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Effectiveness and duration of additional immune defense provided by SARS-CoV-2 infection before and after receiving the mRNA COVID-19 vaccine BNT162b2

Nagashige Shimada [a,c], Satoshi Sugawa [b], Satoshi Murakami [b,*], Masahiro Shinoda [a], Shinichiro Ota [a], Miwa Morikawa [a], Hiroaki Takei [a,c], Yusuke Serizawa [a], Hidenori Takahashi [a], Mio Toyama-Kousaka [a], Hiroto Matsuse [c], Masaharu Shinkai [a]

[a] Department of Respiratory Medicine, Tokyo Shinagawa Hospital, Shinagawa, Tokyo, Japan
[b] Core Diagnostics, Abbott Japan LLC, Mita, Tokyo, Japan
[c] Division of Respiratory Medicine, Department of Internal Medicine, Toho University Ohashi Medical Center, Tokyo, Japan

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ABSTRACT

Background: Our investigation focused whether infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) before or after receiving the mRNA COVID-19 vaccine can increase immune protection. And we also investigated relationship of infection acquired.

Methods: Three shots of the mRNA coronavirus disease 2019 (COVID-19) vaccine BNT162b2 were administered to 736 healthcare workers at Tokyo Shinagawa Hospital. Serum samples were collected before the first shot (P1), at one month (P2), and at six months (P3) after the second shot and at one month after the third shot (P4). The presence of infection was assessed using IgG against the nucleocapsid (IgG (N) and RBD in the spike protein of SARS-CoV-2. We defined infection before P2 as natural infection (NI) and infection between P2 and P3 as breakthrough infection (BI) and compared susceptibility to further infection between the NI (−) and NI (+) groups and between BI (−) and BI (+) groups. Events in 485 participants who had a complete dataset of IgG (N) and IgG (RBD) from P1 to P4 were analyzed.

Results: The presence of SARS-CoV-2 infection before P2 were examined by examining the titers of IgG (N)P1, IgG (N) P2, and IgG (RBD) P1 that exceeded the cutoff values. Consequently, 35 participants (7.22 %) were categorized into the NI (+) group, whereas 450 (92.8 %) were categorized into the NI (−) group. Between P2 and P3, the NI (−) group showed a higher rate of SARS-CoV-2 infection than the NI (+) group; however, there was no significant difference in the infection rate between P3 and P4. The infection rate was significantly lower in the BI (+) group than in the BI (−) group. Pre-primary vaccination infection significantly increased IgG (RBD) levels between P1 and P3. Post-primary vaccination infection significantly increased IgG (RBD) levels between P3 and P4.

Conclusions: Infection with SARS-CoV-2 before or after receiving the mRNA COVID-19 vaccine can increase immune protection; however, the duration of this effect may be limited.

1. Background

Severe acute respiratory syndrome coronavirus 2 (SARS- CoV-2), a highly transmissible and pathogenic coronavirus that emerged in late 2019 in Wuhan, China, has been the cause of a global pandemic of acute respiratory disease known as “coronavirus disease 2019” (COVID-19) [1]. Globally, the number of accumulated cases and deaths as of February 2023 have exceeded 758 million and 6.8 million people, respectively [2]. Vaccination was implemented in late 2020 and the total vaccine doses have exceeded 13 billion [2]. BNT162b2, a COVID-19 vaccine developed by Pfizer-BioNTech uses mRNA technology to stimulate the production of antibodies against the spike protein of SARS-

Abbreviations: RBD, receptor-binding domains; N, nucleocapsid; NI, natural infection; BI, breakthrough infection; P1, Point 1 prior to the first shot; P2, Point 2 one month after the second shot; P3, Point 3 six months after the second shot; and P4, Point 4 one month after the third shot.

* Corresponding author at: Core Diagnostics, Abbott Japan LLC, Mita, Tokyo, Japan.
E-mail address: satoshi.murakami@abbott.com (S. Murakami).

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CoV-2 [3]. In a clinical trial involving 43,548 participants, BNT162b2 was found to be 95 % effective in preventing COVID-19 [3]. However, the challenges for this vaccine include waning antibodies after vaccination and the emergence of viral variants that circumvent part of the immune protection provided by the vaccine. Levin et al. reported a substantial decrease in IgG levels six months after the administration of a second dose of the BNT162b2 vaccine [4]. Bansal D et al. compared the time from the last dose of the primary vaccination schedule to the time by which anti-S IgG antibody titers fell into the lowest quartile (range of values collected), participants vaccinated with the mRNA-1273 vaccine had median anti-S-antibody level of 13,720.9 AU/mL (IQR 6426.5 to 30,185.6 AU/mL) BNT162b2 (median, 7570.9 AU/mL; IQR, 3757.9 to 16,577.4 AU/mL). The median time to reach the lowest quartile was 7.63 months (IQR, 6.3–8.4 months) for the Pfizer vaccine recipients, but more than 50 % of the Moderna vaccine recipients did not reach the lowest quartile by the end of the follow-up period [5]. Hacisuleyman et al. identified vaccine breakthrough infections (BIs) in two of 417 participants who had been administered two shots of mRNA COVID-19 vaccines: one vaccinated twice with mRNA-1273 (Moderna), and the other twice with BNT162b2 [6]. In both cases, three mutations (T95I, del142–144, and D614G) were identified in SARS-CoV-2, indicating a potential risk of BI by viral variants [6]. Although Bernal et al. reported that the effectiveness of two doses of BNT162b2 was 93.7 % (95 % CI, 91.6 to 95.3) among persons with the alpha variant and 88.0 % (95 % CI, 85.3 to 90.1) among those with the delta variant of the virus [7], Tang et al. reported the effectiveness of BNT162b2 against the delta variant after 14 days from the second shot was 51.9 % [8], suggesting the possibility for the attenuated effectiveness of BNT162b2 to the emerging variants. Callaway posed a concern that the fast-spreading omicron SARS-CoV-2 variant could weaken COVID-19 vaccine protection by illustrating that vaccine effectiveness dropped to 8.8 % at 25 weeks or more from the second BNT162b2 shot [9]. Hall et al. reported that infection-acquired immunity that is boosted with vaccination provided protection from infection for a longer duration than the protection from two doses of the BNT162b2 vaccine. Previous infection history is an essential factor in protective immunity [10]. In daily practice, there is a question of when people with a history of COVID-19 should be vaccinated.

Our investigation focused whether infection with SARS-CoV-2 before or after receiving the mRNA COVID-19 vaccine can increase immune protection. And we also investigated relationship of infection-acquired immunity and dynamics of IgG receptor-binding domains (RBD).

2. Materials and methods

2.1. Study design

This study was conducted at the Tokyo Shinagawa Hospital. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee at Tokyo Shinagawa Hospital (approval no. 20-A-34). A total of 736 healthcare workers at Tokyo Shinagawa Hospital, including physicians, nurses, physical therapists, medical technologists, and administrative personnel, participated in this study after providing written informed consent. All participants were intramuscularly administered the mRNA COVID-19 vaccine BNT162b2 (Pfizer, NY, USA) thrice, with intervals of three weeks and eight months between the three doses, as shown in Figure 1. Sera were obtained from the participants at four time points as shown in Figure 1: Point 1 (P1), prior to the first shot; Point 2 (P2), one month after the second shot; Point 3 (P3), six months after the second shot; and Point 4 (P4), one month after the third shot. Serum samples were tested for IgG against the nucleocapsid protein (IgG [N]) and against the RBD of the spike protein (IgG [RBD]) of SARS-CoV-2. Participant information, including age and sex, was used after anonymization.

2.2. Serological tests

IgG (N) was measured using an ARCHITECT SARS-CoV-2 IgG assay on Architect i2000 CS5100 (Abbott Laboratories, Abbott Park, IL, USA), and IgG (RBD) was measured using an ARCHITECT SARS-CoV-2 IgG II Quant assay on Architect i2000 CS5100 (Abbott Laboratories, Abbott Park, IL, USA). Both assays are based on chemiluminescent microparticle immunoassays (CLIA). According to the package insert of the IgG (N) assay, cutoff index is 1.4 signal/cutoff (S/C) and CV% at a mean index of 0.04 S/C of 50 negative controls is 5.9 %. According to the package insert of the IgG (RBD) assay, cutoff index is 50.0 AU/mL and lowest concentration at which CV% is within 20 % is 7.8 AU/mL. The positive agreement of ARCHITECT SARS-CoV-2 IgG assay (based on $\geq$ 14-days post-symptom onset) and negative agreement were 100.0 % (95.9 % to 100.0 %) and 99.6 % (99.1 % to 99.9 %), respectively. The positive agreement of ARCHITECT SARS-CoV-2 IgG II Quant assay (based on $\geq$ 15-days post-symptom onset) and negative agreement were 97.6 % (87.4 % to 99.9 %) and 99.6 % (99.1 % to 99.8 %), respectively. [11,12].

2.3. Definitions of natural infection and BI

We defined SARS-CoV-2 infection prior to P2 as “natural infection (NI)” and infection of SARS-CoV-2 between P2 and P3 as “BI).” The NI (+) group was excluded from the BI grouping to rule out the effects of SARS-CoV-2 infection before vaccination. In this study, 736 healthcare workers were enrolled; however, owing to the withdrawal of consent or protocol deviation between P1 and P4, we analyzed events in 485 participants who had a complete dataset of IgG (N) and IgG (RBD) from P1 to P4. After excluding 35 NI (+) participants, 450 participants were analyzed for comparison between BI groups (Figure 2).

The participants were categorized into the NI (+) group if they met

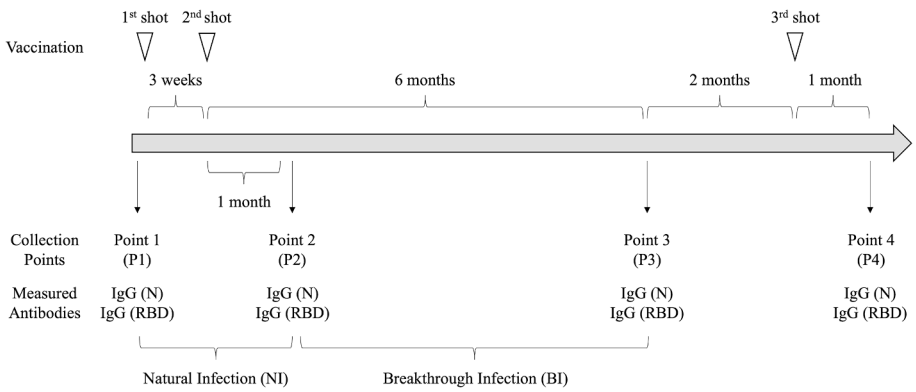

Fig. 1. Timeline of vaccination and specimen collection.

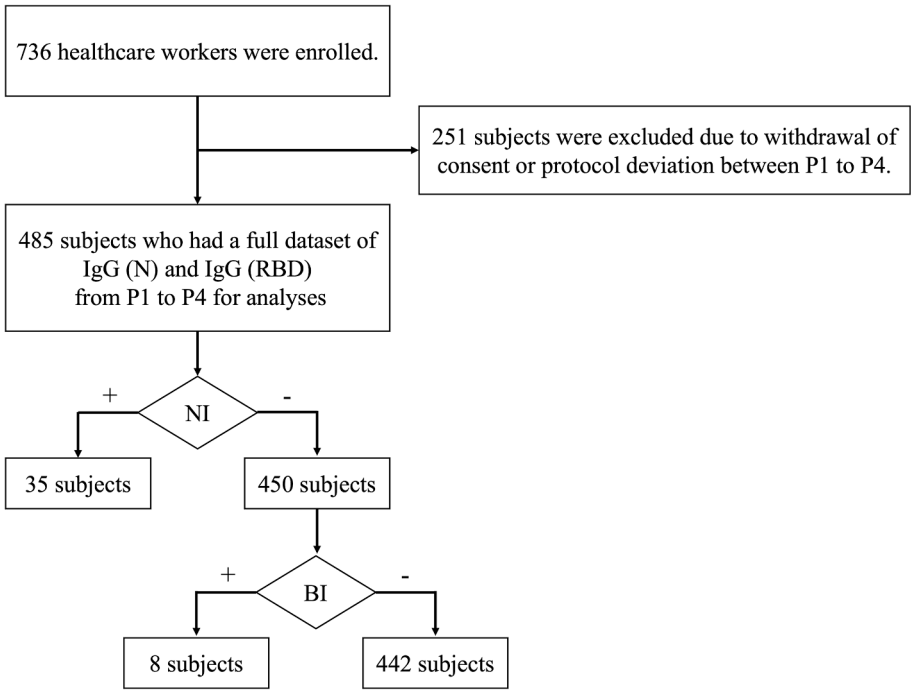

Fig. 2. Study flow diagram.

one of the following criteria: (i) IgG (N) $_{P1}$ was ≥ 1.4 S/C and IgG (RBD)$_{P1}$ was ≥ 50 AU/mL, (ii) IgG (N) $_{P2}$ was ≥ 1.4 S/C, IgG (RBD)$_{P2}$ was ≥ 50 AU/mL. The others were categorized as the NI (−) group. The 442 participants categorized into the NI (−) group, were further categorized into the BI (+) group if IgG (N) $_{P3}$ was ≥ 1.4 S/C or into the BI (−) group if IgG (N) $_{P3}$ was < 1.4 S/C (Figure 2).

2.4. Statistical analysis

Data were analyzed using JMP 16.0.0 (SAS Institute Inc., Cary, NC, USA). Statistical significance was set at $p < 0.05$. To evaluate changes in IgG (N), we used the delta of IgG (N)$_{P3}$ and IgG (N)$_{P2}$, i.e., $IgG(N)_{P3} - IgG(N)_{P2}$, or delta of IgG (N)$_{P4}$ and IgG (N)$_{P3}$, i.e., $IgG(N)_{P4} - IgG(N)_{P3}$. To evaluate changes in IgG (RBD), we used the ratio of IgG (RBD)$_{P3}$ to IgG (RBD)$_{P2}$. This was because the baseline level of IgG (N) was too small to obtain a stable ratio, whereas the baseline level of IgG (RBD) was sufficiently large, and IgG (RBD) levels were diverse. To compare two independent groups, the mean value of IgG (RBD) level, we used the Student's *t*-test when the continuous dependent variable was normally distributed and the Mann-Whitney *U* test when it was not normally distributed. Significance was tested using the $\chi 2$ test or the Fisher's exact test. To examine the correlation between IgG (RBD)$_{P2}$ and IgG (N)$_{P3}$–IgG (N)$_{P2}$, we used Spearman's rank correlation coefficient because the two variables were nonparametric.

3. Results

3.1. Infection of SARS-CoV-2 before P2 (NI)

We examined the presence of SARS-CoV-2 infection before P2 by examining the titers of IgG (N)$_{P1}$, IgG (N) $_{P2}$, and IgG (RBD) $_{P1}$ that exceeded the cutoff values (Supplementary Figures S1A,1B,1C). Consequently, 35 participants (7.22 %) were categorized into the NI (+) group, whereas 450 (92.8 %) were categorized into the NI (−) group.

3.2. Infection of SARS-CoV-2 between P2 and P3 (BI)

Eight participants were categorized into the BI (+) group as their IgG (N)$_{P3}$ was ≥ 1.4 S/C. The remaining 442 participants were classified into the BI (−) group.

3.3. Participant background and IgG titers

The participants' background and titers of IgG (N) and IgG (RBD) at the four time points are presented in Table 1. The female-to-male ratio was approximately 7:3 for all participants in the NI (+), NI (−), BI (+), and BI (−) groups. The median age of the participants was 33.0 years. The NI (+) and BI (+) groups tended to be slightly younger than the NI (−) and BI (−) groups, however, there was no significant difference. IgG (N) remained consistently low from P1 to P4 in all participants in the NI (−) and BI (−) groups (P1: 0.07 [0.03–0.20], P2: 0.07 [0.03–0.18], P3: 0.05 [0.02–0.13], P4: 0.07 [0.03–0.29]). However, in the NI (+) group, it increased significantly depending on the time of infection, and then decreased (P1: 2.08 [0.23–4.13], P2: 2.61 [1.58–3.71], P3: 0.89 [0.45–1.52], P4: 1.04 [0.53–1.89]). In the BI (+) group, it also increased significantly according to the time of infection(P1: 0.11 [0.03–0.23], P2: 0.11 [0.04–0.13], P3: 2.93 [2.60–3.73], P4: 1.09 [0.78–1.21]). In all participants, IgG (RBD) increased after the second vaccination, then decreased, and then increased after the third vaccination (P1: 4.40 [3.00–7.40], P2: 12604.40 [7721.80–19393.50], P3: 1018.70 [647.70–1741.80], P4: 19157.50 [13108.00– 32051.00]). In the NI (+) group, IgG (RBD) levels were significantly higher between P1 and P3 (P1: 298.90 [4.10–846.30], P2: 19614.90 [16057.10–28916.95], P3: 2356.10[1468.10–5059.05]) than that in the NI (−) group (P1: 4.30 [2.90–6.80], P2: 12145.70 [7492.30–18600.50], P3: 967.05 [635.35–1534.02] ($p < 0.001$). However, this difference was not observed at P4 stage (N1 (+): 17806.00 [11914.85–25894.65], N1 (−): 17806.00[11914.85–25894.65] ($p = 0.145$)(Figure 3A). In the BI (+) group, IgG (RBD) increased at P3 (P3: 21345.75 [17617.57–34468.75]) ($p < 0.001$), reflecting post-infection, and was higher at P4 than that in the BI (−) group (BI(+):,27967.55 [22300.97–45946.22], BI(−) 19088.05 [13218.97–32125.32]: although the difference was smaller ($p = 0.037$) (Figure 3B).

Table 1
Patient background and IgG titer.

		Total n = 485	NI (−) n = 450	NI (+) n = 35	*p* value	BI (−) n = 442	BI (+) N = 8	*p* value
Sex								
Number (%)	Female	352 (72.6)	328 (72.9)	24 (68.6)	0.560	322 (72.9)	6 (75.0)	1.000
	Male	133 (27.4)	122 (27.1)	11 (31.4)		120 (27.1)	2 (25.0)	
Age								
median [IQR*] (years)	Total	33.0 [27.0–46.0]	34.0 [27.0–46.0]	30.0 [26.5–39.5]	0.134	34.0 [27.0–46.0]	26.5 [25.8–38.0]	0.146
	Female	33.0 [21.0–70.0]	33.0 [27.0–46.0]	29.0 [25.8–35.8]	0.068	33.5 [27.0–46.0]	26.0 [25.3–37.3]	0.157
	Male	34.0 [22.0–77.0]	34.0 [27.0–45.8]	35.0 [28.5–41.0]	0.948	34.0 [27.0–46.3]	32.0 [29.5–34.5]	0.686
IgG (N) (S/C)								
median [IQR*]	P1	0.08 [0.03–0.20]	0.07 [0.03–0.20]	2.08 [0.23–4.13]		0.07 [0.03–0.20]	0.11 [0.03–0.23]	0.616
	P2	0.08 [0.03–0.23]	0.07 [0.03–0.18]	2.61 [1.58–3.71]		0.07 [0.03–0.18]	0.11 [0.04–0.13]	0.975
	P3	0.05 [0.02–0.16]	0.05 [0.02–0.13]	0.89 [0.45–1.52]	<0.001	0.05 [0.02–0.12]	2.93 [2.60–3.73]	<0.001
	P4	0.09 [0.03–0.37]	0.07 [0.03–0.29]	1.04 [0.53–1.89]	<0.001	0.07 [0.03–0.28]	1.09 [0.78–1.21]	0.004
IgG (RBD) (AU/mL)								
median [IQR*]	P1	4.40 [3.00–7.40]	4.30 [2.90–6.80]	298.90 [4.10–846.30]	<0.001	4.35 [2.82–6.80]	3.90 [3.20–6.45]	0.852
	P2	12604.40 [7721.80–19393.50]	12145.70 [7492.30–18600.50]	19614.90 [16057.10–28916.95]	<0.001	12276.85 [7534.93–18600.50]	7866.25 [7339.02–9960.52]	0.196
	P3	1018.70 [647.70– 1741.80]	967.05 [635.35–1534.02]	2356.10 [1468.10–5059.05]	<0.001	955.50 [634.35–1497.90]	21345.75 [17617.57–34468.75]	<0.001
	P4	19157.50 [13108.00–32051.00]	19525.75 [13258.13–32183.93]	17806.00 [11914.85–25894.65]	0.145	19088.05 [13218.97–32125.32]	27967.55 [22300.97–45946.22]	0.037

* IQR, interquartile range; RBD, receptor-binding domain; NI, natural infection; BI, breakthrough infection; P1, Point 1 prior to the first shot; P2, Point 2 one month after the second shot; P3, Point 3 six months after the second shot; and P4, Point 4 one month after the third shot; IgG (N), IgG against the nucleocapsid protein.

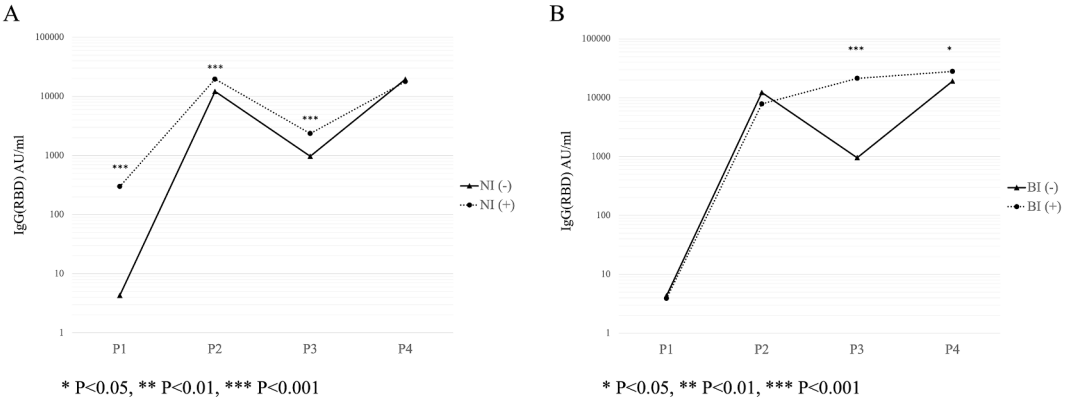

Fig. 3. Changes in IgG (RBD) (A) IgG (RBD) levels in the NI (+) group were significantly higher between P1 and P3 than that in the NI (−) group. However, the difference disappeared at P4. **(B)** In the BI (+) group, IgG (RBD) increased at P3, reflecting post-infection, and was significantly higher at P4 than that in the BI (−) group. RBD, receptor-binding domain; NI, natural infection; BI, breakthrough infection; P1, Point 1 prior to the first shot; P2, Point 2 one month after the second shot; P3, Point 3 six months after the second shot; and P4, Point 4 one month after the third shot.

3.4. Influence of presence or absence of NI on SARS-CoV-2 infection after the second vaccination

We examined the changes in IgG titers between P2 and P3 and between P3 and P4 to determine the presence of SARS-CoV-2 infection after the second vaccination. The 99th percentile distribution of the delta values of IgG $(N)_{P3}$ and IgG $(N)_{P2}$ was 2.57 S/C. Assuming that the samples that exceeded the 99th percentile of the delta distribution showed significant changes, we plotted those that exceeded the 99th percentile in a white circle. We calculated the 99th percentile of the IgG $(RBD)_{P3}$ and IgG $(RBD)_{P2}$ ratio distributions, which was 2.23 S/C. We plotted cases with values that exceeded the 99th percentile and with IgG $(N)_{P3} \geq 1.4$ and IgG $(N)_{P2} < 1.4$ in a gray circle. The other parameters were plotted in triangles. White and gray circles represented seven participants. Additionally, one participant had IgG $(N)_{P3} \geq 1.4$ and IgG $(N)_{P2} < 1.4$. Eight participants were considered to be infected between P2 and P3 after the second vaccination (Figure 4A).

Similarly, the differences between IgG $(N)_{P4}$ and IgG $(N)_{P3}$ levels were evaluated. The difference between IgG $(N)_{P4}$ and IgG$(N)_{P3}$ levels exceeded the 99th percentile (7.04) in five participants; 35 participants had IgG $(N)_{P4} \geq 1.4$ and IgG $(N)_{P3} < 1.4$; and in one, the IgG$(N)_{P4}$ level greatly increased to 8.15 at P4, although it had already exceeded 1.4 at P3. At least 41 participants were considered to be infected between P3 and P4 (Figure 4B).

In the NI (−) group, the difference between IgG $(N)_{P3}$ and IgG $(N)_{P2}$ exceeded the 99th percentile (2.68) in five participants. In two

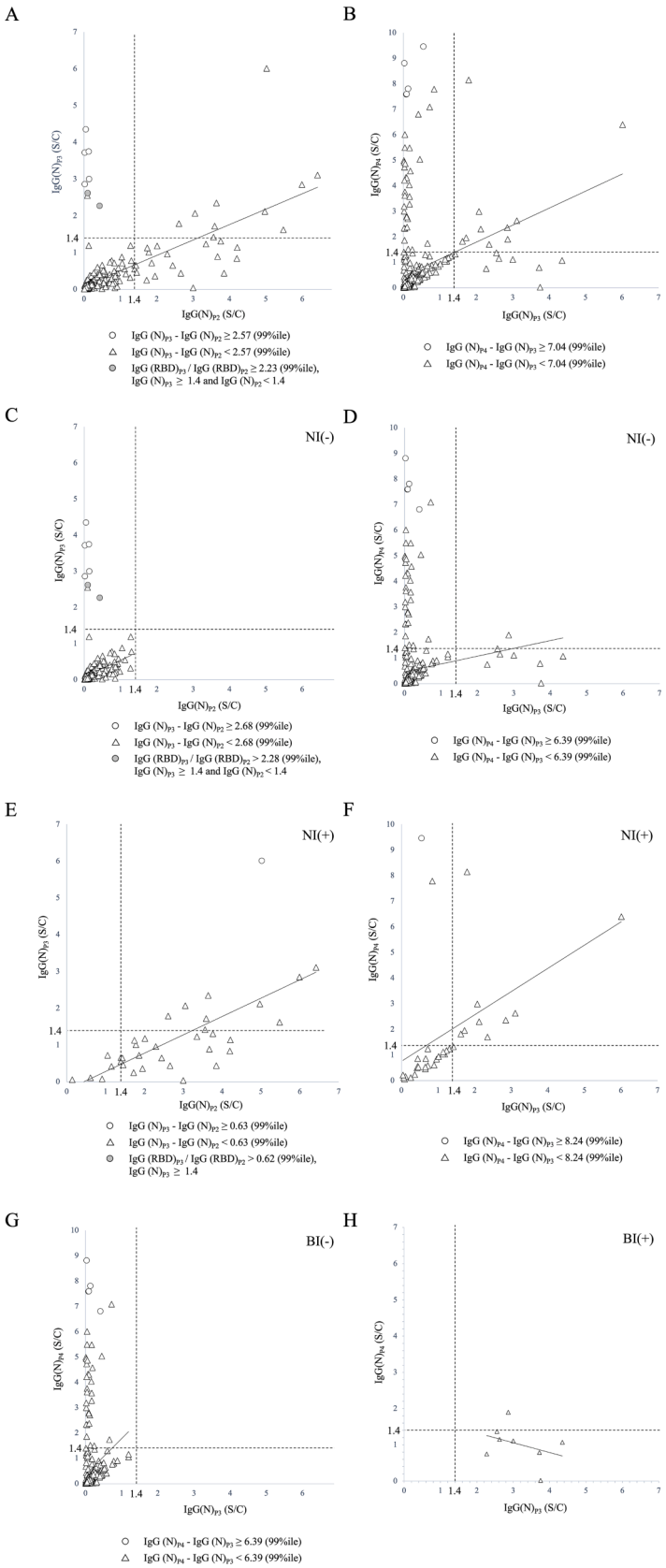

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Fig. 4. Comparison of IgG(N) between P2 and P3 and between P3 and P4 in all participants and NI (+)/ (−) groups and BI (+)/ (−) groups. (A) The evaluation of the difference between IgG $(N)_{P2}$ and IgG $(N)_{P3}$. Eight participants (plotted in white and gray circles were seven participants, and one participant had IgG $(N)_{P3} \geq 1.4$ and IgG $(N)_{P2} < 1.4$) were considered infected between P2 and P3 after the second vaccination. **(B)** The evaluation of the difference between IgG $(N)_{P4}$ and IgG $(N)_{P3}$. The difference between IgG $(N)_{P4}$ and IgG$(N)_{P3}$ exceeded the 99th percentile in five participants (white circle). Another 35 participants had IgG $(N)_{P4} \geq 1.4$ and IgG $(N)_{P3} < 1.4$. The IgG$(N)_{P4}$ level was significantly increased in one participant, reaching 8.15 despite already surpassing 1.4 at P3. Forty-one participants were considered infected between P3 and P4. **(C)** The evaluation of the difference between IgG $(N)_{P2}$ and IgG $(N)_{P3}$ in the NI (−) group. The difference between IgG $(N)_{P3}$ and IgG $(N)_{P2}$ exceeded the 99th percentile in five participants (white circle). IgG $(RBD)_{P3}$ / IgG $(RBD)_{P2}$, IgG $(N)_{P3} \geq 1.4$, and IgG $(N)_{P2} < 1.4$ exceeded the 99th percentile in two participants (two of the above five cases fulfilled this condition and were excluded; gray circle). One additional participant had IgG $(N)_{P3} \geq 1.4$ and IgG $(N)_{P2} < 1.4$. Eight participants (1.78 %) were estimated to be infected after vaccination between P2 to P3. **(D)** The evaluation of the difference between IgG $(N)_{P3}$ and IgG $(N)_{P4}$ in the NI (−) group. The difference between IgG $(N)_{P4}$ and IgG $(N)_{P3}$ exceeded the 99th percentile in five participants between P3 and P4 (white circle). A total of 33 participants had IgG $(N)_{P4} \geq 1.4$ and IgG $(N)_{P3} < 1.4$. Thirty-eight participants (8.44 %) were estimated to be infected after vaccination between P3 to P4. **(E)** The evaluation of the difference between IgG $(N)_{P2}$ and IgG $(N)_{P3}$ in the NI (+) group. The difference between IgG $(N)_{P3}$ and IgG $(N)_{P2}$ exceeded the 99th percentile in one participant (white circle), which was maintained at a persistently high level and increased gradually between P2 and P4. Therefore, this participant was less likely to be reinfected and no participants were reinfected between P2 and P3. In no participants, the IgG $(RBD)_{P3}$ / IgG $(RBD)_{P2}$ exceeded the 99th percentile or IgG $(N)_{P3} \geq 1.4$. **(F)** The evaluation of the difference between IgG $(N)_{P3}$ and IgG $(N)_{P4}$ in the NI (+) group The difference between IgG $(N)_{P4}$ and IgG $(N)_{P3}$ exceeded the 99th percentile in one participant (white circle). Furthermore, two participants demonstrated a great distribution shift toward the upper-left quadrant. Three participants (8.57 %) were estimated to be infected between P3 to P4. **(G)** The evaluation of the difference between IgG $(N)_{P3}$ and IgG $(N)_{P4}$ in the BI (−) group. The IgG $(N)_{P4}$ and IgG $(N)_{P3}$ surpassed the 99th percentile in five participants (white circle). Thirty-eight participants, including the above five, had IgG $(N)_{P4}$ levels ≥ 1.4. Thirty-eight participants (8.60 %) were deemed infected between P3 to P4. **(H)** The evaluation of the difference between IgG $(N)_{P3}$ and IgG $(N)_{P4}$ in the BI (+) group, IgG(N) did not increase from P3 to P4 in all participants. There were no indications of any infections between P3 and P4. RBD, receptor-binding domain; NI, natural infection; BI, breakthrough infection; P1, Point 1 prior to the first shot; P2, Point 2 one month after the second shot; P3, Point 3 six months after the second shot; and P4, Point 4 one month after the third shot; IgG (N), IgG against the nucleocapsid protein.

participants, IgG $(RBD)_{P3}$ / IgG $(RBD)_{P2}$ exceeded the 99th percentile (2.28), IgG $(N)_{P3}$ was ≥ 1.4, and IgG $(N)_{P2}$ was < 1.4 (these two participants from the among the aforementioned five were then excluded). One additional participant had IgG $(N)_{P3} \geq 1.4$ and IgG $(N)_{P2} < 1.4$. Eight participants (1.78 %) were estimated to be infected after vaccination between P2 to P3 (Figure 4C). Between P3 and P4, the difference between IgG $(N)_{P4}$ and IgG $(N)_{P3}$ exceeded the 99th percentile (6.39) in five participants. Additional 33 participants had IgG $(N)_{P4} \geq 1.4$ and IgG $(N)_{P3} < 1.4$. Moreover, 38 participants (8.44 %) were estimated to be infected after vaccination between P3 to P4 (Figure 4D).

In the NI (+) group, the difference between IgG $(N)_{P3}$ and IgG $(N)_{P2}$ exceeded the 99th percentile (0.63) in one participant. There were no participants in which IgG $(RBD)_{P3}$ / IgG $(RBD)_{P2}$ values exceeded the 99th percentile (0.62) and IgG (N)P3 was ≥ 1.4 (Figure 4E). The difference between IgG $(N)_{P3}$ and IgG $(N)_{P2}$ exceeded the 99th percentile (0.63) in one participant; this was maintained a persistently high level and gradually increased between P2 and P4. Therefore, the patient was less likely to have been reinfected. Therefore, no participant was reinfected between P2 and P3. Between P3 and P4 (Figure 4F), the difference between IgG $(N)_{P4}$ and IgG $(N)_{P3}$ exceeded the 99th percentile (8.24) in one participant. Furthermore, two participants demonstrated a significant distribution shift toward the upper-left quadrant, as shown in Figure 4F. Three participants (8.57 %) were estimated to have been infected between P3 and P4.

3.5. Influence of presence or absence of BI on SARS-CoV-2 infection between P3 and P4

To determine whether BI affects the susceptibility to infection after P3, we compared the distributions of IgG $(N)_{P4}$ and IgG $(N)_{P3}$ between the BI (−) and BI (+) groups. In the BI (−) group, the difference between IgG $(N)_{P4}$ and IgG $(N)_{P3}$ exceeded the 99th percentile (6.39) in five participants. In 38 participants, including the previous five, values of IgG $(N)_{P4}$ were ≥ 1.4. Therefore, these 38 (8.60 %) participants were estimated to have been infected between P3 and P4 (Figure 4G). In the BI (+) group, the IgG(N) levels did not increase from P3 to P4 in any participant. There were no signs of infection between P3 and P4 (Figure 4H).

3.6. IgG (RBD) titers and susceptibility to infection of SARS-CoV-2 after 2nd vaccination (between P2 and P3)

To determine whether IgG $(RBD)_{P2}$ titers are associated with susceptibility to BI, we plotted the distribution of delta of IgG $(N)_{P3}$ and IgG $(N)_{P2}$ on the y-axis against IgG $(RBD)_{P2}$ on the x-axis. The Spearman's rank correlation coefficient showed that the association between the two parameters was not significant ($p = 0.351$) (Figure 5).

4. Discussion

Our study explored the effect of infection-acquired immunity on IgG (RBD) and its role in providing protection against infections. Additionally, we explored the effect of SARS-CoV-2 infection occurring before and after vaccination on the establishment of immune protection. The following are the noteworthy findings of our study. In the NI (+) group,

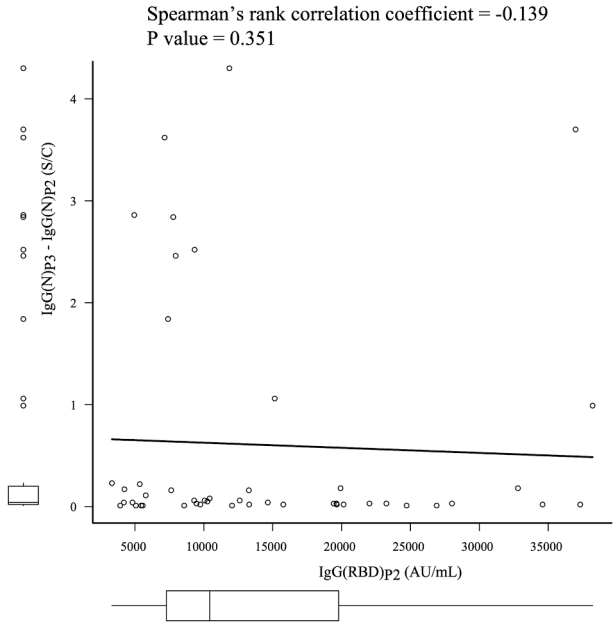

Fig. 5. Two-dimensional distribution to show the association between IgG $(RBD)_{P2}$ titer and breakthrough infection between P2 and P3. The X-axis represents IgG $(RBD)_{P2}$, and the Y-axis represents the amount of change from IgG $(N)_{P3}$ to IgG $(N)_{P2}$. Using Spearman's rank correlation coefficient, the association between the two parameters was not significant ($p = 0.351$). RBD, receptor-binding domain; IgG (N), IgG against the nucleocapsid protein; P1, Point 1 prior to the first shot; P2, Point 2 one month after the second shot; P3, Point 3 six months after the second shot; and P4, Point 4 one month after the third shot.

there were no incidents of reinfection in the six months following the second vaccination. In contrast, the NI (−) group had eight participants (1.78 %) with infection after the second vaccination, indicating a significant decrease in infection rates in the NI (+) group. However, six months after receiving their second vaccination, three patients (8.57 %) in the NI (+) group and 38 patients (8.47 %) in the NI (−) group were reinfected. No significant differences were observed between the two groups. The results showed that hybrid immunity (a combination of immunity through vaccination and infection) was effective for approximately six months. However, their effectiveness decreased over time. IgG (RBD) over time was higher in the NI (+) group than in the NI (−) group, with a significant difference from the second vaccination to six months. Nevertheless, the difference diminished and became non-significant one month after the booster dose (the third shot) of vaccination, approximately nine months after the second vaccination. This could indicate that the decrease in IgG (RBD) antibody levels in the NI (+) group led to an increase in the reinfection rate. Comparison of BI (+) group to the BI (−) group revealed no instances of infection in the BI (+) group between 6 and 9 months after the second vaccination (P3 and P4). In contrast, in the BI (−) group, 38 patients were identified as infected. A significant difference in the IgG (RBD) levels was observed, although the difference between the two groups decreased. This study showed no relationship between vaccine IgG (RBD) levels and post-vaccine infection six months after the second vaccination.

It has been reported that previous SARS-CoV-2 infection and hybrid immunity (previous infection combined with previous vaccination) provide greater protection against reinfection and more sustained protection against hospital admission or severe disease than vaccination alone [13], even with variants such as the Delta and Omicron strains [14]. Hybrid immunity was attenuated to approximately 50 % efficacy against symptomatic infections with BA.1 and BA.2, but its efficacy against severe, critical, or fatal COVID-19 was reported to be $>$ 96 % [15].

Some reports on antibodies against the spike protein found that individuals with hybrid immunity had the highest titers of IgG anti-S antibodies and the slowest decline in antibody levels compared with individuals vaccinated alone or those naturally infected. Individuals with only NI initially had higher IgG anti-spike antibody titers than those with only NI, but the difference in antibody titers disappeared at approximately 5 to 10 months. Antibodies decline fastest in individuals who receive vaccinations only [16,17]. In vitro, the inhibition rates against the wild-type and mutant strains were highest for hybrid immunity. Vaccination resulted in higher inhibition rates against wild-type and mutant strains than NI initially, but there was no difference at 10 months [16]. However, it has also been reported that the antibody level of individuals with hybrid immunity is equivalent to that of individuals vaccinated for only approximately 3–5 months [18]. Moriyama et al. reported that although the number of neutralizing antibodies among individuals who recovered from COVD-19 was significantly reduced, the quality of neutralization was maintained. This means that the maturation of the antibody response to SARS-CoV-2 enhances the ability to cross-neutralize circulating variants, suggesting that a decrease in the antibody titer does not imply a decrease in protection [19]. Hybrid immunity redirects vaccine-induced immunodominance, resulting in a robust functional humoral immune response in the most highly conserved region of the SARS-CoV-2 spike antigen [20]. According to that study, there were apparent differences in SARS-CoV-2 specific serum antibodies following vaccination between previously infected and naïve individuals, with antibody responses of greater magnitude and epitope specificity in individuals vaccinated after a previous infection. Although the antibody levels were similar, several important differences persisted in the FcR responses and shifts in immune response coordination. In previously infected individuals, class-switching and FcR binding were enhanced, and potentially functionally optimized antibodies, particularly targeting the S2-domain of the highly conserved segment of the spike protein, were generated [20]. A previous study demonstrated the polyfunctionality and proliferative capacity of T-cells, indicating long-term protective immunity after SARS-CoV-2 infection [21]. In this study, hybrid immunity effectively prevented disease onset until six months after the second vaccination compared with vaccination alone. However, although the number of BIs in this study was small, the effect of hybrid immunity fell to the same infection rate as that of vaccination alone six months after the second vaccination. From the perspective of antibodies, although there was a significant difference in RBD antibody levels between the NI (+) and NI (−) groups, the attenuation rate appeared to be similar. Unlike previous reports, it was unclear whether the decay rate was rapid. It has been reported that the amount of RBD antibodies after infection depends on the severity [22]. Therefore, almost 100 % of the participants with NIs were asymptomatic or mildly infected, which may have affected the immune response. Even if there are defense mechanisms other than antibodies, as mentioned above, it is possible that the rapid decline in RBD antibodies could have resulted in the reduced efficacy of hybrid immunity. However, no severe cases of BI occurred in patients with hybrid immunity, including those who received vaccination only.

Correlations between the delta of IgG $(N)_{P3}$ and IgG $(N)_{P2}$ and IgG $(RBD)_{P2}$ were not significant. This result suggests that IgG $(RBD)_{P2}$ titers and IgG (RBD) titers only after one month after the second vaccination are not associated with susceptibility to BI. The proportion of antibody responses above 4160 AU/ml is a threshold that was previously shown to correspond to a 95 % probability of viral neutralization [23,24]. This was because the IgG (RBD) required for efficacy was obtained after the second vaccination session. However, it has been reported that the higher the anti-spike antibody titer, the lower the risk of Omicron BA.5 infection [23], and sufficient attention should be paid to its attenuation.

In this study, IgG (RBD) levels in infected participants increased after vaccination and were significantly higher after booster vaccination than in non-infected participants. BI rates were also significantly lower in post-vaccination infected individuals than those in uninfected individuals. Participants who were infected before vaccination had antibody levels similar to those of uninfected participants after the booster vaccination. Although there are few reports on the immunity of infected people after vaccination, it has been reported that the IgG (RBD) titer in infected individuals after vaccination was higher than that in non-infected individuals, and high titers were maintained for 3–5 months [25]. In this study, IgG (RBD) levels after booster vaccination in uninfected participants recovered up to the post-second vaccination. This result was similar to that of previous reports [25], and the IgG titer likely decreased over time following infection.

Our study confirmed that natural and BIs can be detected by the delta of IgG (N) titers and the ratio of IgG (RBD) titers. We have previously reported that IgG (N) and IgG (RBD) are valid for infection control [26,27]. These results illustrate the clinical utility of serological tests in monitoring SARS-CoV-2 infection. It has also been reported that mRNA vaccines could significantly increase antibodies against the nucleocapsid (N) protein [28]. Clinical, serological, and RT-PCR findings should be evaluated comprehensively.

This study has several limitations. First, the absolute number of BIs was small. This may lead to an underestimation or overestimation. Second, because the study was conducted in a younger population with no underlying medical conditions, generalization of the results is limited and would require study of a broader population. However, a noteworthy finding is that the persistence of hybrid immunity in the population of this study appears to be shorter than that previously reported due to the surrounding environment. The study was conducted as population with no underlying medical conditions, this statement is not supported by the data, as medical conditions were not assessed in the study sample. Due to the emergency situation, it was not realistic to formulate a research plan to understand the health status of the subjects. Therefore, breakthrough infection could not be confirmed. In addition, the total number of participants and the number of people infected after

vaccination were not large enough to conduct multivariate analysis.

5. Conclusions

Hybrid immunity can prevent disease onset compared with immunity from vaccination alone, but it is necessary to pay close attention to its persistence, especially in mutant strain epidemics and in people who may be exposed to a high frequency of SARS-CoV-2. When planning vaccinations, we suggest that people with hybrid immunity can wait for approximately half a year after the last vaccination (or the last infection). Subsequently, the risk of a BI could increase, especially the environmental risk of reinfection, and booster vaccination should be considered.

Infection with SARS-CoV-2 before or after receiving the mRNA COVID-19 vaccine can increase immune protection and prevent disease onset. However, the duration of this effect may be limited, particularly in terms of the environmental risk of reinfection.

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CRediT authorship contribution statement

Nagashige Shimada: Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Satoshi Sugawa:** Writing – original draft, Visualization, Validation, Data curation. **Satoshi Murakami:** Writing – original draft, Visualization, Validation, Data curation. **Masahiro Shinoda:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition, Data curation, Conceptualization. **Shinichiro Ota:** Data curation. **Miwa Morikawa:** Data curation. **Hiroaki Takei:** Data curation. **Yusuke Serizawa:** Data curation. **Hidenori Takahashi:** Data curation. **Mio Toyama-Kousaka:** Data curation. **Hiroto Matsuse:** Data curation. **Masaharu Shinkai:** Writing – review & editing, Funding acquisition, Data curation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Masahiro Shinoda reports financial support was provided by Abbott Japan Co Ltd. Satoshi Murakami reports a relationship with Abbott Japan Co Ltd that includes: employment. Satoshi Sugawa reports a relationship with Abbott Japan Co Ltd that includes: employment. Authors without Satoshi Sugawa and Satoshi Murakami received joint research funding from Abbott Japan Ltd. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper].

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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