Interests**

365 Matsushima, Noda, Sato, and Yoshida are employees of Sysmex Corporation.
366

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380

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- 467

468 **Figure Legends**

469 **Figure 1:** Correlations of neutralizing titers with ages and S1-binding-IgG and -IgM levels. A.
470 Correlation between neutralizing titers (NT_{50s}) and ages (on day 28 post-1st shot). The age range of
471 the study participants was 21 to 72 (average 41.8 y.o.). A correlation is negligible between NT_{50} values
472 and ages (Spearman's $\rho=-0.22$; 95% CI -0.34 to -0.09). The geometric mean NT_{50} of the values from
473 all participants ($n=225$) was 375.2-fold (range 25.6-2,680-fold), greater by a factor of 2.3 than the
474 geometric mean NT_{50} from 65 COVID-19-convalescent patients (geometric mean=163.0-fold; range
475 20.0-1470-fold) shown as references on the far right (human COVID-19-convalescent serum: HCS).
476 B. A high correlation is identified (Spearman's $\rho=0.71$; 95% CI 0.63 to 0.77) between NT_{50} values and
477 S1-binding-IgG levels in samples obtained on day 28 post-1st dose. C. Moderate correlation is seen
478 between neutralizing titers and S1-binding-IgM levels (Spearman's $\rho=0.43$; 95% CI 0.31 to 0.53). One
479 participant, who had been infected with SARS-CoV-2 with PCR-positivity documented, is indicated
480 as a solid-red solitary circle. This participant was excluded from all analyses at later timepoints.

481

482 **Figure 2:** Correlations of neutralizing titers with injection-site pain scores and systemic fever grades.

483 A. No correlation was seen between NT₅₀ values and injection-site pain (Spearman's $\rho=0.14$; 95% CI
484 0.00 to 0.26). The injection-site pain following the 2nd BNT162b2 dose was scored by using the short-
485 form McGill Pain Questionnaire²⁶. B. Correlation was negligible between NT₅₀ values and systemic
486 fever grades (Spearman's $\rho=0.26$; 95% CI 0.13 to 0.38). A solid-red circle indicates a person with
487 previous SARS-CoV-2 infection documented.

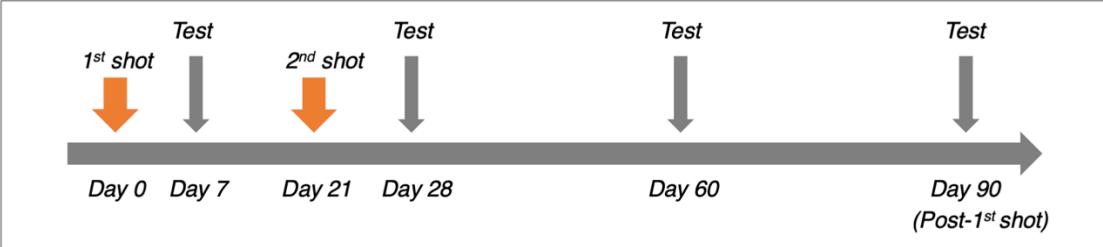
488

489 **Figure 3:** Kinetics of neutralizing activity and S1-binding-IgG and -IgM levels. Time-course analyses
490 of neutralizing activity for 90 days were conducted. The 1st vaccine was administered on day 0, and
491 the 2nd vaccine on day 21. Blood samples from vaccinated individuals were obtained on days 7, 28,
492 60, and 90 post 1st shot as illustrated in Table 1. A. Neutralizing activity is shown as NT₅₀ (50%
493 neutralizing titer). The NT₅₀ value of 20-fold is the detection limit and values determined to be less
494 than 20-fold were treated as 20-fold. B and C. Kinetics of S1-binding-IgG and -IgM levels are shown.
495 The average values of each data point are shown in black solid circles, which are connected with solid
496 black lines. One participant, who had been infected with SARS-CoV-2 with PCR-positivity
497 documented, is indicated as a solid-red solitary circle in B and C. This participant was excluded from
498 all analyses at later timepoints. D. Decline of neutralizing activity, S1-binding-IgG and -IgM over 90
499 days post-1st shot. The solid-black lines consist of predicted values estimated by mixed effects model,
500 and the shaded areas denote corresponding 80% prediction intervals. The dashed horizontal lines in
501 the upper three panels denote the NT₅₀ detection limit of 20-fold. NT₅₀ values determined to be less
502 than 20-fold were treated as 20-fold. The lowest detection limit for S1-binding-IgG and -IgM
503 quantification shown as dashed horizontal lines in the two lower panels was 0.1 SU/ml and the values
504 lower than 0.1 SU/ml were calculated as 0.1 SU/ml.

505

506 **Figure 4:** Blockade of the infectivity and replication of SARS-CoV-2 variants by vaccinees' serums.
507 The activity of vaccinees' serums to block the infectivity and replication of 5 SARS-CoV-2 variants
508 (alpha variants: SARS-CoV-2_{QHN001} and SARS-CoV-2_{QK002}; a beta strain: SARS-CoV-2_{TY8-612}; a delta
509 strain: SARS-CoV-2₁₇₃₄; and a kappa strain: SARS-CoV-2₅₃₅₆) was evaluated. A Wuhan strain 05-2N¹⁹
510 was employed as a reference SARS-CoV-2. Six serums were from elite responders (NT₅₀>1,500-fold)
511 and 12 serums were from randomly-selected moderate responders (NT₅₀=200~1,500-fold). The NT₅₀
512 titers of each serum against 6 SARS-CoV-2 strains are shown in red circles (for 6 elite responders)
513 and in black circles (for 12 moderate responders). D043 is a serum from a COVID-19-convalescent
514 patient³⁹ and served as an internal control in the assays.

Table 1: Study protocol and demographic characteristics of the participants.

 A horizontal timeline diagram showing the study protocol. It starts at Day 0 with a grey arrow pointing to the right. An orange downward arrow labeled '1st shot' points to the arrow at Day 0. A grey downward arrow labeled 'Test' points to the arrow at Day 7. Another orange downward arrow labeled '2nd shot' points to the arrow at Day 21. A grey downward arrow labeled 'Test' points to the arrow at Day 28. A grey downward arrow labeled 'Test' points to the arrow at Day 60. A final grey downward arrow labeled 'Test' points to the arrow at Day 90, which is also labeled '(Post-1 st shot)'.						
		Day7 (Post 1 st dose)		Day28	Day60	Day90
All		225		220	211	210
Age	20-39	97	43.1	92	84	84
	40-59	110	48.9	110	109	108
	≥60	18	8.0	18	18	18
	(Average)	(41.8 y.o.)				
Gender	Men	68	30.2	68	63	61
	Women	157	69.8	52	148	149
Job	Physicians	36	16.0			
	Nurses	125	55.6			
	Others	64	28.4			

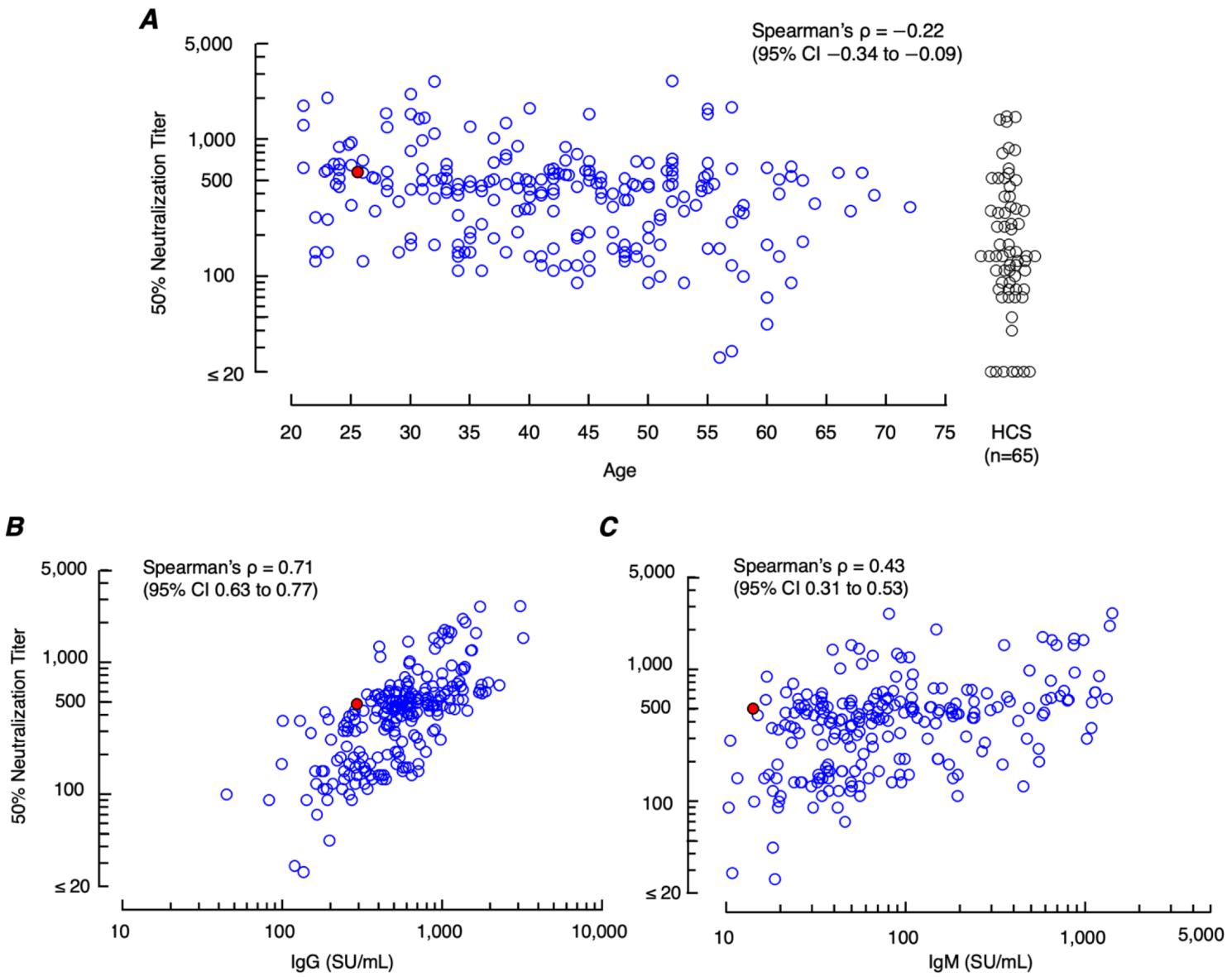


Figure 1

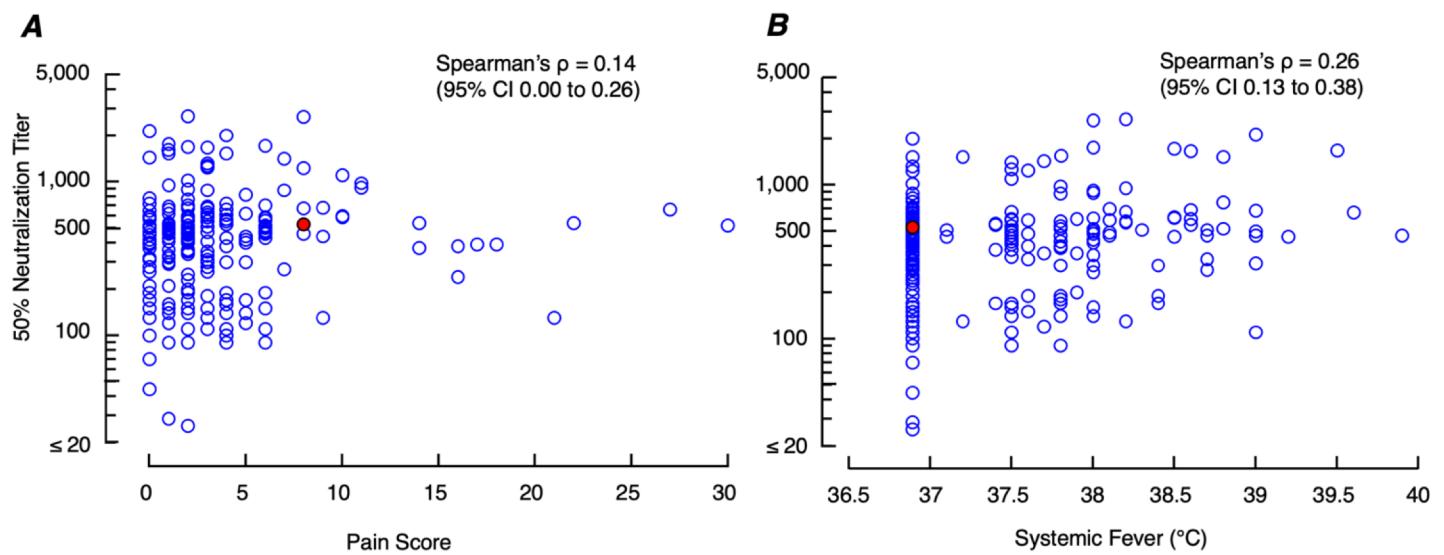


Figure 2

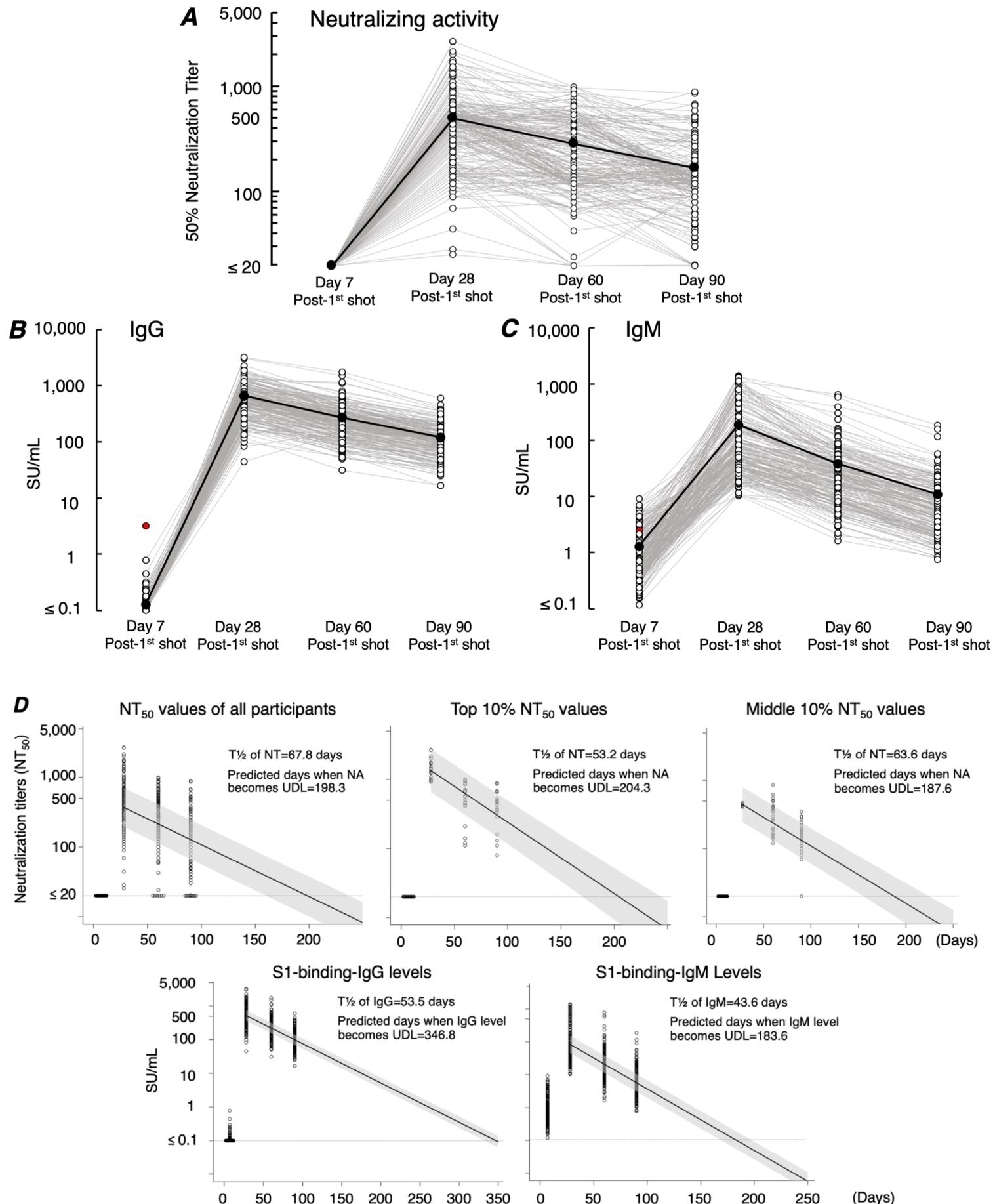


Figure 3

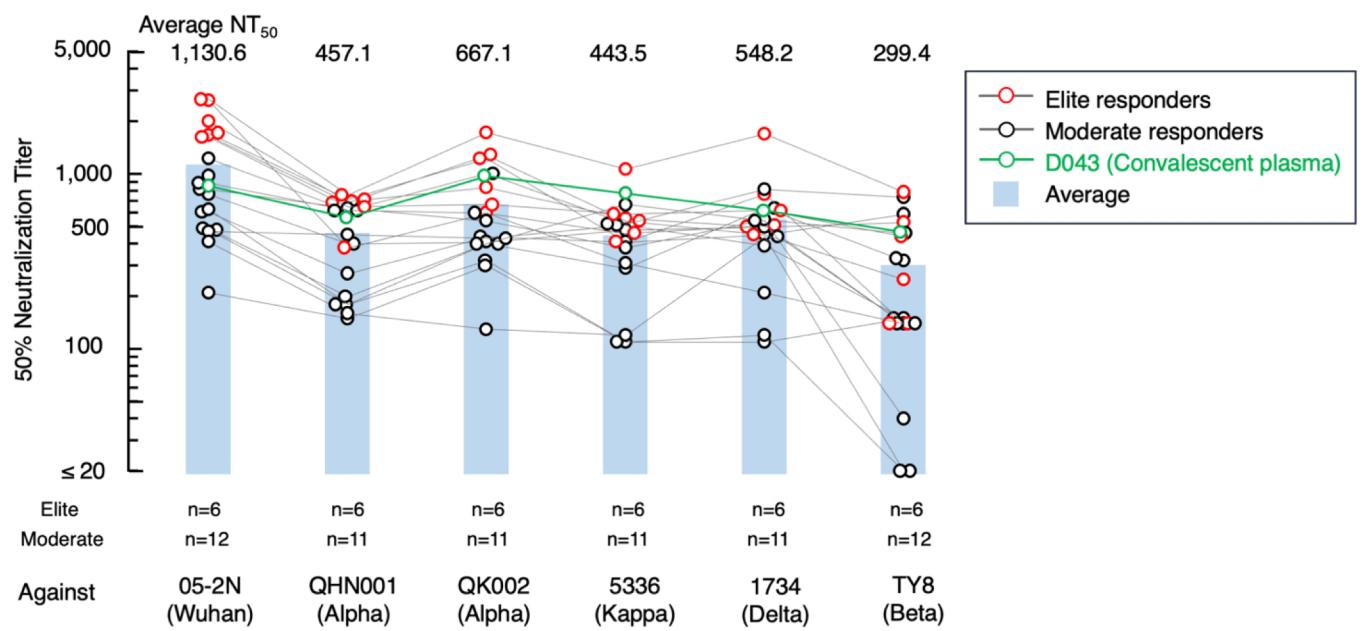


Figure 4

Supplementary appendix

Supplement to: Maeda K, Amano M, Uemura Y et al. Correlates of Neutralizing/SARS-CoV-2-S-1-binding Antibody Response with Adverse Effects and Immune Kinetics in BNT162b2-Vaccinated Individuals.

Contents

Supplemental figures (Figure S1 to S5)

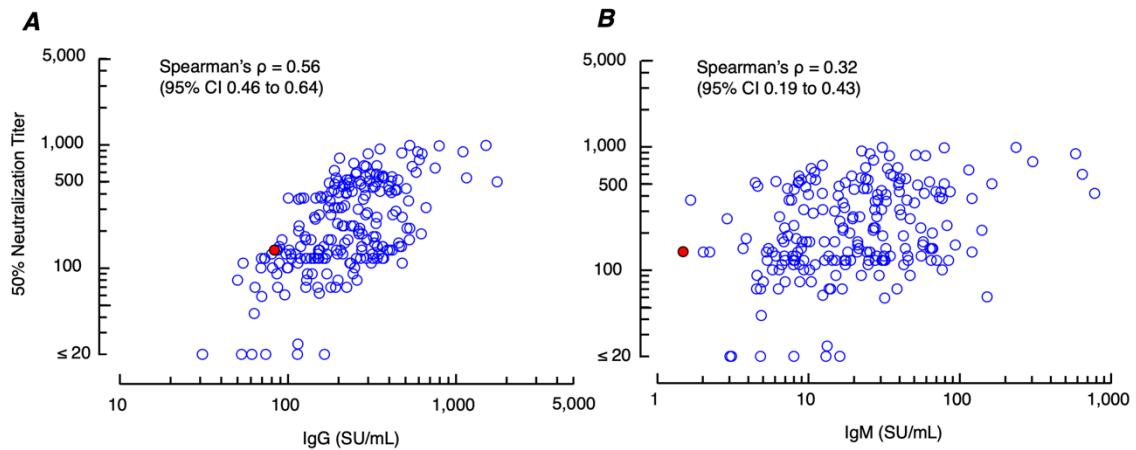


Figure S1: A. Correlation between neutralizing activity and IgG levels on day 60 post 1st shot. Moderate correlation is identified (Spearman's $\rho=0.56$; 95% CI 0.46 to 0.64) between NT₅₀ values and S1-binding-IgG levels in samples obtained on day 60 post-1st dose. B. Correlation between neutralizing activity and S1-binding-IgM levels on day 60 post-1st dose. Low correlation is seen between neutralizing titers and S1-binding-IgM levels (Spearman's $\rho=0.32$; 95% CI 0.19 to 0.43). A red-solid circle denotes a person with previous SARS-CoV-2 infection documented.

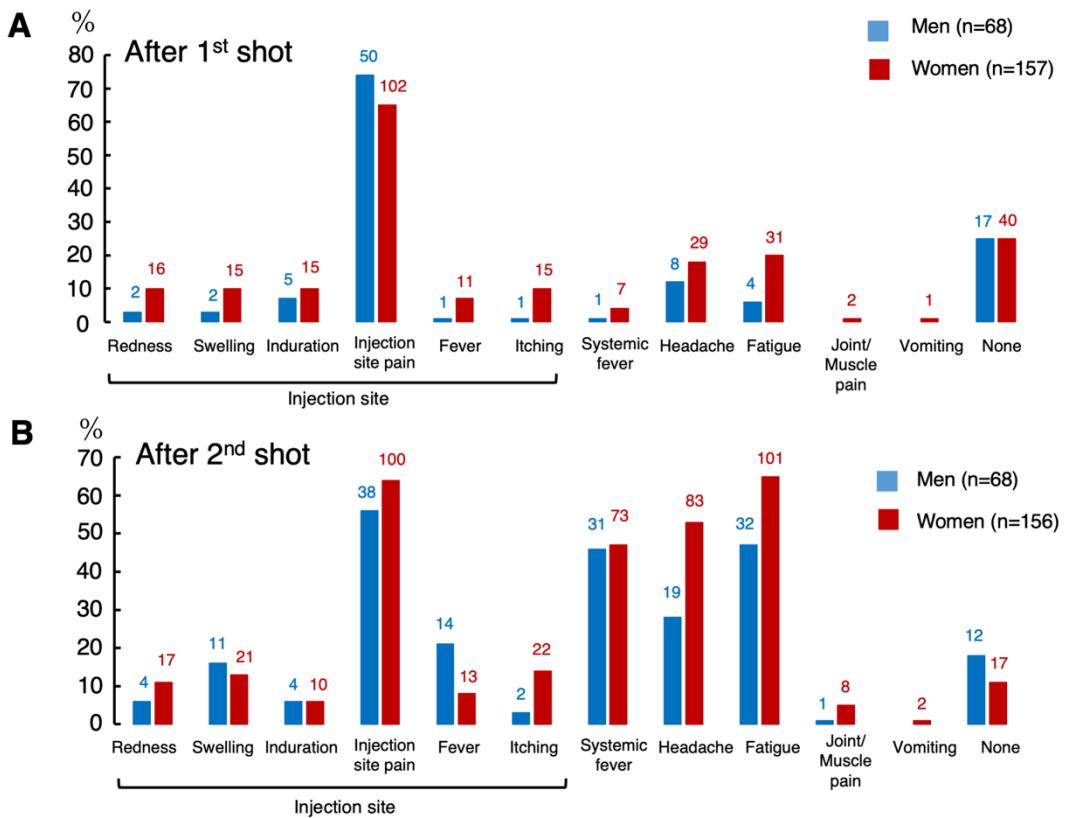


Figure S2: Incidences of adverse effects reported after the 1st vaccination (A) and the 2nd vaccination (B). A total of 225 (Men: 68, Women: 157) participants reported after the 1st shot, and a total of 224 (Men: 68, Women: 156) participants after the 2nd shot. Systemic fever of $\geq 37.1^{\circ}\text{C}$ and pain scores of ≥ 1 were taken into the analyses. The number at the top of each bar denotes the number of individuals reporting each AE.

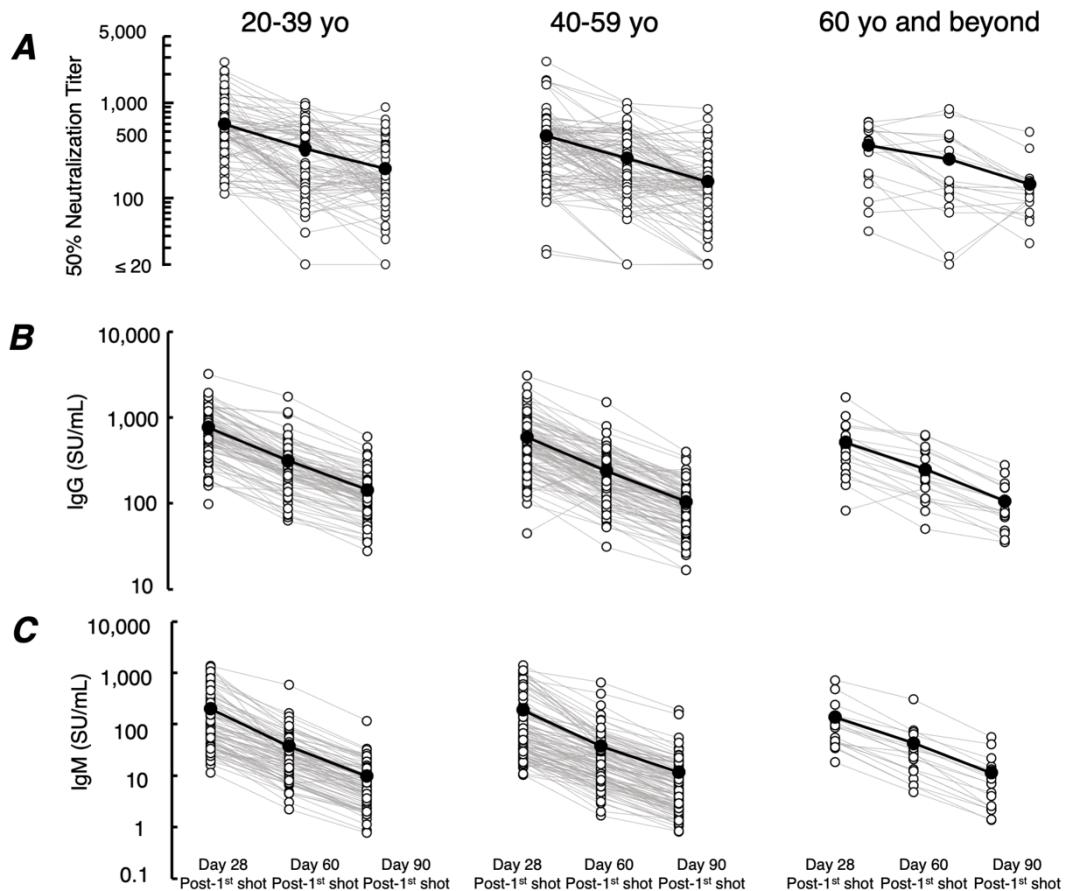


Figure S3: Decline of neutralization titers and S1-binding antibody levels in three age groups. The chronological decline rates of neutralizing titers (A), S1-binding-IgG (B), and -IgM levels (C) among three age groups; (i) 20-39 yo, (ii) 40-59 yo, and (iii) 60's and beyond, were evaluated. No significant difference was identified among the three age groups in the decline rates of neutralizing titers, IgG, or IgM levels ($p=0.596$, 0.163 , and 0.106 , respectively). Statistical analysis was conducted with the mixed-effects model including time, age category and time-age category interaction term and intercept as a random effect. A solitary open circle on day 28 in the 20-39 yo subgroup illustrated in B denotes a participant who did not provide blood sample for unknown reason on days 60 and 90 post-1st shot.

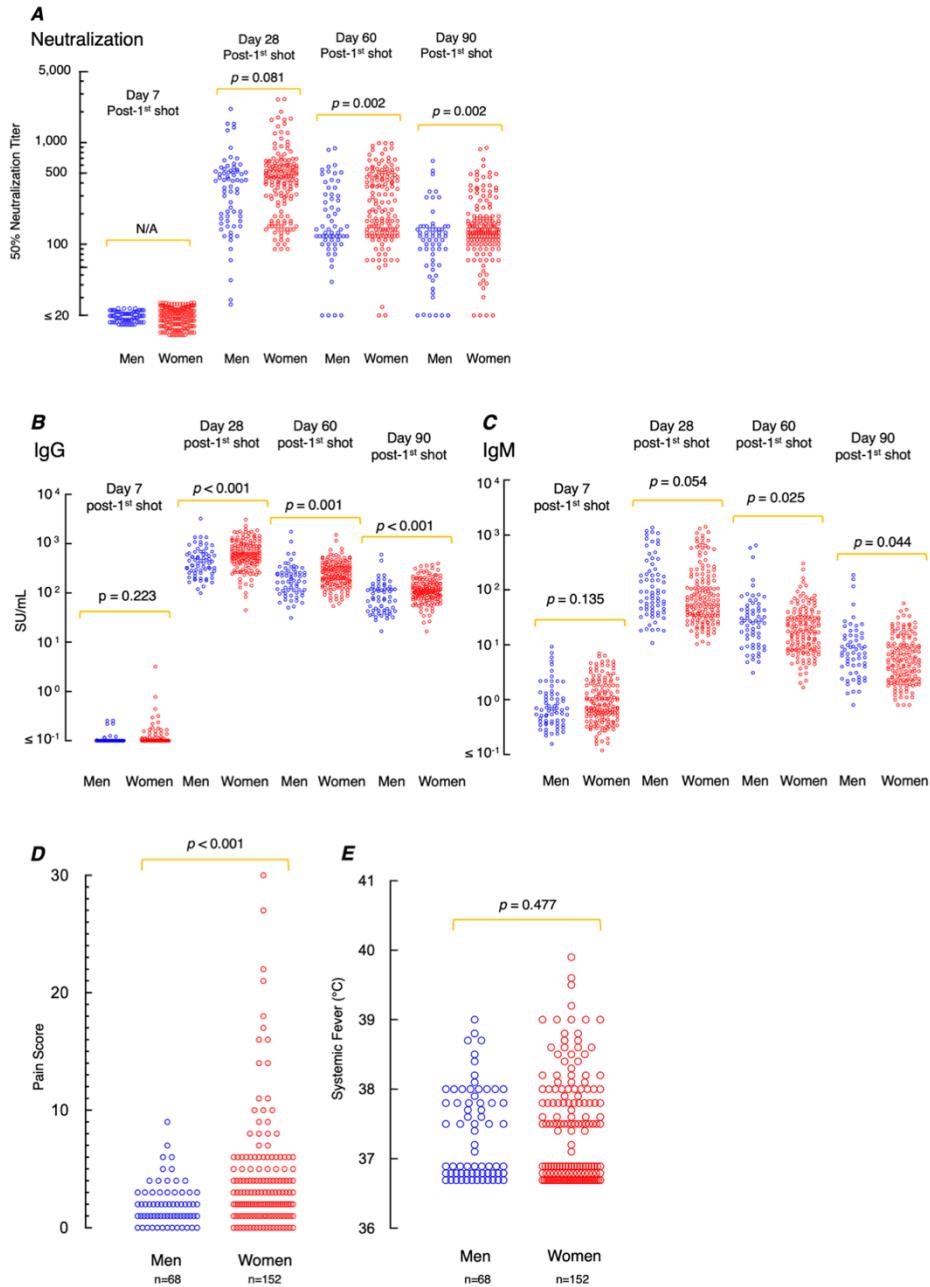


Figure S4: Neutralization activity, S1-binding-IgG and -IgM levels, and pain scores are greater in women than in men. A. Neutralization activity was greater in women than in men at two timepoints (days 60 and 90 post-1st shot). B and C. S1-binding-IgG levels were greater in women at all three timepoints (days 28, 60, and 90 post-1st shot) and S1-binding-IgM levels greater in women at two timepoints (day 60 and 90 post-1st shot). D, E. Injection-site pain scores and systemic fever grades in men and women. Scores of injection-site pain were greater in women than in men ($p < 0.001$)(D), while no difference was seen in systemic fever grades between the two groups ($p = 0.477$)(E). Statistical significance was evaluated using Wilcoxon rank sum test. N.A., not applicable.

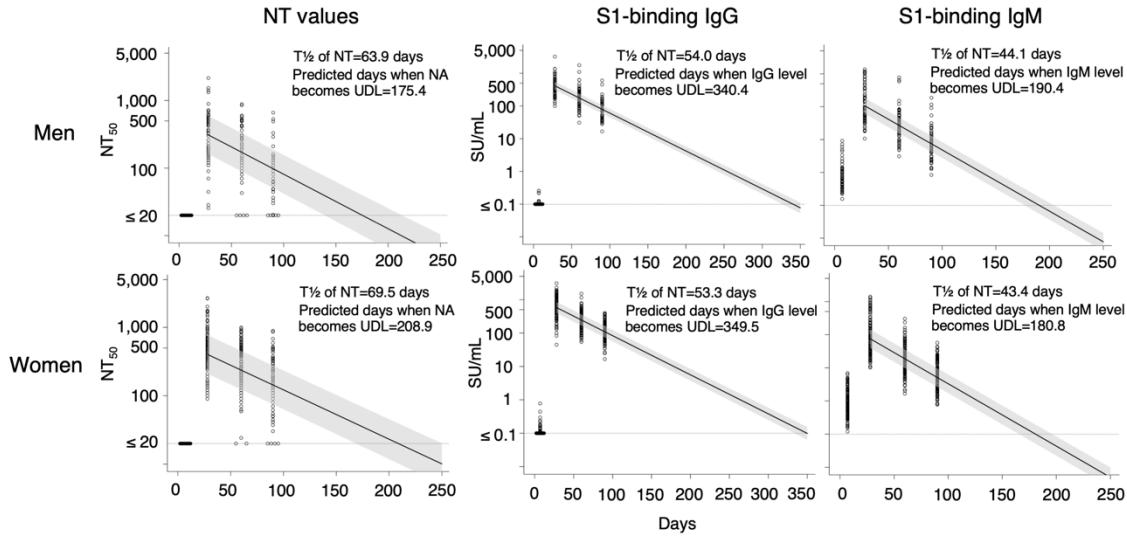


Figure S5: No difference in the decline rates of the neutralization activity, S1-binding-IgG and -IgM levels between men and women. Decline rates of neutralizing activity, S1-binding-IgG and -IgM over 90 days post-1st shot were compared. The solid lines consist of predicted values estimated by mixed effects model, and the shaded areas denote corresponding 80% prediction intervals. The dashed horizontal lines denote the NT₅₀ detection limit (20-fold). NT₅₀ values determined to be less than 20-fold were treated as 20-fold. The lowest detection limit for S1-binding-IgG and -IgM quantification was 0.1 SU/ml and the values lower than 0.1 SU/ml were calculated as 0.1 SU/ml. No significant difference was identified in the three indicators between men and women. Statistical analysis was conducted with the mixed-effects model including time, age category and time-age category interaction term and intercept as a random effect.