Neutralizing antibodies to SARS-CoV-2 variants of concern including Delta and Omicron in subjects receiving mRNA-1273, BNT162b2, and Ad26.COV2.S vaccines

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Abstract
SARS-CoV-2 vaccines have contributed to the control of COVID-19 in some parts of the world. However, the constant emergence of variants of concern (VOCs) challenges the effectiveness of SARS-CoV-2 vaccines over time. In particular, Omicron contains a high number of mutations in the spike (S) protein gene, on which most vaccines were developed. In this study, we quantitated neutralizing antibodies in vaccine recipients at various times postvaccination using S protein-based pseudoviruses derived from wild type (WT) SARS-CoV-2 and five VOCs including Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529). We found that two-dose mRNA-1273 and BNT162b2 vaccines elicited robust neutralizing antibodies against WT, Alpha, Beta, Gamma, and Delta, but waned after 6 months with a faster decline observed for BNT162b2. Both mRNA-1273 and BNT162b2 elicited weak neutralizing antibodies against Omicron. One dose of Ad26.COV2.S vaccine induced weaker neutralizing antibodies against WT and most VOCs than mRNA-1273 and BNT162b2 did but moderate neutralizing antibodies against Delta and Omicron, which lasted for 6 months. These results support current recommendations of the Centers for Disease Control and Prevention for a booster 5 months after full immunization with an mRNA-based vaccine and the use of an mRNA-based vaccine 2 months after Ad26.COV2.S vaccination.

KEYWORDS
COVID-19, neutralizing antibodies, SARS-CoV-2, vaccine efficacy, variants of concern (VOCs)

1 | INTRODUCTION

The COVID-19 pandemic caused by SARS-CoV-2 has persisted for more than 2½ years since the report of the first case in December 2019.1,2 A number of variants of concern (VOCs) have subsequently emerged including Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529), first reported in the United Kingdom, South Africa, Brazil, India, and Botswana, respectively.3–12 The most common mutations in these VOCs are in the spike (S) protein including: Δ69, Δ114Y, N501Y, D614G, and P681H for Alpha; L18F, Δ242, K417N, E484K, N501Y, D614G, and A701V for Beta; L18F, K417T, E484K, N501Y, and D614G for Gamma; T95I, L452R, T478K, D614G, and P681R for Delta; and A67V, Δ69-70, T95I, G142A, Δ143-145, Δ211, L212I, ins214EPE, G339A, S371L,
S373P, S375P, L417A, A440L, G446S, S477A, T478L, G484A, G493A, Gly496Ser, G498A, A501T, T505H, T547L, A614G, H655T, A679L, P681H, A764L, A796T, A856L, G954H, A969L, and L981P for Omicron.9,10,13 In November 2021, the Omicron variant was first reported and has since spread throughout the world.14,15 SARS-CoV-2 mutations impact virus characteristics, including cell tropism, virus replication and production, transmissibility, and antigenicity. VOCs emerge in response to the changing immune profile of the human population and the selective pressure this puts on viral fitness.16 The S protein is required for cell entry and hence is an ideal target for vaccine development. The S protein binds with high affinity to ACE2, the receptor for SARS-CoV-2, which is widely distributed in human tissues.17,18 Incidentally, similar to the SARS-CoV-2 nucleocapsid, the S protein is an ideal biomarker for diagnosis.19,20

Vaccination has played a significant role in controlling the COVID-19 pandemic.21 Numerous SARS-CoV-2 vaccines have been approved or are under development, including mRNA-based vaccines, viral vector-based vaccines, inactivated vaccines, and recombinant subunit vaccines, and so on.22–29 The mRNA-based vaccines Moderna mRNA-1273 and Pfizer-BioNTech BNT162b2 were granted emergency use authorization by the US Food and Drug Administration (FDA) in late 2020 and have since been widely used all over the world.30 mRNA-1273 and BNT162b2 are two-dose vaccines based on the S protein gene sequence but with different immunization intervals (28 vs. 21 days, respectively) and different doses (100 vs. 30 µg, respectively).31–33 Adenovirus serotype 26 (Ad26) vector-based vaccine Janssen Ad26.COV2.S has also played a crucial role in preventing COVID-19 disease.34,35 Ad26.COV2.S is a single dose vaccine of 5 × 10^10 viral particles.35

Because of high mutation rates in the S protein in VOCs, there is emerging evidence of reduced neutralizing activity by antibodies produced in vaccinated subjects against some SARS-CoV-2 VOCs.7,12,36,37 In this study, we used a pseudovirus neutralization assay18 to evaluate the states of neutralizing antibodies against SARS-CoV-2 wild type (WT) and different VOCs in plasma samples collected at various time points, from study participants who received mRNA-1273, BNT162b2, and Ad26.COV2.S under emergency use authorization.

## 2 | MATERIALS AND METHODS

### 2.1 | Study design and samples collection

The study was approved by the Institutional Review Board (IRB) of the University of Pittsburgh (STUDY20090114, STUDY20120181, STUDY20030228, STUDY20060154). All study participants provided written informed consent. All participants received a SARS-CoV-2 vaccine mRNA-1273 (100 µg/dose), BNT162b2 (30 µg/dose), or Ad26.COV2.S (5 × 10^10 viral particles/dose) under emergency use authorization. Data on this cohort has been previously described.38 In this study, a total 281 plasma samples were collected from 74 participants, of which 24 received mRNA-1273, 26 received BNT162b2, and 24 received Ad26.COV2.S (Table 1). Up to four blood samples were collected from each participant: Bleed 1 was collected on the same day before the first dose of vaccine (prevaccination); Bleed 2 was collected at a median of 21–31 days after the first dose of vaccine, which was also the day of the second dose of vaccine for mRNA-1273 and BNT162b2; Bleed 3 was collected at a median of 45–63 days after the first dose of vaccine; and Bleed 4 was collected at a median of 182–184 days after the first dose of vaccine (Figure 1A). In total, 90 samples from 24 mRNA-1273 participants, 103 samples from 26 BNT162b2 participants, and 88 samples from 24 Ad26.COV2.S participants were collected.

### 2.2 | Cell lines and cell culture

HEK293/hACE2, a cell line derived from HEK293T cells with overexpression of human ACE2 from Dr. Patrick Moore and obtained from BEI Resources, and HEK293T cell line purchased from ATCC were maintained in Dulbecco’s modified Eagle medium (DMEM) with

| Participant | mRNA-1273 | BNT162b2 | Ad26.COV2.S | Total |
|-------------|-----------|----------|-------------|------|
| Number of subjects (n) | 24 | 26 | 24 | 74 |
| Age (median, range) | 48 (20–75) | 41 (21–71) | 49 (25–76) | 46 (20–76) |
| Gender | | | | |
| Female (%) | 16 (67) | 23 (88) | 7 (29) | 46 (62) |
| Male (%) | 8 (33) | 3 (12) | 17 (71) | 28 (38) |
| Race | | | | |
| White (%) | 21/24 (88) | 24/26 (92) | 18/24 (75) | 63/74 (85) |
| Asian (%) | 2/24 (8) | 0/26 (0) | 5/24 (21) | 7/74 (9) |
| Black (%) | 0/24 (0) | 1/26 (4) | 1/24 (4) | 2/74 (3) |
| >1 (%) | 1/24 (4) | 0/26 (0) | 0/24 (0) | 1/74 (1) |
| N/A (%) | 0/24 (0) | 1/26 (4) | 0/24 (0) | 1/74 (1) |
10% heat-inactivated fetal bovine serum (FBS) and 1% Penicillin-Streptomycin 100× Solution. Cells were maintained at 37°C with 5% CO₂. HEK293T cells were used for pseudovirus packaging, while HEK293/hACE2 cells were used for the pseudovirus antibody neutralization assay.

### 2.3 Spike (S) proteins of SARS-CoV-2 WT and VOCs

Plasmid expressing S proteins of SARS-CoV-2 WT and VOCs including Alpha, Beta, Gamma, Delta, and Omicron were used in this study. Plasmids encoding Alpha, Beta, Gamma, and Delta S protein genes were gifts from Dr. David Nemazee while the Omicron S protein gene plasmid was a gift from Dr. Tongqing Zhou. The S protein genes were cloned from the original plasmids into pcDNA3.1(+) by restriction digestion and T4 ligation. Constructs were verified by restriction analysis and DNA sequencing. The appropriate expression of the S protein by each plasmid was confirmed by Western blotting as previously described.

### 2.4 Packaging of pseudoviruses

Pseudoviruses containing the S proteins from SARS-CoV-2 WT and VOCs were packaged as previously described (Figure 1B). Each S protein gene plasmid and reporter backbone plasmid were cotransfected into HEK293T cells with jetOPTIMUS transfection reagent (117-15: Polypus) according to the manufacturer’s instructions. The supernatant was collected at 36 h posttransfection, centrifuged at 1,500 rpm in 4°C, filtered with a 45 µm filter. WT and VOCs
pseudoviruses were titrated and normalized by relative luminescence unit (RLU) and stored at −80°C for further use.

2.5 | Antibody neutralization assay

All manipulations of the blood samples were performed in a BSL-2+ biosafety cabinet. Based on the normalized virus titers, equivalent amounts of SARS-CoV-2 pseudoviruses were used in the infection assay for all variants. Plasma samples were heat-inactivated at 56°C for 30 min. To determine the neutralizing antibody titer of a plasma sample, pseudovirus at 50 µl was incubated with the sample diluted at 1:50; 1:250; 1:1,250; 1:6,250; 1:31,250 in 96-well plate for 1 h at 37°C, followed by infection of HEK293T-ACE2 cells as previously described (Figure 1C). Briefly, cells were seeded one day before infection and infected at a confluence at 70%-90%. Wells infected with untreated pseudoviruses and uninfected cells were set as positive and negative controls, respectively, which were applied to every plate in triplicate. All the infection assays were performed in triplicate. At 60 h postinfection, 65 µl Reporter Lysis Buffer (E397A; Promega) were added to each well for cell lysis. After a 5 min centrifugation, 25 µl lysate supernatant were mixed with 25 µl Luciferase reagent (E1501; Promega) in a LumaPlate-96 white plate, followed by measuring luciferase activity on a Turner BioSystems Instrument. The results were used to calculate the 50% pseudovirus antibody neutralization titer (pVNT$_{50}$) and geometric mean titer (GMT) with 95% confidence intervals (CI) for each sample (Figure 1D).

2.6 | Statistical analysis

GraphPad Prism 9 was used for data analysis. One-way analysis of variance (ANOVA) was applied for grouped analyses followed by Tukey’s multiple comparisons between the groups. GMTs with 95% CIs were presented as neutralization titers. $p<0.05$ was considered as significant.

3 | RESULTS

3.1 | mRNA-1273, BNT162b2, and Ad26.COV2.S vaccines elicited robust neutralizing antibodies against SARS-CoV-2 WT

Consistent with the original study describing the immune responses of the studied participants using a live virus neutralization assay, the mRNA-1273, BNT162b2, and Ad26.COV2.S vaccines elicited neutralizing antibodies against WT pseudovirus in most participants after the first dose of immunization (Figure 2). However, the GMTs of Bleed 2 samples from BNT162b2 subjects after the first dose were lower than those from mRNA-1273 and Ad26.COV2.S subjects (52 vs. 183 and 153, respectively, $p = 0.0246$ by one-way ANOVA test in Figure 3A). After the second dose of vaccine, the GMTs of Bleed 3 samples from both mRNA-1273 and BNT162b2 immunized subjects were significantly increased by 3.8- and 9.0-fold, reaching 698 and 467, respectively, while the GMT from the one-dose Ad26.COV2.S immunized subjects at Bleed 3 had no further increase, remaining at 102 (Figure 2). It was clear that, after the full two-dose vaccination, mRNA-1273 and BNT162b2 immunized subjects had higher titers of neutralizing antibodies than one-dose Ad26.-COV2.S immunized subjects had ($p = 0.0033$ by one-way ANOVA test in Figure 3A). These results were consistent with the reported higher protective efficacies of mRNA-1273 and BNT162b2 than that of Ad26.COV2.S.$^{34,42-44}$

3.2 | Duration of neutralizing antibodies against SARS-CoV-2 WT after full mRNA-1273, BNT162b2, or Ad26.COV2.S immunization

To measure the durability of the immune response, we examined neutralizing antibody titers 6 months after mRNA-1273, BNT162b2, or Ad26.COV2.S immunization. There was a slight 1.9-fold drop of GMT to 368 for the mRNA-1273 subjects (Figure 2A). However, a significant 8.8-fold drop of GMT to 53 was observed for the BNT162b2 subjects (Figure 2B). In contrast, no drop of GMT was observed for the Ad26.COV2.S subjects maintaining at 157 (Figure 2C). This is again consistent with the original study describing the immune responses of these participants using a live virus neutralization assay, which also demonstrated a waning of immunity over time in the BNT162b2 immunized subjects.$^{38}$

3.3 | Neutralization against different SARS-CoV-2 VOCs for recipients of mRNA-1273, BNT162b2, and Ad26.COV2.S vaccines

Neutralizing antibodies against five variants including Alpha, Beta, Gamma, Delta, and Omicron were measured in the plasma samples from subjects who received mRNA-1273, BNT162b2, and Ad26.-COV2.S vaccines. After the first dose of vaccine (Bleed 2), mRNA-1273 induced robust neutralizing antibodies against Alpha, Beta, and Gamma VOCs similar to WT (GMTs 498, 137, and 662 vs. 183, respectively, in Figure 4A); however, the titers of neutralizing antibodies were slightly lower against Delta (GMT 70 vs. 183) but were significantly lower against Omicron (GMT 12 vs. 183) with a reduction of 15.3-fold (Figure 4A). After the second dose (Bleed 3), mRNA-1273 immunized subjects had high GMTs against Alpha, Beta, and Gamma with titers similar to or higher than the GMT against WT (1,586, 594, and 2,074 vs. 698, respectively, in Figure 4A); however, a 2.1-fold drop against Delta and a sharp 15.2-fold drop against Omicron were observed (698 vs. 325 and 46, respectively) (Figure 4A). At 6 months after mRNA-1273 immunization (Bleed 4), GMTs against Alpha, Beta, and Gamma remained high compared to WT (631, 487, and 950 vs. 368, respectively, in Figure 4A). However,
the GMT against Delta at 6 months had a further 2.1-fold drop from 325 to 157, and maintained a low level against Omicron at 115 (Figure 4A). These results indicate that the mRNA-1273 vaccine can induce equivalent neutralizing antibodies against Alpha, Beta, and Gamma as against WT. However, it induced lower levels of neutralizing antibodies against Delta and Omicron as shown in the lower neutralizing antibody titers after the first dose vaccine, full immunization, and 6 months after full immunization (Figure 4A).

The induction of neutralizing antibodies by BNT162b2 against Alpha, Beta, Gamma, and Delta was similar to that of WT manifesting lower neutralizing antibody titers after the first dose vaccine, full immunization, and 6 months after full immunization (Figure 4A).

The Ad26.COV2.S vaccine induced moderate neutralizing antibodies against all VOCs similar to WT (Figure 4C). While these single-dose Ad26.COV2.S immunized subjects had lower GMTs against Alpha, Beta, Gamma, and Delta than fully immunized mRNA-1273 and BNT162b2 subjects had (Bleed 3 in Figure 3B–E), their levels were maintained at 6 months after immunization (Bleed 4 in Figure 3B–E). Compared to fully immunized mRNA-1273 and BNT162b2 subjects, Ad26.COV2.S induced moderate but slightly higher neutralizing antibodies against Omicron (GMT 391 vs. 46 and 129, respectively, in Bleed 3, \( p = 0.007 \) by one-way ANOVA test in Figure 3F). At 6 months after immunization, Ad26.COV2.S subjects had slightly higher GMTs against Omicron than mRNA-1273 and BNT162b2 subjects had (376 vs. 115 and 46, respectively, in Bleed 4, \( p = 0.0004 \) by one-way ANOVA test in Figure 3F). Hence, while Ad26.COV2.S vaccination did not achieve the highest titers, the response demonstrated longevity.

4 | DISCUSSION

Vaccination plays an important role in reducing the spread, morbidity, and mortality of infectious diseases. The COVID-19 pandemic prompted the unprecedented rapid development of vaccines against
FIGURE 3  (See caption on next page)
FIGURE 4  Differences of neutralizing antibody titers in subjects immunized with mRNA-1273, BNT162b2, and Ab26.COV2.S against SARS-CoV-2 wild type (WT) and variants of concern (A) mRNA-1273. (B) BNT162b2. (C) Ad26.COV2.S. Plasma samples were examined at different times after immunization (Bleed 2, 3, and 4). Multiple groups were compared by one-way analysis of variance test. geometric mean titers and change folds compared with WT were listed on the top of each group. The dashed line represents limitation of detection.

FIGURE 3  Comparison of neutralizing antibody titers in subjects immunized with mRNA-1273, BNT162b2, and Ab26.COV2.S against SARS-CoV-2 wild type (WT) and variants of concern. (A) WT. (B) Alpha. (C) Beta. (D) Gamma. (E) Delta. (F) Omicron. The geometric mean titers (GMTs) against WT, Alpha, Beta, Gamma, Delta, and Omicron in participants immunized with mRNA-1273, BNT162b2, and Ab26.COV2.S are presented. Each dot represents a sample. Differences of neutralizing antibody titers among mRNA-1273, BNT162b2, and Ad26.COV2.S were compared in each Bleed (Bleed 2, 3, and 4). Multiple groups were compared by one-way analysis of variance test. Tukey's test was performed for post-hoc comparison. Significant p values were listed at the top of each panel. No significant difference was labeled as ns. The dashed line represents limitation of detection.
SARS-CoV-2. The first three vaccines against SARS-CoV-2 used in the US were mRNA-1273, BNT162b2, and Ad26.COV2.S, which have since contributed to the control of the pandemic.\(^{32,34,44}\) While Ad26.COV2.S is a conventional adenovirus vector-based vaccine, both mRNA-1273 and BNT162b2 are novel type of mRNA vaccines.\(^{32,34,44}\) This is the first time that this RNA-based vaccine technology was successfully applied against infectious disease.\(^{30,46}\)

Since S protein is essential for SARS-CoV-2 entry into cells, neutralizing antibodies against S protein in immunized subjects are likely to play a role in the effective control of SARS-CoV-2 infection and COVID-19 disease.\(^{47}\) The emergence of SARS-CoV-2 VOCs has challenged the effectiveness the mRNA-1273, BNT162b2, and Ad26.COV2.S vaccines, which were all developed based on the WT S protein gene sequence. The original SARS-CoV-2 has since evolved to harbor high numbers of mutations in new VOCs.\(^{13}\) In this study, we used lentiviral pseudoviruses of SARS-CoV-2 WT and 5 other VOCs including Alpha, Beta, Gamma, Delta, and Omicron to quantify neutralizing antibodies in the plasma samples of mRNA-1273, BNT162b2, and Ad26.COV2.S immunized subjects. Our results showed that all three vaccines elicited neutralizing antibodies against SARS-CoV-2 WT and most of the VOCs tested after full immunization, which are consistent with the success of these vaccines in controlling COVID-19 during the early phase of the pandemic.\(^{48}\) However, the efficacies of these vaccines against Delta and Omicron VOCs have been challenged. We have compared the results of our study with those of others for their neutralizing antibody titers after full immunization, durability, and effectiveness against the Delta and Omicron VOCs of these vaccines (Table 2).

We observed overall higher levels of neutralizing antibodies in fully immunized mRNA-1273 and BNT162b2 subjects than those immunized with the Ad26.COV2.S vaccine (Table 2). These results are consistent with other studies showing robust neutralizing antibodies against WT and most VOCs following BNT162b2 and mRNA-1273 immunization\(^{72–74}\) as well as those of another study of the same studied subjects using a live virus neutralization assay.\(^{38}\) Between the two RNA vaccines, BNT162b2 induced lower levels of neutralizing antibody titers against WT and most of VOCs than mRNA-1273 did after the first and second doses of immunization. Furthermore, neutralizing antibody titers of BNT162b2 subjects declined rapidly 6 months after immunization. The lower neutralizing antibody titers of BNT162b2 subjects could be due to the lower dose used compared to that of mRNA-1273 (30 vs. 100 µg) or the different dosing interval. Indeed, in an early study of mRNA-1273 in which three different doses at 25, 100, and 250 µg were administered twice, subjects immunized with a higher dose developed higher antibody titers than those immunized with a lower dose.\(^{75}\) Meanwhile, mRNA-1273 produced higher levels of binding and neutralizing antibodies, which declined slightly over time but remained elevated in all participants 3 months after the second dose immunization.\(^{76}\) Nevertheless, we observed some reduction of neutralizing antibodies in mRNA-1273 vaccinated subjects 6 months after immunization. These results support the current CDC recommendations for a booster dose 5 months after two-dose mRNA-1273 or BNT162b2 immunization (https://www.cdc.gov/coronavirus/2019-ncov/vaccines/booster-shot.html).

Ad26.COV2.S encodes a stabilized S protein.\(^{35}\) Despite the lower levels of neutralizing antibodies than those of mRNA-1273 and BNT162b2 fully immunized subjects, we found that Ad26.COV2.S induced stable neutralizing antibodies 1 month after immunization, and the titers were sustained up to 6 months. Our results are consistent with those of another study that elevated neutralizing antibodies with GMTs ranging from 212 to 354 against SARS-CoV-2 WT were detected in 90% participants 29 days after the first dose of Ad26.COV2.S.\(^{35}\) Furthermore, in Ad26.COV2.S phase 1/2a and 2 clinical trials, durable and stable S protein binding and neutralizing antibodies, which lasted for more than 6 months, were detected in 18–55 and >65 years old participants.\(^{38}\) In another study, durable humoral and cellular immune responses were also observed for at least 8 months following Ad26.COV2.S immunization.\(^{71}\) However, despite these in vitro findings in vaccine recipients, the effectiveness of Ad26.COV2.S vaccination, even with boosting remains low.\(^{77}\)

The emergence of Delta and Omicron VOCs has posed significant challenges for the control of COVID-19. Our results showed that mRNA-1273 and BNT162b2 immunized subjects had reduced levels of neutralizing antibodies against Omicron, which further decreased against Delta and Omicron after 6 months of immunization (Table 2, Figure 4). It was reported that compared to WT, neutralizing antibodies against Omicron from BNT162b2 immunized subjects decreased 43 times by 3 months and were not detected in the period of 6–12 months after immunization.\(^{50}\) In fact, the two-dose series of either mRNA vaccine without boost was not effective at preventing infection with Omicron.\(^{78}\)

In contrast, low but moderate neutralizing antibodies were detected in Ad26.COV2.S immunized subjects, not only against WT, but also VOCs, including Delta and Omicron, which were sustained for at least 6 months (Table 2). In Janssen’s final analysis of efficacy and safety of single-dose Ad26.COV2.S, the results showed that Ad26.COV2.S’ protective effect varied according to the variant.\(^{79}\) In the 39,185 participants, vaccine efficacy against moderate to severe–critical COVID-19 at least 28 days after administration was 52.9% (433 cases in the vaccine group vs. 883 in the placebo group).\(^{79}\) One-dose Ad26.COV2.S provided protection against most sequenced variants with the observed vaccine efficacies against WT, Alpha, Beta, Gamma, and Delta at 58.2%, 70.2%, 51.9%, 36.5%, and 5.7%, respectively, after 28 days of administration.\(^{79}\) In Janssen’s another study, it was demonstrated that one-dose of Ad26.COV2.S induced 8-month durable humoral and cellular immune responses with detectable pseudovirus neutralizing antibodies against all the tested pseudoviruses, including but not limited to WT, Alpha, Beta, Gamma, and Delta.\(^{80}\)

It is unclear why the medium levels of neutralizing antibody titers against Omicron in the Ad26.COV2.S immunized subjects were
| Vaccine   | Study            | Subjects (n) | Dose | WT titer | Durability (month) | Delta titer | Omicron titer |
|----------|------------------|--------------|------|----------|--------------------|-------------|---------------|
| mRNA-1273 | This study       | 74           | 2    | High (PsV)| ~5–7               | High        | Low           |
| mRNA-1273 | [49]             | 48           | 2    | High (PsV)| ~6–8               | High        | Med           |
| mRNA-1273 | [50]             | 239          | 2    | High (PsV)| ~6                 | High        | Low           |
| mRNA-1273 | [51]             | 54           | 2    | High (LV) | N/A                | N/A         | Low           |
| mRNA-1273 | [52]             | 120          | 2    | High (PsV)| ~9                 | N/A         | Low           |
| mRNA-1273 | [53]             | 46           | 2    | High (PsV)| N/A                | High        | Low           |
| mRNA-1273 | [54]             | 54           | 3    | High (LV) | N/A                | N/A         | Low           |
| mRNA-1273 | [71]             | 30           | 2    | High (PsV)| N/A                | N/A         | Med           |
| mRNA-1273 | [55]             | 30           | 2    | High (PsV)| N/A                | N/A         | Low           |
| mRNA-1273 | [56]             | 54           | 3    | High (PsV)| ~6–8               | N/A         | High          |
| mRNA-1273 | [72]             | 30           | 3    | High (PsV)| N/A                | N/A         | High          |
| BNT162b2  | This study       | 74           | 2    | High (PsV)| ~4–6               | High        | Low           |
| BNT162b2  | [49]             | 48           | 2    | High (PsV)| ~4–6               | High        | Low           |
| BNT162b2  | [73]             | 19           | 2    | Med (LV)  | N/A                | N/A         | Low           |
| BNT162b2  | [50]             | 239          | 2    | High (PsV)| ~4–6               | Med         | ND            |
| BNT162b2  | [74]             | 32           | 2    | High (PsV)| ~6                 | N/A         | Low           |
| BNT162b2  | [58]             | 39           | 2    | High (PsV)| N/A                | N/A         | Low           |
| BNT162b2  | [59]             | 40           | 2    | Low (LV)  | N/A                | Low         | ND            |
| BNT162b2  | [60]             | 171          | 2    | High (LV) | ~8–9               | Med         | Low           |
| BNT162b2  | [61]             | 54           | 2    | High (LV) | N/A                | N/A         | Low           |
| BNT162b2  | [62]             | 234          | 2    | Med (LV)  | N/A                | N/A         | Low           |
| BNT162b2  | [63]             | 39           | 2    | High (PsV)| N/A                | Med         | Low           |
| BNT162b2  | [64]             | 50           | 2    | Med (LV)  | N/A                | Med         | Low           |
| BNT162b2  | [51]             | 54           | 3    | High (PsV)| N/A                | N/A         | Med           |
| BNT162b2  | [83]             | 30           | 3    | High (PsV)| N/A                | High        | Med           |
| BNT162b2  | [60]             | 171          | 3    | High (PsV)| N/A                | High        | High          |
| BNT162b2  | [61]             | 234          | 3    | High (PsV)| N/A                | N/A         | Med           |
| BNT162b2  | [74]             | 32           | 3    | High (PsV)| N/A                | N/A         | High          |
| BNT162b2  | [62]             | 22           | 3    | High (rV) | ~4                 | N/A         | High          |
| BNT162b2  | [53]             | 46           | 3    | High (PsV)| N/A                | High        | Med           |
| BNT162b2  | [63]             | 39           | 3    | High (PsV)| N/A                | High        | High          |
| BNT162b2  | [82]             | 48           | 3    | High (PsV)| N/A                | High        | High          |
| BNT162b2  | [59]             | 40           | 3    | High (LV) | N/A                | High        | Med           |
| Ad26.COV2.S | This study    | 74           | 1    | Med (PsV)| ~6–8               | Med         | Med           |
| Ad26.COV2.S | [50]            | 239          | 1    | Low (PsV)| ~4–6               | Low         | Low           |
observed in this study but not others. Similarly, only low neutralizing antibody titers against Delta were detected in a live virus neutralization assay with the same Ad26.COV2.S immunized subjects in a separate study. In those studies, the viruses were incubated with the plasma samples for a short time (1 h) and used to infect cells. Hence, the inhibitory effect of the neutralizing antibodies is primarily due to the blocking effect on virus entry. In the current study, the pseudoviruses were incubated with the plasma samples for a short time but the infection was performed in the presence of the plasma samples. In this case, the inhibitory effect of the neutralizing antibodies could exert not only on virus entry but also on cell-to-cell spread as reported by other studies. More investigations are required to clarify these discrepancies.

It is interesting that we observed low titers of neutralizing antibodies in a small subset of subjects before immunization with any SARS-CoV-2 vaccines (Figure 2). Similar results have also been observed. It is highly possible that these subjects have developed cross-reactive antibodies following prior exposure to other coronaviruses. It has also been reported that other infections or prior immunizations with other vaccines could generate cross-reactive antibodies against SARS-CoV-2.

In conclusion, our findings indicate that both mRNA-1273 and BNT162b2 elicited robust neutralizing antibodies against WT, Alpha, Beta, and Gamma after full immunization but waned after 6 months with a faster decline observed for the BNT162b2 immunized subjects. Both mRNA-1273 and BNT162b2 elicited some but minimal neutralizing antibodies against Omicron. Ad26.COV2.S vaccine induced lower neutralizing antibodies against WT and VOCs, including Delta and Omicron, which lasted for up to 6 months. To prevent moderate to severe COVID-19, mRNA-1273, BNT162b2, and Ad26.COV2.S recipients are advised to receive a booster.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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