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## **Strong decay of SARS-CoV-2 spike antibodies after two BNT162b2 vaccine doses and high antibody response to a third dose in nursing home residents**

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**Running title :** Strong boost vaccine antibody response in residents

**Key words:** BNT162b2 vaccine; SARS-CoV-2 spike antibodies; nursing home residents; boost vaccine dose.

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**Brief summary** - The 6-month post-vaccine decay of antibodies and the high antibody response after the third BNT162b2 vaccine dose validate a boost vaccine dose less than 6 months after the second dose in residents.

**Authors' contributions:** Hubert Blain has full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

*Design and conduct of the study:* Blain, Tuaillon

*Collection, management, analysis, and interpretation of the data:* all authors

*Drafting of the manuscript:* Blain, Tuaillon

*Critical revision of the manuscript:* all authors

*Decision to submit the manuscript for publication:* Blain

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1 **Abstract**

2 **Objectives:** To measure the antibody decay after two BNT162b2 doses and the antibody  
3 response after a third vaccine dose administered 6 months after the second one in nursing home  
4 residents with and without prior COVID-19.

5 **Design:** Cohort study.

6 **Setting and Participants:** 418 residents from 18 nursing homes.

7 **Methods:** Blood RBD-IgG (IgG II Quant assay, Abbott Diagnostics; upper limit: 5680 BAU)  
8 and nucleocapsid-IgG (Abbott Alinity) were measured 21-28 days after the second BNT162b2  
9 dose, as well as 1-3 days before and 21-28 days after the third vaccine dose. RBD-IgG levels  $\geq$   
10 592 BAU/mL were considered as high antibody response. Residents with prior positive RT-  
11 qPCR on a nasopharyngeal swab or with N-IgG levels above 0.8 S/CO were considered as prior  
12 COVID-19 residents.

13 **Results:** In prior COVID-19 residents (n=122), RBD-IgG median levels decreased by 82% in  
14 167 days on average. In the same period, the number of residents with a high antibody response  
15 decreased from 88.5% to 54.9% ( $p < .0001$ ) and increased to 97.5% after the third vaccine dose  
16 ( $p = 0.02$  vs the first measure). In residents without prior COVID-19 (n=296), RBD-IgG median  
17 levels decreased by 89% in 171 days on average. The number of residents with a high antibody  
18 response decreased from 29.4 to 1.7% ( $p < .0001$ ) and increased to 88.4% after the third vaccine  
19 dose ( $p < .0001$  vs the first measure)

20 **Conclusions and Implications:** The strong and rapid decay of RBD-IgG levels after the second  
21 BNT162b2 dose in all residents and the high antibody response after the third dose validate the  
22 recommendation of a third vaccine dose in residents less than 6 months after the second dose,  
23 prioritizing residents without prior COVID-19. The slope of RBD-IgG decay after the third

24 BNT162b2 dose and the protection level against SARS-CoV-2 B.1.1.529 (omicron) and other  
25 variants of concern provided by the high post-boost vaccination RBD-IgG response require  
26 further investigation in residents.  
27

## 28 **Introduction**

29 Nursing home (NH) residents are at high risk for severe COVID-19. Vaccination using the  
30 mRNA COVID-19 vaccine is safe and effective in adults but is less documented in older  
31 persons.<sup>1-3</sup> The humoral response observed in NH residents 21 days after the first BNT162b2  
32 vaccine dose<sup>4</sup>, and 14 days<sup>5,6</sup> and 6 months following the second vaccine dose,<sup>7-9</sup> has been  
33 shown to be lower than that observed in healthcare professionals. In addition, outbreaks of the  
34 SARS-CoV-2 variants B.1.1. 4-7 (alpha) and B.1.617.2 (delta) have been reported in nursing  
35 homes (NHs) less than 6 months after the full BNT162b2 vaccination of most residents.<sup>8,11</sup> The  
36 low humoral response observed after two BNT162b2 doses, associated with reduced vaccine  
37 protection some months after the second vaccine dose, has led to recommendations in the US and  
38 in other countries such as France of an additional vaccine dose at least 5 months after the second  
39 vaccine dose in persons moderately or severely immunocompromised.<sup>12,13</sup> In France, more than  
40 95% of residents had received a second BNT162b2 dose in September 2021.<sup>14</sup>  
41 The 6-month post-vaccine antibody decay after the second vaccine dose and the antibody  
42 response to the third dose have not been quantified in the same NH residents, which does not  
43 enable the comparison of the antibody response after 2 and 3 BNT162b2 doses.  
44 We compared IgG levels against the SARS-CoV-2 Receptor-Binding Domain (RBD-IgG) (i)  
45 after two doses of BNT162b2, (ii) just before the third vaccine dose, and (iii) after the third dose.  
46 We distinguished those with and without prior COVID-19.

## 47 **Methods**

48 Since March 2019, around 6,000 residents from 122 nursing homes are being prospectively  
49 followed in the Montpellier area (French Occitanie region). All residents, with or without prior  
50 COVID-19 (outbreaks of wild type SARS-CoV-2 between March 2020 and February 2021; of

51 SARS-CoV-2 B.1.1.7 between February 2021 and October 2021; of B.1.617.2 between July  
52 2021 and October 2021), were offered two vaccine doses in January-February 2021, and a third  
53 dose 6 months later. A follow-up of post-vaccine antibody response was proposed in residents  
54 from fifteen nursing homes having faced a COVID-19 outbreak in 2020. Blood testing was  
55 performed 21-28 days after the second vaccine dose, as well as 1-3 days before and 21-28 days  
56 after the third dose, to assess RBD-IgG (IgG II Quant assay, Abbott Diagnostics; upper limit:  
57 5680 BAU) and nucleocapsid-IgG (Abbott Alinity). RBD-IgG levels  $< 149$  BAU/mL or  $\geq 592$   
58 BAU/mL were considered as low or high responses, respectively.<sup>15,16</sup> Residents with a history of  
59 positive RT-qPCR on a nasopharyngeal swab or with N-IgG levels above 0.8 S/CO were  
60 considered as prior COVID-19 residents. All participants (or their legal representative) provided  
61 informed consent. The study was approved by our University Hospital institutional review board.  
62 RBD-IgG levels were compared by using the Friedman and Cochran tests, and the multinomial  
63 mixed model for repeated measures. The statistical significance threshold was set at 5%.  
64 Analyses were performed using the SAS Enterprise Guide, v7.3 (SAS Institute Inc).

## 65 **Results**

66 The characteristics of the residents included in the 15 nursing homes are shown in Table 1. Of  
67 the 418 studied residents, 296 had no previous COVID-19 infection (mean age  $88 \pm 8$  years, 74%  
68 women), and 122 had prior COVID-19 ( $88.6 \pm 8$  years, 81% women). The mean lapse of time  
69 between the first and the second RBD-IgG measures were  $171 \pm 11$  and  $167 \pm 11$  days,  
70 respectively.

71 In prior COVID-19 residents, RBD-IgG median levels decreased by 82% between the first and  
72 second measures (4,318 BAU/mL to 773 BAU/mL), and the number of residents with a high

73 antibody response decreased from 88.5% to 54.9% ( $p < .0001$ ). After the third dose, RBD-IgG  
74 median levels increased significantly (to 3,596 BAU/mL), and the number of residents with a  
75 high RBD-IgG level was higher after the third and after the second dose (97.5% vs 88.5%,  
76 respectively)( $p = 0.02$ )(Table 2, Figure).

77 In residents without prior COVID-19, RBD-IgG median levels between the first and second  
78 measures decreased by 89% (348 BAU/mL to 35 BAU/mL), and the number of residents with a  
79 high antibody response decreased from 29.4% to 1.7% ( $p < .0001$ ). After the third vaccine dose,  
80 88.4% of residents had a high antibody response vs 29.4% after the second dose, and 4.7% had a  
81 low antibody response vs 27.4% ( $p < .0001$ )(Table, Figure).

82

### 83 **Discussion**

84 This preliminary study shows that 6 months after the second BNT162b2 dose, 88.8% of residents  
85 without prior COVID-19 have an RBD-IgG level below 149 BAU, and only 54.9% of those with  
86 prior COVID-19 have an RBD-IgG level above 592 BAU. The 82-89% decrease in RBD-IgG  
87 level observed in both groups in less than 6 months may partly explain the occurrence of  
88 outbreaks of the SARS-CoV-2 variants B.1.1. 4-7 and, more recently, of the Delta variant  
89 B.1.617.2 in nursing homes (NHs) in which most of the residents had been vaccinated with two  
90 doses of the BNT162b2 vaccine.<sup>8,11</sup> This tends to validate, on an immunological basis, the  
91 recommendation of a third BNT162b2 dose at least 5 months after the second one in NH  
92 residents. The RBD-IgG response after 3 vaccine doses is significantly higher than that observed  
93 after two doses, and is excellent, largely above 592 BAU, in 97.5% of the residents with prior  
94 COVID-19 and in 88.4% of those without. Limitations of the study include the relatively small



95 sample size, with a possible lack of representativeness, and the lack of clinical data in residents  
96 with prior COVID-19. Whether the severity of the initial infection has impacted the antibody  
97 response to the vaccine cannot be analyzed in this study. One other limitation is the lack of  
98 neutralization assays. However, we used an automated quantitative assay to measure the RBD  
99 IgG level that correlates well with virus neutralization.<sup>17,18</sup> We did not assess T-cell contribution  
100 to vaccine-induced immunity. The present study focuses on antibodies and not on clinical  
101 disease. Even if associations have been shown between RBD-IgG levels and protection against  
102 SARS-CoV-2 in healthcare workers and NH residents<sup>8,11, 19-21</sup>, whether the high level of RBD  
103 IgG found after a booster BNT162b2 dose in most residents is associated with high protection  
104 against COVID-19 remains to be determined.

105 Because the administration of a booster BNT162b2 dose as well as the vaccination of previously  
106 infected individuals generate an anti-B.1.1.529 neutralizing response, with titers 5 to 31 fold  
107 lower against Omicron than against Delta,<sup>22</sup> further studies are needed to assess whether the high  
108 RBD-IgG response found in most of the residents after the third vaccine dose will be sufficient to  
109 prevent SARS-CoV-2 B.1.1.529 in those with and without prior COVID-19 and, if so, for what  
110 duration.

## 111 **Conclusions and Implications**

112 The strong and rapid decay of RBD-IgG levels 6 months after the second BNT162b2 dose  
113 observed in most of the residents (with very low levels observed after the second dose in  
114 residents without prior COVID-19), as well as the high antibody response after the third dose in  
115 the residents (observed in the present study and in a recent unpublished study)<sup>23</sup> support, on an  
116 immunological basis, the recommendation to offer a third vaccine dose in nursing home residents  
117 less than at least 5 months after the second dose, prioritizing those without prior COVID-19.

118 Whether the slope of RBD-IgG decay after the third BNT162b2 dose will be similar to that  
119 found after the second vaccine dose, and whether the high RBD-IgG response found after the  
120 third vaccine dose will be associated with a high protection level against SARS-CoV-2 B.1.1.529  
121 and other variants of concern require further investigation in nursing home residents.

122

123

124 **Figure.** Individual change in RBD-IgG levels between 21-28 days after the second BNT162b2  
125 vaccine dose (Post-V2), 1-3 days before the third vaccine dose (Pre-V3), and 21-28 after the  
126 third vaccine dose (Post-V3) of residents with or without confirmed prior COVID-19

127 **Legend:** Between March 2019 and September 2021, residents from nursing homes facing a  
128 COVID-19 outbreak had repeated RT-qPCR testing until no new cases were diagnosed.  
129 Residents from 18 nursing homes having faced a COVID-19 outbreak in 2020 were proposed to  
130 undergo blood testing (i) to follow their post-BNT162b2 vaccine response using SARS-CoV-2  
131 Receptor-Binding Domain (RBD-IgG) levels (IgG II Quant assay, Abbott Diagnostics; upper  
132 limit: 5680 BAU) and (ii) to determine if they had prior COVID-19, using IgG antibodies against  
133 the SARS-CoV-2 nucleocapsid protein (N Protein-IgG) (Abbott Alinity; positive level when  
134 above 0.8 S/CO). Blood testing when an informed consent was obtained was performed 21-28  
135 days after the second vaccine dose, 1-3 days before the third dose, and 21-28 days after the third  
136 dose in order to make comparisons possible in every resident. To make the illustration more  
137 readable, the change in values of RBD-IgG levels 21-28 days after the second vaccine dose,  
138 before the third dose administered 6 months after the second dose, and 21-28 days after the third  
139 vaccine dose are presented for 100 residents with and 100 residents without prior COVID-19.

140 These residents were drawn at random from the 122 with and the 296 without confirmed prior  
141 COVID-19.

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<b>Nursing homes</b>	<b>Studied residents, N (%)</b>	<b>Female/male gender, N (%)</b>	<b>Age, Mean (SD)</b>	<b>Prior COVID-19, N (%)</b>
1	38 (9.1)	28 (73.7)/ 10 (26.3)	86.5 (8.7)	9 (23.7)
2	38 (9.1)	34 (89.5)/ 4 (10.5)	92.1 ( 5.6)	15 (39.5)
3	37 (8.8)	27 (73.0)/ 10 (27.0)	86.4 (8.4)	4 (10.8)
4	14 (3.3)	10 (71.4)/ 4 (28.6)	83.1 (10.6)	2 (14.3)
5	25 (6.0)	17 (68.0)/ 8 (32.0)	84.6 (7.4)	10 (40.0)
6	28 (6.7)	19 (67.9)/ 9 (32.1)	86.8 (7.0)	8 (28.6)
7	26 (6.2)	20 (76.9)/ 6 (23.1)	87.0 (6.8)	7 (26.9)
8	21 (5.0)	15 (71.4)/ 6 (28.6)	86.7 (8.7)	6 (28.6)
9	32 (7.7)	23 (71.9)/ 9 (28.1)	86.6 (7.7)	9 (28.1)
10	34 (8.1)	28 (82.3)/ 6 (17.7)	91.2 (7.3)	26 (76.5)
11	11 (2.6)	10 (90.9)/ 1 (9.1)	92.8 (5.4)	3 (27.3)
12	20 (4.8)	15 (75.0)/ 5 (25.0)	91.9 (7.2)	9 (45.0)
13	27 (6.5)	21 (77.8)/ 6 (22.2)	87.1 (8.8)	6 (22.2)
14	37 (8.8)	27 (73.0)/ 10 (27.0)	90.4 (5.6)	8 (21.6)
15	30 (7.2)	23 (76.7)/ 7 (23.3)	89.3 (8.2)	0 (0.0)
<b>Total</b>	<b>418 (100)</b>	<b>317 (75.8)/ 101 (24.2)</b>	<b>88.7 (8.0)</b>	<b>122 (29.2)</b>

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218  
219

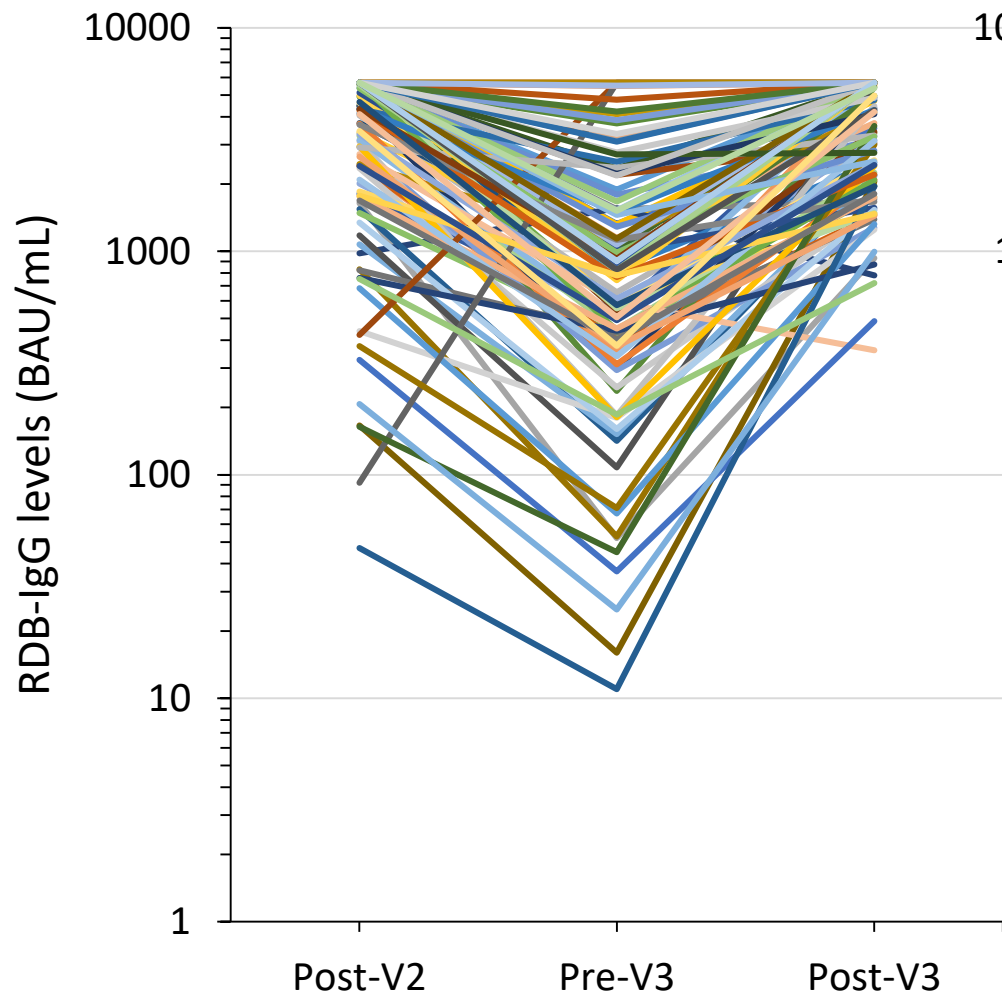
Table 1. Demographic characteristics of the residents studied in 15 nursing homes

220 Table 2: RBD-IgG levels 4-5 weeks after the second BNT162b2 vaccine dose (A), 1-3 days  
 221 before the third vaccine dose (B), and 4-5 weeks after the third vaccine dose (C) of residents with  
 222 (n=122) or without (n = 296) confirmed prior COVID-19 infection.  
 223

	After the second vaccine dose (A)	Before the third vaccine dose (B)	After the third vaccine dose (C)	P value
<b>Prior COVID-19 residents (n=122)</b>				<.0001
RBD IgG level, median (IQRs), BAU	4318 (1772 ; 5680)	773 (339 ; 1742)	3596 (1751 ; 5680)	
RBD IgG Level, No. (%)				<.0001 <sup>b</sup>
- < 149 BAU	5 (4.1)	16 (13.1)	0	<.001 <sup>c</sup> A vs B
- 150 - 591 BAU	9 (7.4)	39 (32.0)	3 (2.5)	and B vs C
- ≥ 592 BAU	108 (88.5)	67 (54.9)	119 (97.5)	.02 <sup>c</sup> A vs C
<b>Without Prior COVID-19 residents (n=296)</b>				<.0001
RBD IgG level, median (IQRs), BAU	348 (120 ; 752)	35 (15 ; 70)	1926 (875 ; 3866 )	
RBD IgG Level, No. (%)				<.0001 <sup>b</sup>
- < 149 BAU	81 (27.4)	263 (88.8)	14 (4.7)	<.0001 <sup>c</sup> A vs B,
- 150 - 591 BAU	128 (43.2)	28 (9.5)	41 (13.8)	B vs C, and A vs
- ≥ 592 BAU	87 (29.4)	5 (1.7)	241 (88.4)	C

224  
 225 <sup>a</sup> Friedman test; <sup>b</sup> Multinomial mixed model; <sup>c</sup> Cochran test

### Prior-COVID-19



### Without prior COVID-19

