COVID-19 mRNA vaccine induced antibody responses and neutralizing antibodies against three SARS-CoV-2 variants

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3

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22 Abstract

23 As SARS-CoV-2 has been circulating for over a year, dozens of vaccine candidates are under
24 development or in clinical use. The BNT162b2 mRNA COVID-19 vaccine induces spike protein-
25 specific neutralizing antibodies associated with protective immunity. The emergence of the B.1.1.7 and
26 B.1.351 variants has raised concerns of reduced vaccine efficacy and increased re-infection rates. Here
27 we show, that after the second dose, the sera of BNT162b2-vaccinated health care workers (n = 180)
28 effectively neutralize the SARS-CoV-2 variant with the D614G substitution and the B.1.1.7 variant,
29 whereas the neutralization of the B.1.351 variant is five-fold reduced. Despite the reduction, 92% of the
30 vaccinees have a neutralization titre of >20 for the B.1.351 variant indicating some protection. The
31 vaccinees' neutralization titres exceeded those of recovered non-hospitalized COVID-19 patients. Our
32 work provides strong evidence that the second dose of the BNT162b2 vaccine induces efficient cross-
33 neutralization of SARS-CoV-2 variants currently circulating in the world.

34

35 Introduction

36 The emergence and spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has
37 caused a pandemic with over 2.7 million deaths¹ and rapid development of multiple vaccine
38 candidates². SARS-CoV-2 infection elicits antibodies against spike protein (S) and nucleoprotein (N)^{3–}
39 ⁵, of which, on the basis of virus challenge studies in animals, the spike protein-specific antibodies are
40 neutralizing and associated with protective immunity^{6,7}. In addition, recent studies of COVID-19
41 patients and vaccinees indicate that previous infections and vaccinations are related to a decreased rate
42 of SARS-CoV-2 infections^{8–10}. Although the persistence of vaccine-induced antibodies is still not
43 known, infection-induced neutralizing antibodies have remained detectable for at least six months after
44 symptom onset¹¹.

45 Currently, European Medicines Agency (EMA) has authorized four vaccines to be used in European
46 Union: two mRNA vaccines (BNT162b2 / Comirnaty by Pfizer-BioNTech and mRNA-1273 by
47 Moderna) and two adenoviral vector-based vaccines (ChAdOx1-S by AstraZeneca-Oxford and
48 COVID-19 Vaccine Janssen by Janssen Biologics B.V. and Janssen Pharmaceutica NV)¹². All four
49 vaccines aim to generate spike protein-specific antibodies and all have been shown to induce anti-S
50 IgG antibodies with neutralizing activity against the first pandemic SARS-CoV-2 Wuhan Hu-1 variant
51 and against the currently circulating D614G variants^{13–15}. The recent emergence of SARS-CoV-2
52 variants of concern, such as B.1.1.7 first identified in the United Kingdom¹⁶ and B.1.351 first identified
53 in South Africa¹⁷, has raised concerns about increased virus transmissibility and reduced vaccine
54 efficacy. These two variants of concern are defined by eight to ten amino acid changes or deletions in
55 the spike protein to which vaccine-induced antibodies are targeted^{17–20}. Both of these variants are now
56 transmitted in several countries (https://cov-lineages.org/global_report.html). Initial studies reported
57 that antibodies produced in response to vaccination and natural infection neutralize the B.1.1.7

variant^{19,21}, whereas neutralization of the B.1.351 is reduced 8 to 13-fold^{18,22,23}. However, it is still unclear whether the B.1.351 variant can escape from humoral and cell-mediated immunity.

Here, we characterize the BNT162b2 vaccine-induced antibody responses among a sequential serum sample cohort of 180 Finnish healthcare workers who, belonging to the group targeted first in Finland, received two doses of COVID-19 vaccine with three weeks interval. SARS-CoV-2 S1-specific IgG, IgA, and IgM antibody responses and neutralization titres for three SARS-CoV-2 variants were determined. We show that two-dose immunization yields high levels of anti-S1 IgG antibodies in 100% of vaccinees. The second vaccine dose induces antibodies for efficient neutralization of D614G and B.1.1.7. variants, whereas the neutralization titres for B.1.351 are lower.

Results

Study subjects

The vaccinee group comprised 180 volunteers (115 from TYKS and 65 from HUH), aged 20 to 65 years (mean 43 and median 41); 149/180 (83%) were females (age 20-65 years) and 31/180 (17%) were males (age 22-60 years). The group of recovered COVID-19 patients comprised 50 volunteers (from HUH), aged 19-93 (mean 43 and median 38); 33 were females and 17 males.

Antibody responses against SARS-CoV-2 S1 and N proteins in vaccinees and convalescent phase patients

In order to monitor the immunological responses of vaccinees, we collected sequential serum samples (0, 3, 6 weeks) from 180 vaccinated health care workers (HCWs). The first vaccinated HCWs received their first vaccination on 28 December 2020, and the last vaccinations for those HCWs in this study were given on 12 February 2021. Serum samples from 50 non-hospitalized, recovered COVID-19

79 patients from 2020 were also included in the analysis. The generation of anti-S1 IgG, IgA, IgM, and
 80 total Ig antibodies after vaccination was analyzed with enzyme immunoassay (EIA). The original
 81 optical density values in the assay were converted to EIA units to minimize inter-assay variation (Fig.
 82 1A). To verify the EIA results and to differentiate those with a previous or concurrent SARS-CoV-2
 83 infection, sera were also analyzed with N protein-specific EIA (Fig. 1B).

84 Before vaccination (0 day sampling) 11/180 (6%) had anti-S1 IgG antibodies (Supplementary Fig. 1A)
 85 indicating that these individuals had undergone a previous SARS-CoV-2 infection. Five of these anti-
 86 S1 positive participants had also anti-N IgG antibodies. Already after the first dose of the vaccine (3
 87 weeks), vaccinees with prior SARS-CoV-2 infection showed clearly increased levels of anti-S1 IgG
 88 antibodies (geometric mean 99). After the second dose of the vaccine (6 weeks), all vaccinees with
 89 prior SARS-CoV-2 infection had very high levels of anti-S1 IgG antibodies (geometric mean 109)
 90 (Supplementary Fig. 1A).

91 Three weeks after the first dose of BNT162b2, vaccinees without prior SARS-CoV-2 infection
 92 (169/180, 94%) developed varying levels of anti-S1 IgG antibodies (geometric mean 47), and moderate
 93 levels of anti-S1 IgA and IgM antibodies (Fig. 1A, Table 1). Total Ig levels for S1 ranged from 1 to 98
 94 EIA units. The EIA levels for anti-N antibodies among the vaccinees remained the same as before the
 95 vaccination (Fig. 1B, Table 1) indicating the absence of SARS-CoV-2 infections in this study group.
 96 Anti-N IgG antibody levels were higher in sera of non-hospitalized COVID-19 patients (geometric
 97 mean 17) than of vaccinees (geometric mean 2) (Fig. 1B, Table 1). However, already after the first
 98 vaccination dose, the geometric mean of anti-S1 IgG and anti-S1 total Ig antibodies of vaccinees
 99 exceeded those of convalescent phase COVID-19 patients, 47 and 37 vs. 20 and 23, respectively (Fig.
 100 1A, Table 1).

101 Six weeks after the first vaccine dose (three weeks after the second vaccine dose) all participants
102 elicited high levels of anti-S1 IgG antibodies together with a modest increase in anti-S1 IgA and IgM
103 antibody levels (Fig. 1A). The vaccinees did not develop anti-N antibodies following vaccination (Fig.
104 1B). Also, based on anti-N antibodies, only one person got infected with SARS-CoV-2 during the 6
105 weeks: participant was anti-N and anti-S1 IgG negative at 0 day sampling, and anti-N and anti-S1 IgG
106 positive at 3 week sampling. The negative control antigen signals were close to the background values
107 (Supplementary Fig. 2). Anti-S1 IgG antibody and total anti-S1 Ig levels induced by two doses of
108 BNT162b2 vaccine were clearly higher than the anti-S1 IgG levels measured from the convalescent
109 phase patient sera, geometric means being 107 and 86 vs. 20 and 23, respectively.

110 **Characterization of SARS-CoV-2 isolates**

111 To analyze the neutralization capacity of the vaccinees' sera, we isolated for microneutralization tests
112 four virus variants circulating in Finland: D614G variants FIN-25 (spring 2020) representing B.1
113 lineage and SR121 (autumn 2020) representing B.1.463 lineage, a variant of concern 85HEL
114 representing B.1.1.7 lineage and a variant of concern HEL12-102 representing B.1.351 lineage. FIN-25
115 isolate was passaged first in VeroE6 cells followed by passaging in VeroE6 cells expressing
116 transmembrane protease serine 2 (VeroE6-TMPRSS2-H10). Other three isolates were passaged in
117 VeroE6-TMPRSS2-H10 cells to avoid the generation of mutations in the vicinity of the furin cleavage
118 site (Fig. 2A). The isolates were sequenced to compare the mutations in SR121, 85HEL (B.1.1.7) and
119 HEL12-102 (B.1.351) variants to FIN-25 that represented the circulating strains in Finland until the
120 emergence of variants of concern. Sequence analysis of SARS-CoV-2 isolates revealed 3 amino acid
121 changes in the spike protein of FIN-25, 4 in SR121, 10 in 85HEL (B.1.1.7) and 9 in HEL12-102
122 (B.1.351) variants compared to original Wuhan Hu1 strain (Fig. 2A). The sequence of FIN-25 that was
123 passaged initially in VeroE6 cells had close to the furin cleavage site a deletion of amino acids 674-678

124 in 45% and R682W mutation at the furin cleavage site in 41% of the virus population, indicating some
125 heterogeneity of the FIN-25 virus stock, which did, however, not affect the growth properties of the
126 virus (data not shown). Sequences of the three other isolates passaged in VeroE6-TMPRSS2-H10 cells
127 only had either aforementioned deletion in the minority of the virus population (8% of SR121) or a
128 completely intact furin cleavage site. Otherwise, all spike protein sequences obtained from the virus
129 propagations were identical to the sequences obtained from the respective original patient sample, all
130 also containing the D614G substitution linked to increased fitness and transmissibility^{24,25}.

131 The availability of the 3-dimensional structure of SARS-CoV-2 spike protein enabled the positioning
132 of the amino acid changes into the structure of the trimeric spike protein (Fig. 2B). Substitutions found
133 in FIN-25 and SR121 spike proteins localize in the stem regions of the trimeric spike protein. The
134 substitutions found in the spike proteins of B.1.1.7 and B.1.351 variants located both to the stem region
135 and on the surface of the trimeric spike protein close to the receptor binding domain (RBD). The three
136 B.1.351 variant substitutions E484K, K417N, and N501Y are in the groove of RBD – ACE2
137 interaction domain. In addition, both the B.1.1.7 and B.1.351 variants had 3 amino acid deletions in the
138 far edges of the 3-dimensional structure (Fig. 2B). Figure 2C shows combined amino acid changes
139 found in the isolates used in this study indicating the accumulation of substitutions on multiple
140 localizations on the trimeric structure of the spike protein. The amino acid changes in the spike protein,
141 especially aforementioned E484K, K417N, and N501Y have recently been reported to affect the
142 neutralizing efficacy of the antibodies²⁶.

143 **Neutralizing antibody titres against SARS-CoV-2 variants**

144 To measure the neutralizing potential of the vaccinees' sera against all four SARS-CoV-2 isolates,
145 neutralizing antibody titres elicited by the BNT162b2 vaccine were analyzed with microneutralization
146 test (MNT). The neutralizing titres with two D614G isolates FIN-25 and SR121 were almost identical

147 both three weeks ($p=0.02$) and six weeks after the first dose ($p=0.11$) (Fig. 3A), indicating that the
148 mutations in FIN-25 spike protein due to initial propagation in VeroE6 cells did not affect the
149 neutralizing titres.

150 Before vaccination (0 day sampling) those 11/180 with a likely SARS-CoV-2 infection based on EIA
151 results, showed increased geometric mean titres (GMT) of 35, 31 and 16 against FIN-25, 85HEL
152 (B.1.1.7) and HEL12-102 (B.1.351) virus isolates, respectively. Following the first and the second
153 vaccination, the GMTs further increased to 435 and 682, to 320 and 640, and to 101 and 132,
154 respectively (Supplementary Fig. 1B). These results with this small group indicate that even one dose
155 of BNT162b2 vaccine induces high MNT titres in those individuals who had suffered a previous
156 COVID-19 infection.

157 None of the vaccinees without a prior SARS-CoV-2 infection (169/180) had neutralizing antibodies
158 before the vaccination (Fig. 3A). Three weeks after the first vaccine dose, neutralizing titres against all
159 four isolates were slightly increased (GMT of 24 for FIN-25, 32 for SR121, 24 for 85HEL (B.1.1.7)
160 and 12 for HEL12-102 (B.1.351)). Six weeks after the first dose of the vaccine (three weeks after the
161 second dose), neutralizing titres were increased to a GMT of 234 against FIN-25, 275 against SR121,
162 240 against 85HEL (B.1.1.7) and 48 against HEL12-102 (B.1.351) (Fig. 3A, Table 1). Three weeks
163 after the first dose 37%, 17%, 37% and 85% of vaccinees had a neutralization titre <20 against FIN-25,
164 SR121, 85HEL (B.1.1.7) and HEL-12-102 (B.1.351) isolates, respectively. After the second vaccine
165 dose, 100% of vaccinees had neutralizing antibodies against FIN-25, SR121 and 85HEL (B.1.1.7),
166 whereas 92% of vaccinees had neutralizing antibodies against the HEL-12-102 (B.1.351) variant.

167 GMTs against all four isolates in vaccinees exceeded the GMTs seen in convalescent phase patient sera
168 (Fig. 3A, Table 1).

Three weeks after the first vaccine dose, the GMT for HEL-12-102 (B.1.351) was 2-fold lower ($p<0.0001$) compared to FIN-25 and 85HEL (B.1.1.7). After the second immunization, the GMT for HEL-12-102 (B.1.351) was 5-fold lower compared to FIN-25 and 85HEL (B.1.1.7) (Fig. 3B).

The MNT titres for two D614G-containing isolates, FIN-25 and SR121, correlated very well, as also did FIN-25 and 85HEL (B.1.1.7) ($r>0.8$, $p<0.0001$) (Fig. 4). MNT titres for FIN-25 and HEL-12-102 (B.1.351) correlated relatively well and highly significantly ($r=0.74$, $p<0.0001$), as did the two variants of concern, 85HEL (B.1.1.7) and HEL-12-102 (B.1.351) ($r=0.75$, $p<0.0001$).

To analyze the effect of age and gender to the antibody responses, the vaccinees were divided into age and gender groups and the S1 IgG EIA and MNT results were compared between the groups (Fig. 5A and B). After the first vaccine dose, anti-S1 IgG antibody levels and neutralization titres decreased significantly in the older age group (55–65 years) compared to younger age groups (20–34 and 35–44 years) (Fig. 5A). However after the second vaccine dose, the neutralization titres were similar between the age groups (GMT 257, 268, 200 and 206 in age groups of 20–34, 35–44, 45–54 and 55–65 years, respectively) (Fig. 5A). We also compared gender-related antibody responses even though male vaccinees were underrepresented, comprising only 17% (29/169) of the vaccinees. After the second dose, female vaccinees had slightly higher neutralization titres than male ($p=0.0412$), although the anti-S1 IgG antibody levels remained at the same level (Fig. 5B).

EIA values correlate with MNT titres

Neutralization tests with live SARS-CoV-2 viruses are very time-consuming, and at the moment the assay requires BSL-3 laboratory conditions, whereas EIA and other similar colorimetric/fluorometric antibody assays are faster and user friendlier. To assess whether EIA values correlate with MNT titres, anti-S1 IgG and total anti-S1 Ig were compared to neutralization titres against FIN-25 (Fig. 6, Supplementary Fig. 3). Both anti-S1 IgG and total anti-S1 Ig EIA measurements correlated very well

192 with MNT titres ($r>0.9$, $p<0.0001$) suggesting that EIA, especially IgG EIA, using spike protein as an
193 antigen could be a useful method to determine COVID-19 immunity.

194 Discussion

195 The emergence of the COVID-19 pandemic in early 2020 prompted a rapid development of various
196 types of vaccines such as mRNA encoding SARS-CoV-2 spike protein, viral vector based (e.g.
197 adenovirus), inactivated virus, virus-like particle, and recombinant protein vaccines. Once the
198 European Union had made agreements with a number of vaccine producers, mass immunization was
199 started in Finland at the end of December 2020, first with the mRNA based Pfizer-BioNTech vaccine
200 and somewhat later the Moderna mRNA and AstraZeneca adenovirus-based vaccines¹². Vaccination of
201 health care professionals within a national vaccination programme in Finland enabled us to start
202 independent of pharmaceutical companies a follow-up study of vaccine-induced immunity. In the
203 present report, we show that two-dose vaccination with the BNT162b2 mRNA COVID-19 vaccine
204 induces very high antibody levels against viral spike protein and high titres of neutralizing antibodies.
205 The vaccine induced good cross-reactivity to D614G and B.1.1.7 variants and, albeit reduced levels,
206 detectable neutralizing antibodies to B.1.351 variant.

207 EIA is a rapid and sensitive method to analyze immune responses against vaccine antigens or different
208 viral proteins in response to infection. The method is easily quantitative and suitable for analyzing
209 different immunoglobulin classes. In this study, we observed that practically all seronegative health
210 care workers (20–65 years of age) responded to the first BNT162b2 vaccine dose and an increase in
211 spike protein-specific antibody responses in IgG antibody class was detectable. The antibody levels
212 varied considerably, however, and relatively few individuals showed increased antibody levels in the
213 IgA and IgM antibody classes. The second vaccine dose, which was given according to the original

214 vaccination protocol three weeks after the first vaccine dose, induced very high levels of spike protein-
215 specific IgG antibodies, while IgA and IgM responses remained low. The vaccinees' IgG antibody
216 levels were on average higher than measured in convalescent phase sera from home-treated patients.
217 Antibody responses have been found generally higher for COVID-19 patients with a more severe
218 disease^{23,27,28}, however, as shown by this study also, BNT162b2 vaccine appears to induce higher
219 antibody levels than those measured in patients^{29,30}. Remarkably, administration of two doses of the
220 mRNA vaccine induced very high antibody responses in 100% of the vaccinees.

221 The global circulation of SARS-CoV-2 and a huge number of infections worldwide have led to the
222 emergence of hundreds of evolutionary lineages and variants of the virus ([https://cov-](https://cov-lineages.org/global_report.html)
223 [lineages.org/global_report.html](https://cov-lineages.org/global_report.html)). The evolutionary speed of SARS-CoV-2 has been relatively slow, at
224 least compared to influenza A viruses, presumably due to a virus-encoded enzyme with proof-reading
225 capability. Within the first 15 months of circulation, up to 30–35 mutations have been identified
226 accumulating into the viral genome. Many of these mutations are silent or appear in places of the
227 genome that are not critical for avoiding immunity induced by vaccination or natural infection.

228 However, a number of variants have raised concern due to mutations accumulating particularly in the
229 S-gene and causing changes in the immunodominant epitopes of the trimeric spike protein. Mapping
230 the spike protein mutations on variants sequenced and used in this study revealed that they occur
231 outside the globular head of the trimeric spike protein. The D614G and B.1.1.7 variant viruses were
232 readily neutralized by the vaccinees' sera, indicating that these mutations are unlikely to impair the
233 neutralizing antibody capacity induced by vaccination or natural infection. However, it should be noted
234 that the neutralizing titre of these sera decreased five-fold against the B.1.351 variant, which denotes
235 that the amino acid changes accumulating in this variant are potentiating the escape of the virus from
236 the humoral immune responses. Despite this, more than 92% of the vaccinees showed measurable

237 neutralizing antibody titres against the B.1.351 variant, suggesting that the spike protein encoded by
238 Pfizer-BioNTech's mRNA vaccine is similar enough to also mount an immune response against the
239 B.1.351 variant.

240 The critical amino acid changes linked to escape from humoral immunity in the B.1.351 variant appear
241 to be K417N, E484K, and N501Y³⁰⁻³². These amino acids are situated in the grooves within the
242 receptor binding site of the trimeric S protein complex. There is no three-dimensional structure
243 presently available for the B.1.351 variant spike protein trimer, but because of its relatively radical
244 amino acid substitutions, conformational changes in the spike structure may prove substantial.

245 Interestingly, the B.1.351 and B.1.1.7 variants have deletions in the tips of the globular S1 domain
246 (amino acids 243-245 and amino acids 69-70 and 244, respectively) which could contribute to the
247 impaired recognition by neutralizing antibodies.

248 It is currently not known how high neutralizing antibody titres against a given virus variant are required
249 for antibody-mediated protection against the COVID-19. However, the clinical efficacy data
250 accumulating from COVID-19 vaccine studies strongly suggest that already one dose of the vaccine
251 provides protection against severe COVID-19, even when neutralizing antibody levels cannot be
252 detected in all vaccinees^{33,34}. This suggests that the first vaccine dose may prime the individual for a
253 rapid induction of protective immunity when contracting the virus in nature and avoiding severe
254 COVID-19. According with previous data^{29,35-38}, we found that individuals with prior SARS-CoV-2
255 infection readily responded to the first vaccine dose with high antibody levels and neutralization titres.

256 Humoural immune response to vaccinations has been shown to decline with age^{39,40}. Consistently, we
257 observed a trend of declining immune response to the COVID-19 mRNA vaccine by age. This trend
258 was not very strong, presumably because the ages of our vaccinees ranged from 20 to 65 years, while
259 age-dependent immunosenescence should be more pronounced in the age group >65 years³⁹. Another

260 explanation might be that the BNT162b2 mRNA vaccine is exceptionally immunogenic and therefore,
261 especially when given two doses, it enables practically all individuals regardless of gender and age, to
262 develop high antibody levels and neutralization titres.

263 In summary, in the present study we show that the Pfizer-BioNTech BNT162b2 COVID-19 mRNA
264 vaccine is highly immunogenic, and particularly after two vaccine doses all vaccinees showed very
265 high humoral immune response to D614G variant viruses. Immunity to a recent B.1.1.7 variant was
266 equally good as compared to the D614G variant, whereas vaccine- and SARS-CoV-2- infection
267 induced immunity against B.1.351 variant was reduced. Despite this, almost all vaccinees showed
268 neutralizing antibodies against the B.1.351 variant, suggesting to provide at least some degree of
269 protection against these variant viruses. In the future, it will be intriguing to study the development and
270 persistence of cell-mediated immunity induced by COVID-19 vaccines. Promising data has been
271 reported at least for the BNT162b2 vaccine which in preliminary studies has induced good cell-
272 mediated immunity^{41,42}. As the use of other types of SARS-CoV-2 vaccines will be increased, it is the
273 responsibility of the scientific community and public health professionals to systematically collect
274 serum and cellular samples for comparative analyses of vaccine-induced immunity, cross-protection
275 and longevity of vaccine-and natural infection-induced immunity.

276 As a whole, all vaccines that have currently obtained market authorization in EU show excellent
277 protective efficacy against severe COVID-19. Thus, it is very likely that immunogenicity results
278 similar to those presented here will be applicable to them as well. The future of SARS-CoV-2 vaccines
279 therefore seems to look bright.

280 **Methods**

281 **Study participants**

282 SARS-CoV-2 vaccinations started in Finland at the end of December 2020 with Pfizer-BioNTech
283 BNT162b2 mRNA (Comirnaty) vaccine. Study participants (n=180) were recruited among healthcare
284 personnel of Turku University Hospital (TYKS, Turku, Finland) (Southwest Finland health district
285 ethical permission ETMK 19/1801/2020) and Helsinki University Hospital (HUU, Helsinki, Finland)
286 (Helsinki-Uusimaa health district ethical permission HUS/1238/2020) prior to receiving an optimal
287 regimen of two doses of BNT162b2 mRNA vaccine at a three-week dosing interval as part of hospital
288 occupational health care. Serum samples were collected before or on the day of the first vaccine dose (0
289 day sample, n=180), 16 to 28 days (mean 20) after the first vaccine dose (3 week sample, n=176), and
290 13 to 33 (mean 23) days after the second vaccine dose (34 to 54 days after the first vaccine dose) (6
291 week sample, n=180).

292 Convalescent phase serum samples (n=50) were collected at HUU from patients with initial RT-qPCR
293 confirmed home-treated COVID-19 infection (Helsinki-Uusimaa health district ethical permission
294 HUS/1238/2020). The patients provided written informed consent and were sampled 14 days – 6
295 weeks after the positive PCR test result. As negative control serum samples (n=40) we used randomly
296 selected diagnostic serum samples collected at TYKS prior to COVID-19 pandemic (Jalkanen et al.
297 2020, submitted).

298 **Expression and purification of SARS-CoV-2 nucleoprotein and S1 antigens**

299 SARS-CoV-2 protein expression was done as described previously (Jalkanen et al. 2020, submitted).
300 Briefly, SARS-CoV-2 N and S sequences were obtained from GenBank (NC_045512.2 and
301 MN908947.3, respectively). N protein was expressed as a fusion protein with glutathione S-transferase

(N-GST) in *Spodoptera frugiperda* (Sf-9) cells. GST alone was produced to be used as a control protein. S1 domain of the spike protein (amino acid residues 16-541) was expressed as a fusion protein with mouse IgG2a Fc and 8xhistidine tag (S1-mFc-8xhis) in human embryonic kidney (HEK293F) cells. Mouse promyostatin (ProMstn)-mFc(IgG2a)-6xhis (later referred as Mstn-mFc) was produced to be used as a control protein. Proteins were purified and buffer was exchanged to PBS. Concentrations of produced proteins were measured with BCA protein assay kit (Thermo Fisher Scientific).

IgG, IgA, and IgM EIA and total Ig EIA for SARS-CoV-2 S1 and N protein antibodies

Enzyme immunoassay (EIA) was performed by coating 96-well microtitre plates (Nunc Maxisorp, Thermo Fisher Scientific) for 16 h at +4°C with GST-N (2.0 µg/ml), S1-mFc (3.5 µg/ml), and corresponding molar amounts of GST (0.7 µg/ml) and Mstn-mFc (2.4 µg/ml) antigens in PBS. The plates were washed with 0.1% Tween-20 in PBS and blocked for 30 min with assay buffer (5% swine serum (BioInd), 0.1% Tween-20 in PBS) before the addition of 50 µl serum dilutions (final dilution 1:300 or 1:1000 for some analyses in assay buffer). After 2h incubation at +37°C, the plates were washed three times followed by addition of 100 µl horseradish peroxidase (HRP) conjugated anti-human antibodies (1:8000 dilution of anti-hIgG HRP (Dako A/S), 1:8000 dilution of anti-hIgA HRP (Invitrogen), 1:4000 dilution of anti-hIgM HRP (Dako A/S), and 1:20 000 dilution of anti-hIg (IgG, IgA, IgM) HRP (Abcam)) for 1 h at +37°C. The plates were washed three times and 100 µl TMB One substrate (Kementec Solutions A/S) was added. The plates were incubated for 20 min at room temperature, 100 µl of 0.2N sulphuric acid was added to stop the reaction and the levels of IgG, IgA, IgM, and total Ig antibodies were measured at 450 nm with Victor Nivo plate reader (Perkin Elmer). Optical density (OD) values were converted into EIA units by comparing the sample OD values to the

324 OD values of positive (marked as 100) and negative control samples (marked as 0). EIA units <1 were
325 marked as 1. Cut-off units for S1-based EIA were calculated as the average of 20 negative samples plus
326 three standard deviations (SDs) and for N-based EIA as the average of 20 negative samples plus six
327 SDs.

328 **Propagation of SARS-CoV-2 isolates**

329 SARS-CoV-2 isolate Fin/25/20 (Gisaid: EPI_ISL_412971) from lineage B.1 was isolated from the
330 nasopharyngeal sample of COVID-19 patient in Finland in February 2020. Swab sample in transport
331 medium was inoculated onto African green monkey kidney epithelial VeroE6 cells at +37°C and 5%
332 CO₂ in culture medium (Eagle's minimum essential medium (EMEM) supplemented with 2% fetal
333 bovine serum (FBS), 0.6 µg/mL penicillin, 60 µg/mL streptomycin, 2 mM L-glutamine, 20 mM
334 HEPES). Virus was propagated in VeroE6 cells for a total of three times. Subsequently, a VeroE6
335 clone expressing TMPRSS2, a serine protease essential for SARS-CoV-2 spike protein integrity, was
336 generated (VeroE6-TMPRSS2-H10)⁴³. Fin/25/20 was further propagated twice in VeroE6-TMPRSS2-
337 H10 cell line (virus isolate named FIN-25). Another 2020 isolate, SR121 from lineage B.1.463, isolated
338 from a patient in Finland in September 2020, was isolated and propagated only in VeroE6-TMPRSS2-
339 H10 cells⁴³. Variants 85HEL of B.1.1.7 lineage and HEL12-102 of B.1.351 lineage were isolated from
340 patients in Finland as described²³ and further propagated only in VeroE6-TMPRSS2-H10 cells.
341 VeroE6-TMPRSS2-H10 cells were maintained in D-MEM (Lonza) supplemented with 10% FBS, 2mM
342 L-glutamine (Gibco) and penicillin/streptomycin. For virus propagation in VeroE6-TMPRSS2-H10
343 cells, D-MEM supplemented with 2% FBS, 2 mM L-glutamine and penicillin/streptomycin was used.
344 Supernatants containing viruses were harvested, cell debris removed with centrifugation at 500 g for 5
345 min, and aliquots stored at -80°C.

346 Fifty-percent tissue culture infective dose (TCID₅₀) of virus stocks was determined with endpoint
347 dilution assay in VeroE6-TMPRSS2-H10 cells. Briefly, 50 000 cells per well were plated on 96-well
348 tissue culture plates (Sarstedt), and the next day media was changed to infection media (2% FBS). Ten-
349 fold virus dilutions in infection media were applied onto cells, and the plates were incubated for 3 days
350 at +37°C and 5% CO₂. Cells were fixed with 4% formaldehyde and stained with crystal violet. Virus
351 dilution resulting in 50% cell death was determined to represent TCID₅₀ value of the stock virus. Virus
352 propagations and end point dilution assays were done in BSL-3 laboratory conditions.

353 **Sequencing of SARS-CoV-2 isolates**

354 For sequencing of virus stocks, the viral RNA was extracted from supernatants using the RNeasy Mini
355 kit (Qiagen) and reverse-transcribed to cDNA with LunaScript RT SuperMix kit (New England
356 Biolabs). Primer pools targeting SARS-CoV-2 were designed using PrimalScheme tool⁴⁴ and PCR was
357 done with PhusionFlash PCR master mix (Thermo Scientific). Sequencing libraries were prepared with
358 NEBNext ultra II FS DNA library kit (New England Biolabs) according to the manufacturer's
359 instructions and sequenced using Illumina Miseq with v3 sequencing kit. Raw sequence reads were
360 trimmed, and low quality (quality score <30) and short (<25 nt) sequences were removed using
361 Trimmomatic⁴⁵. The trimmed sequence reads were assembled to the reference sequence
362 (NC_045512.2) using BWA-MEM⁴⁶ algorithm implemented in SAMTools version 1.8⁴⁷. Sequences of
363 four SARS-CoV-2 isolates used in this study were deposited in GenBank: FIN-25 (GenBank
364 MW717675), SR121 (GenBank MW717676), 85HEL (GenBank MW717677) and HEL-12-102
365 (GenBank MW717678).

366 **Illustration of amino acid changes in SARS-CoV-2 spike protein**

367 SARS-CoV-2 spike structure in closed conformation obtained through cryo-electron microscopy, pdb
368 accession 6VXX⁴⁸, was used for illustration of the residue differences between the employed SARS-
369 CoV-2 variants in YASARA (available at <http://www.yasara.org/>).

370 **Microneutralization test**

371 Neutralizing antibodies were measured using a microneutralization test (MNT). Serum samples were
372 serially diluted two-fold, starting at 1:20 dilution, in 2% FBS in DMEM and incubated with 100
373 TCID₅₀ of SARS-CoV-2 isolate in 96-well tissue culture plates (Sarstedt) for 1h at +37°C. VeroE6-
374 TMPRSS2-H10 cells were added (40 000–50 000 cells per well) and the plates were incubated at
375 +37°C, 5% CO₂ for 3 days. Cells were fixed with 4% formaldehyde and stained with crystal violet.
376 MNT titres were calculated as the reciprocal dilution resulting in 50% inhibition of cell death. MNT
377 assays were done at the BSL-3 laboratory conditions.

378 **Statistical analysis**

379 Geometric means with geometric standard deviations (SD) were calculated with GraphPad Prism 8
380 software. Statistical significance of differences between variants were analyzed with Wilcoxon
381 matched pairs signed-rank test, and two-tailed p-values <0.05 were considered significant. Differences
382 between age and gender groups were tested with two-tailed Mann-Whitney U test.

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495 **Author contributions**

496 P.J., L.K., J.L., A.K. and I.J. designed the experiments; P.J., P.K., M.H., S.M., R.L. and L.K. did
497 microneutralization tests and analyzed the data; P.J., A.R. and S.T. did EIA tests and analyzed the data;
498 H.K.H., S.H.P., P.T., I.L., A.N., T.M., H.V., L.I., J.L. and A.K. recruited vaccinees and patients and
499 collected their sera and data; A.P., R.N., P.J. and O.R. produced antigens for EIA; P.Ö., S.K., J.H. and
500 O.V. isolated and characterized virus strains; J.H. produced VeroE6-TMPRSS2-H10 cell line; T.S. did
501 sequencing and T.S., J.H. and P.K. analyzed sequences and structures; P.J. analyzed all data sets; P.J.,
502 L.K. and I.J. wrote the manuscript and all co-authors contributed to the edition of the text.

503 **Competing Interests**

504 The authors declare no competing interests.

505 **Figure legends**

506 **Fig. 1 Antibody responses against SARS-CoV-2 S1 and N proteins in BNT162b2 vaccinated**
507 **health care workers and non-hospitalized recovered COVID-19 patients.**

508 **A.** Anti-S1 and **B.** anti-N IgG, IgA, IgM, and total Ig antibody levels were measured with EIA. Serum
509 samples from BNT162b2 vaccinated participants (n=169) were collected before vaccination (0d), and
510 three (3wk) and six (6wk) weeks after the first dose of the vaccine. All vaccinees received the second
511 dose of the vaccine three weeks after the first dose. Convalescent phase patient samples (Conv, n=50)
512 were collected 14 days to 6 weeks after the positive RT-qPCR test result. Data is represented as
513 geometric means and geometric standard deviations (SD). Cut-off values are indicated with dashed
514 lines.

515 **Fig. 2 Genetic analysis of virus variants and spike protein structure prediction.**

516 **A.** Schematic presentation of S gene and amino acid changes in FIN-25 (B.1 lineage), SR121
517 (B.1.463), 85HEL (B.1.1.7) and HEL12-102 (B.1.351) virus isolates used in the present study. **B.**
518 Trimeric SARS-CoV-2 S protein, referred to as the spike, structure in closed conformation (pdb:
519 6VXX). Amino acid substitutions (yellow) and deletions (green) as compared to the original spike
520 structure. **C.** Collective presentation of all amino acid changes found in virus isolates. Space filling
521 model indicating amino acids changes (yellow) and deletions (green) on the surface of a trimeric S
522 protein. Side and top views are shown.

523 **Fig. 3 Neutralization of B.1.1.7 and B.1.351 variants by BNT162b2 vaccinees' sera and COVID-**
524 **19 patient sera.**

525 **A.** Neutralization titres for D614G variants FIN-25 and SR121, and 85HEL (B.1.1.7) and HEL12-102
526 (B.1.351) variants before (0d), three (3wk) and six weeks (6wk) after the first dose of BNT162b2

527 vaccine and neutralization titres of convalescent sera of non-hospitalized patients. Values above the
528 groups indicate geometric mean titres (GMTs) and data is shown as geometric means and geometric
529 SDs. Neutralization titres <20 were plotted as 10. **B.** Neutralization titres 3 weeks (3wk) and six weeks
530 (6wk) after the first dose of the vaccine. Statistical differences between the virus isolates were analyzed
531 with Wilcoxon matched pairs signed-rank test. Two-tailed p-values *<0.05, **<0.01, ****<0.0001
532 were considered significant.

533 **Fig. 4 Correlation of MNT titres against SARS-CoV-2 isolates.**

534 MNT titres of BNT162b2 vaccinees (n=169) against FIN-25 were compared with MNT titres against
535 85HEL (B.1.1.7) and HEL12-102 (B.1.351) variants including 0d, 3wk and 6wk samples. Comparison
536 between two D614G virus isolates, FIN-25 and SR121, was done with sera from 86 BNT162b2
537 vaccinees. Correlation co-efficient (r) was calculated with Pearsons correlation test and p-values <0.05
538 were considered significant. Each dot may represent multiple samples.

539 **Fig. 5 Antibody responses against SARS-CoV-2 S1 protein and neutralization of B.1.1.7 and**
540 **B.1.351 variants by age and gender.**

541 **A.** BNT162b2 vaccinated health care workers were divided into four age groups. Age specific
542 differences of anti-S1 IgG antibody levels and neutralization titres against FIN-25 virus isolate were
543 analyzed. Sera was collected three weeks (3w) and six weeks (6wk) after the first vaccine dose. **B.**
544 Gender-specific differences in antibody responses and neutralization titres. IgG antibody levels are
545 represented as EIA units. Differences between age and gender groups were tested with two-tailed
546 Mann-Whitney U test. Two-tailed p-values *<0.05, **<0.01, ****<0.0001 were considered significant.

547 **Fig. 6 Correlation of anti-S1 antibody levels with SARS-CoV-2 neutralization titres.**

548 Anti-S1 IgG and total Ig antibody levels were determined with EIA and neutralization titres of
549 BNT162b2 vaccinated health care workers (n=169) against FIN-25 virus isolate were obtained with
550 microneutralization test (MNT). All sequential serum samples (0d, 3wk and 6wk) were included in the
551 analysis. Spearman's rank correlation coefficient (r) is indicated.

552 **Table 1 Antibody responses in BNT162b2 vaccinated health care workers (HCW) and non-**
553 **hospitalized convalescent phase COVID-19 patients.** HCW samples were collected before
554 vaccination (0d), and three (3wk) and six (6wk; three weeks after the second vaccine dose) weeks after
555 the first vaccine dose. Geometric mean (GM) and number of positive samples for anti-S1 IgG and total
556 Ig, and anti-N IgG antibodies and neutralizing antibodies is indicated. In microneutralization test
557 (MNT) neutralization titre >20 was considered positive and for calculation of geometric means a value
558 of 10 was given for values of <20.

		0d		3wk		6wk		Convalescent	
		GM	Positive (n/n)	GM	Positive (n/n)	GM	Positive (n/n)	GM	Positive (n/n)
EIA	anti-S1 IgG	1	0% (0/169)	47	96% (160/167)	107	100% (169/169)	20	62% (31/50)
	anti-S1 tot Ig	2	4 % (6/169)	37	96 % (160/167)	86	100 % (169/169)	23	82 % (41/50)
	anti-N IgG	2	4% (6/169)	2	7% (11/167)	2	5% (9/169)	17	66% (33/50)
MNT	FIN-25	10	0% (0/169)	24	63% (106/167)	234	100% (169/169)	55	86% (43/50)
	SR121	10	0% (0/84)	32	83% (70/84)	275	100% (86/86)	86	96% (48/50)
	85HEL	10	0% (1/169)	24	63% (106/167)	240	100% (169/169)	74	96% (48/50)
	HEL12-102	10	0% (0/169)	12	15% (25/167)	48	92% (156/169)	16	56% (28/50)

559

560 **Figure 1**

563 **Figure 2**

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569

570

Figures

Figure 1

Figure 1

Antibody responses against SARS-CoV-2 S1 and N proteins in BNT162b2 vaccinated health care workers and non-hospitalized recovered COVID-19 patients. A. Anti-S1 and B. anti-N IgG, IgA, IgM, and total Ig antibody levels were measured with EIA. Serum samples from BNT162b2 vaccinated participants (n=169) were collected before vaccination (0d), and three (3wk) and six (6wk) weeks after the first dose of the vaccine. All vaccinees received the second dose of the vaccine three weeks after the first dose. Convalescent phase patient samples (Conv, n=50) were collected 14 days to 6 weeks after the positive RT-qPCR test result. Data is represented as geometric means and geometric standard deviations (SD). Cut-off values are indicated with dashed lines.

Figure 2

Figure 2

Genetic analysis of virus variants and spike protein structure prediction. A. Schematic presentation of S gene and amino acid changes in FIN-25 (B.1 lineage), SR121 (B.1.463), 85HEL (B.1.1.7) and HEL12-102 (B.1.351) virus isolates used in the present study. B. Trimeric SARS-CoV-2 S protein, referred to as the spike, structure in closed conformation (pdb: 6VXX). Amino acid substitutions (yellow) and deletions (green) as compared to the original spike structure. C. Collective presentation of all amino acid changes found in virus isolates. Space filling model indicating amino acids changes (yellow) and deletions (green) on the surface of a trimeric S protein. Side and top views are shown.

Figure 3

Figure 3

Neutralization of B.1.1.7 and B.1.351 variants by BNT162b2 vaccinees' sera and COVID 19 patient sera. A. Neutralization titres for D614G variants FIN-25 and SR121, and 85HEL (B.1.1.7) and HEL12-102 (B.1.351) variants before (0d), three (3wk) and six weeks (6wk) after the first dose of BNT162b2 vaccine and neutralization titres of convalescent sera of non-hospitalized patients. Values above the groups indicate geometric mean titres (GMTs) and data is shown as geometric means and geometric SDs.

Neutralization titres <20 were plotted as 10. B. Neutralization titres 3 weeks (3wk) and six weeks (6wk) after the first dose of the vaccine. Statistical differences between the virus isolates were analyzed with Wilcoxon matched pairs signed-rank test. Two-tailed p-values *<0.05, **<0.01, ****<0.0001 were considered significant

Figure 4

Figure 4

Correlation of MNT titres against SARS-CoV-2 isolates. MNT titres of BNT162b2 vaccinees (n=169) against FIN-25 were compared with MNT titres against 85HEL (B.1.1.7) and HEL12-102 (B.1.351) variants

including 0d, 3wk and 6wk samples. Comparison between two D614G virus isolates, FIN-25 and SR121, was done with sera from 86 BNT162b2 vaccinees. Correlation co-efficient (r) was calculated with Pearsons correlation test and p-values <0.05 were considered significant. Each dot may represent multiple samples.

Figure 5

Figure 5

Antibody responses against SARS-CoV-2 S1 protein and neutralization of B.1.1.7 and B.1.351 variants by age and gender. A. BNT162b2 vaccinated health care workers were divided into four age groups. Age specific differences of anti-S1 IgG antibody levels and neutralization titres against FIN-25 virus isolate were analyzed. Sera was collected three weeks (3w) and six weeks (6wk) after the first vaccine dose. B. Gender-specific differences in antibody responses and neutralization titres. IgG antibody levels are represented as EIA units. Differences between age and gender groups were tested with two-tailed Mann-Whitney U test. Two-tailed p-values $* < 0.05$, $** < 0.01$, $**** < 0.0001$ were considered significant.

Figure 6

Figure 6

Correlation of anti-S1 antibody levels with SARS-CoV-2 neutralization titres. Anti-S1 IgG and total Ig antibody levels were determined with EIA and neutralization titres of BNT162b2 vaccinated health care workers (n=169) against FIN-25 virus isolate were obtained with microneutralization test (MNT). All sequential serum samples (0d, 3wk and 6wk) were included in the analysis. Spearman's rank correlation coefficient (r) is indicated.

Supplementary Files

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