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1 **TITLE:** Neutralization heterogeneity of United Kingdom and South-African SARS-CoV-2
2 variants in BNT162b2-vaccinated or convalescent COVID-19 healthcare workers

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22 **Running title:** COVID-19 infection or vaccine-elicited NAb

23 **ABSTRACT**

24 There are concerns about neutralizing antibodies (NAbs) potency against SARS-CoV-2
25 variants. Despite decreased NAb titers elicited by BNT162b2-vaccine against VOC202012/01
26 and 501Y.V2 (SA) strains, 28/29 healthcare workers (HCW) had a NAb titer $\geq 1:10$. In contrast,
27 six months after COVID-19 mild forms, only 9/15 (60%) of HCW displayed detectable NAbs
28 against SA strain.

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45 INTRODUCTION

46 In the gene encoding the Spike (S) protein of SARS-CoV-2, various mutations have been
47 reported[1,2] and recently, the United Kingdom (UK) and South Africa (SA) have faced a rapid
48 increase in COVID-19 cases mediated by the emergence of new variants (VOC-202012/01 for
49 UK and 501Y.V2 for SA)[3,4]. The spreading of these variants has increased rapidly in other
50 countries and recent observations suggests that they are significantly more transmissible than
51 previously circulating variants. It is still not fully known if the pathogenicity is either increased,
52 although some elements have been recently released with likely enhanced disease severity for
53 the UK strain[5].

54 These variants harbor a specific pattern of deletion and mutations including amino-acid
55 replacements at key sites in the S Receptor Binding Domain (RBD) (K417N, E484K, N501Y
56 for the SA strain and only N501Y for the UK strain) and in the N-terminal domain (Δ 69/70 and
57 Δ Y144 deletions for the UK strain and L18F, D80A, D215G and Δ 242-244 for the SA strain).

58 In the era of the COVID-19 vaccination, the question remained whether these variants could
59 escape the neutralizing response elicited by mRNA-vaccine. Two recent studies performed on
60 engineered SARS-CoV-2 viruses containing only some mutations from the newly emerged UK
61 and SA variants showed weaker neutralization capacity of vaccine-elicited sera[6,7]. Another
62 study tested SARS-CoV-2-S pseudoviruses bearing either the Wuhan reference strain or the
63 UK spike protein with BNT162b2 vaccine-elicited sera showed a slightly reduced but overall
64 largely preserved neutralizing titers against the UK pseudovirus[8]. However, none of these
65 studies was performed on clinical isolates harboring the full genomic mutations background of
66 UK and SA strains. Thus, the question remained whether a replicating virus with the full set of
67 S mutations, which may potentially interfere with antibody binding would be neutralized
68 efficiently by convalescent COVID-19 or BNT162b2-immune sera, especially in the healthcare
69 workers (HCW), a particularly exposed population to SARS-CoV-2 infection.

70 To answer this question, we performed a virus neutralization test (VNT), with a strict 100%
71 inhibition criterion, on sera from HCW with either previous mild forms of COVID-19 or
72 BNT162b2 immunization using three clinical isolates of SARS-CoV-2 variants: a D614G strain
73 (D614G) which became the dominant form of the virus circulating globally in the second part
74 of 2020[2], a UK strain (UK, lineage B.1.1.7) and a SA strain (SA, lineage B.1.351).

75

76 **MATERIALS AND METHODS**

77 **Study population and serum specimen**

78 Convalescent sera were recovered six months after symptom's onset from symptomatic HCW
79 with a positive RT-PCR result. BNT162b2-vaccine elicited sera were recovered three weeks
80 after the first injection and seven days after the booster immunization. This retrospective study
81 was carried out in accordance with the Declaration of Helsinki without addition to standard of
82 care procedures. Data collection were declared to the Sorbonne Université Data Protection
83 Committee under number 2020-025. Written informed consent for participation in this study
84 was obtained from all participants.

85 **Virus neutralization test**

86 The neutralizing activity of the various serum specimen was assessed with a whole virus
87 replication assay as previously described (9) using three SARS-CoV-2 clinical isolates D614G,
88 UK and SA (GenBank accession number MW322968, MW633280 and MW580244
89 respectively). Microscopy examination was performed on day 4 to assess the cytopathic effect
90 (CPE). Neutralizing antibody (NAb) titers are expressed as the highest serum dilution
91 displaying 100% (NT₁₀₀), 90% (NT₉₀) or 50% (NT₅₀) inhibition of the CPE. A same known
92 positive control serum was added to each experiment to assess the repeatability.

93 **Statistical analysis**

94 NT₅₀ or NT₉₀ were inferred by non-linear regression using a four-parameter variable slope
95 model using GraphPad Prism 8.0.2 software. Geometric mean titer (GMT) with 95%
96 confidence interval (95%CI) were calculated for NT₁₀₀, NT₅₀ and NT₉₀ (Figure 1 and Table S1).
97 Difference in distribution of NT₁₀₀ between UK strain or SA strain with the D614G strain was
98 performed with a two-tailed Mann-Whitney-U test. A probability value of $p < 0.05$ was
99 considered statistically significant. No statistical comparisons were made between post
100 vaccines and mild COVID-19 groups.

101

102 RESULTS

103 We studied two sets of serum samples from HCW: a convalescent group of 15 participants with
104 SARS-CoV-2 proven infection on March 2020 and a vaccinated group of 29 participants
105 without history of clinical COVID-19. The median [IQR] age was 50 [32 – 66] years and 40%
106 (6/15) were male for the convalescent group. The median age was 55 [38 – 65] and 31% (9/29)
107 were male for the vaccinated group. Convalescent sera were collected 6 months after the
108 symptom's onset (184 [182 – 189] days). Three weeks after the first injection of the BNT162b2
109 vaccine, 52% (13/25) of HCW harbored NT₁₀₀ \geq 1:5 against the D614G strain, 24% (6/25) were
110 neutralizing against the UK strain and only two (8%) had detectable NAbs against the SA strain
111 (Figure 1A). Seven days after the booster immunization, all but one HCW displayed
112 ~~neutralizing activity~~ NT₁₀₀ against the three SARS-CoV-2 clinical strains with a GMT of 117.3
113 (95%CI, 90.4 to 152.0) against the D614G strain, 45.1 (95%CI, 34.3 to 59.3) against the UK
114 strain and 22.9 (95%CI, 16.6 to 31.6) against the SA strain. The NT₁₀₀ against UK and SA
115 strains were significantly reduced compared to NT₁₀₀ against the D614G strain 7 days after the
116 second injection of BNT162b2 vaccine (respectively, $p < 0.0001$ and $p < 0.0001$) (Figure 1B).
117 Six months after the symptom's onset, all the 15 HCW of the convalescent group harbored
118 NT₁₀₀ against the D614G strain (GMT of 21.0; 95%CI, 11.8 to 37.1) and the UK strain (GMT

119 of 14.5; 95%CI, 8.8 to 23.9) without statistical difference between the respective NT₁₀₀ (p =
120 0.40). However, only 60% (9/15) serum samples of these HCW displayed a neutralizing activity
121 against the SA strain with a NT₁₀₀ GMT of 3.3 (95%CI, 1.8 to 6.1; p < 0.0001) (Figure 1C).

122

123 **DISCUSSION**

124 In this work we assessed the neutralizing activity of sera from 15 convalescent COVID-19 or
125 29 BNT162b2-vaccinated HCW against the two rapidly spreading SARS-CoV-2 variants of
126 concern VOC202012/01 and 501Y.V2 and the globally circulating variant D614G using a VNT
127 with whole replicating clinical strains. Based on a very strict criterion of 100% inhibition of
128 CPE to determine NT₁₀₀, we show that, three weeks after a single dose of BNT162b2, these
129 NT₁₀₀ remain inexistent or low among HCW especially against the UK and SA variants and
130 could questioned the extend of the dosing interval of BNT162b2 in some countries in order to
131 vaccinate as many people as possible. This observation was confirmed with less strict/restrictive
132 criteria of NT₉₀ or NT₅₀ (Table S1). However, we were not able to follow participants more
133 than three weeks after the first injection because all of them received a second dose of
134 BNT162b2 according to the French guidelines. Nevertheless, seven days after the booster
135 immunization all but one vaccinated HCW develop NAbs against the three strains with a highest
136 neutralizing activity against the strain closely related to the Wuhan ancestral strain, the D614G
137 strain. Despite a 2.60-fold reduction of NT₁₀₀ GMT against the UK and a 5.12-fold reduction
138 against the SA strains in comparison with the D614G most of the participants have displayed a
139 neutralizing activity \geq 1:10 which could be at least indicative of a potential protection against
140 severe COVID-19 even with these variants. Although the correlates of protection are not
141 already known, there is probably a certain degree of protection before the NAbs are detectable.
142 Based on NT₁₀₀, NT₉₀ and NT₅₀ values, we also demonstrate a lack of serum neutralizing
143 activity against SA strain in up to 40% of HCW recovered from mild form of COVID-19 six

144 months after the symptom's onset associated with a 6.32 to 7.17-fold reduction of GMT in the
145 HCW with detectable NAb. This finding, and the recent report describing a severe case of
146 reinfection by the SA variant four months after a first COVID-19 infection[9], highlights the
147 need of vaccination even in people who had recovered from a previous COVID-19, especially
148 during the increased circulation of the SARS-CoV-2 variants. Nevertheless, correlates of
149 immunity to the SARS-CoV-2 are not well defined, only few studies have tried to assess these
150 correlates in other human coronaviruses with experimental challenges on volunteers. They
151 showed an association between serum NAb titers pre-exposure and viral excretion[10]. Further
152 studies are required to determine the SARS-CoV-2 correlates of vaccine-induced protection
153 based on NAb and T cell responses. A limitation of our work is that we were not able to assess
154 potential cellular response differences against the three strains in the vaccinated or convalescent
155 groups although it has been described generation of a robust CD4+ and CD8+ responses against
156 the Wuhan ancestral strain[11]. The long-term evaluation regarding the lasting of NAb induced
157 by vaccination is needed to assess the durability of protection against SARS-CoV-2 variants.

158

159 **CONCLUSION**

160 In conclusion, in BNT162b2-vaccinated participants with two dose regimen, despite
161 heterogeneity neutralizing capacity against the three SARS-CoV-2 variants, most of the sera
162 harbored at least a NAb titer $\geq 1:10$. Although immune protection correlates need to be defined,
163 our findings suggests a certain humoral protection activity either on UK or SA variants after
164 two doses of mRNA-vaccine. We also show that six months after SARS-CoV-2 infection
165 leading to mild forms of COVID-19, an important proportion of HCW displayed no neutralizing
166 activity against SA strain. This result supports a strong recommendation for SARS-CoV-2
167 vaccination of previously infected subjects.

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174 UK and SA strains.

175 **Competing interests:** Authors declare that they have no competing interests.

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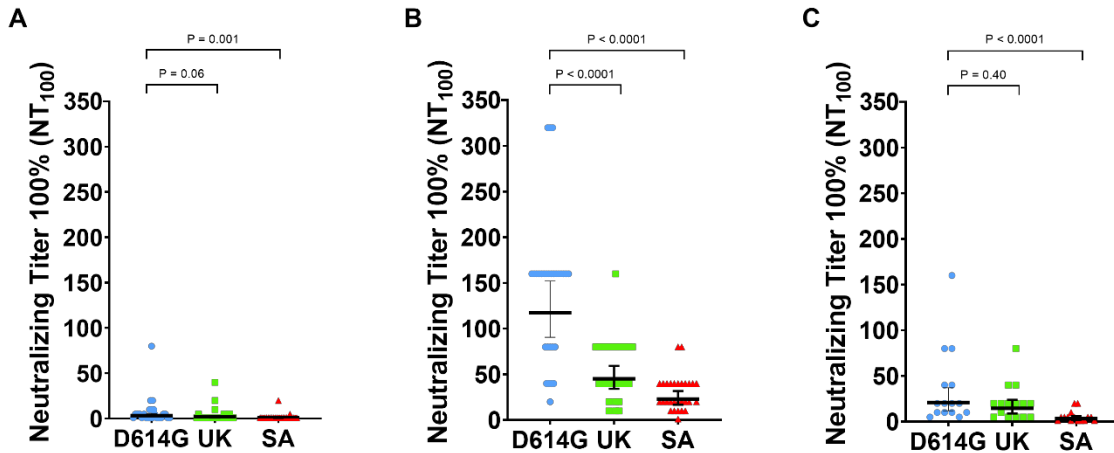
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257 **Fig. 1. Neutralizing antibody (NAb) titer with 100% inhibition (NT₁₀₀) against clinical**
 258 **strains of D614G, United-Kingdom (UK) and South African (SA) SARS-CoV-2 variants**
 259 **of 29 BNT162b2-vaccine elicited sera and 15 convalescent sera recovered from healthcare**
 260 **workers (HCW). (A) NT₁₀₀ against the three clinical isolates of BNT162b2-vaccine elicited**
 261 **HCW sera recovered three weeks after first injection. (B) NT₁₀₀ against the three clinical isolates**
 262 **of BNT162b2-vaccine elicited HCW sera recovered seven days after second injection. (C)**
 263 **NT₁₀₀ against the three clinical isolates of convalescent COVID-19 HCW sera recovered 6**
 264 **months after the symptom's onset. NT₁₀₀ against D614G strain are in blue dot, NT₁₀₀ against**
 265 **UK strain are in green square and NAb titer against SA strain are in red triangle. Black**
 266 **horizontal lines indicate geometric median titer (GMT) of NT₁₀₀. Whiskers indicate 95%**
 267 **confidence interval. Two-tailed P values were determined using the Mann-Whitney test and are**
 268 **reported on each panel.**

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272 **Table S1. Geometric median titer (GMT) of neutralizing antibody (NAb) with 50% (NT₅₀) or 90% (NT₉₀) inhibition against clinical strains**
 273 **of D614G, United-Kingdom (UK) and South African (SA) SARS-CoV-2 variants of 29 BNT162b2-vaccine elicited sera and 15 convalescent**
 274 **sera recovered from healthcare workers (HCW). 95% confidence interval were written between parentheses.**

275

	D614G strain		UK strain		SA strain		GMT ratio D614G/UK		GMT ratio D614G/SA	
	NT50	NT90	NT50	NT90	NT50	NT90	NT50	NT90	NT50	NT90
BNT162b2 - 1 st injection	5.38 (3.0 to 9.6)	4.90 (2.8 to 8.7)	3.46 (2.0 to 5.7)	2.89 (1.8 to 4.7)	1.47 (1.0 to 2.2)	1.39 (1.0 to 2.0)	1.56	1.70	3.66	3.53
BNT1622 - 2 nd injection	174.2 (133.5 to 227.2)	157.9 (122.4 to 203.8)	72.34 (52.8 to 99.1)	55.89 (39.2 to 79.6)	40.55 (28.5 to 57.7)	31.99 (22.4 to 45.8)	2.41	2.83	4.30	4.94
Convalescent	35.40 (20.2 to 62.1)	30.88 (17.1 to 55.7)	28.11 (16.5 to 47.8)	20.04 (12.1 to 33.2)	4.94 (2.4 to 10.1)	4.43 (2.3 to 8.5)	1.26	1.54	7.17	6.97

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