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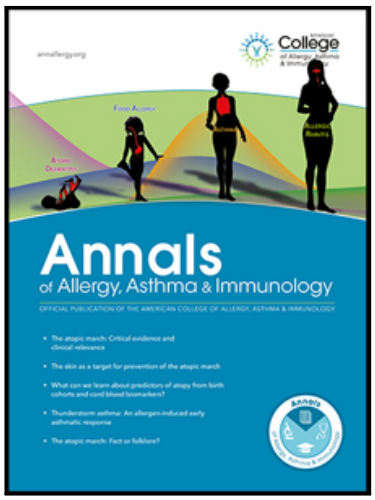

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PII: S1081-1206(22)01829-4
DOI: https://doi.org/10.1016/j.anai.2022.10.003
Reference: ANAI 4110

To appear in: *Annals of Allergy, Asthma Immunology*

Received date: 5 August 2022
Revised date: 19 September 2022
Accepted date: 3 October 2022

Please cite this article as: Samuel M. Ailsworth BA , Behnam Keshavarz PhD , Nathan E. Richards MD , Lisa J. Workman BA , Deborah D. Murphy RN , Michael R. Nelson MDPhD , Thomas A.E. Platts-Mills MDPhD , Jeffrey M. Wilson MDPhD , Enhanced SARS-CoV-2 IgG durability following COVID-19 mRNA booster vaccination and comparison of BNT162b2 with mRNA-1273, *Annals of Allergy, Asthma Immunology* (2022), doi: https://doi.org/10.1016/j.anai.2022.10.003

Enhanced SARS-CoV-2 IgG durability following COVID-19 mRNA booster vaccination and comparison of BNT162b2 with mRNA-1273

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Conflicts of interest: none

Funding source: UVA Manning COVID-19 Research Fund (JW), Salary support from the AAAAI Faculty Development Award (JW) and NIH R37-AI20565 (TPM)

Keywords: COVID-19, SARS-CoV-2, vaccines, mRNA vaccines, IgG, durability, BNT162b2**,** mRNA-1273

Abbreviations: GM – geometric mean; IgG – immunoglobulin G; FDA – Food and Drug Administration; S-RBD – SARS-CoV-2 spike receptor-binding domain; UVA – University of Virginia; VDH – Virginia Department of Health; CDC – Centers for Disease Control and Prevention; HHS – United States Department of Health and Human Services.

Word count: 3088

Figures: 4

Tables: 1

22-08-0517R1

Abstract

Background: BNT162b2 (Pfizer/BioNTech, Cominarty) and mRNA-1273 (Moderna, Spikevax) are mRNA vaccines that elicit antibodies against the SARS-CoV-2 spike receptor-binding domain (S-RBD) and have been approved by the Food and Drug Administration (FDA) to combat the COVID-19 pandemic. Because vaccine efficacy and antibody levels waned over time

after the two-shot primary series, the FDA authorized a booster (third) dose for both mRNA vaccines to adults in the fall of 2021.
Objective: We sought to assess the magnitude and durability of S-RBD IgG after the booster mRNA vaccine dose in comparison to the primary series. We also compared S-RBD IgG levels after BNT162b2 and mRNA-1273 boosters and explored effects of age and prior infection.
Methods: Surrounding receipt of the second and third homologous mRNA vaccine doses, adults in an employee-based cohort provided serum and completed questionnaires, including information about prior COVID-19 infection. IgG to S-RBD was measured using an ImmunoCAP-based system. A subset of samples were assayed for IgG to SARS-CoV-2 nucleocapsid by commercial assay.
Results: 228 subjects had samples collected between 7 and 150 days after their primary series vaccine, and 117 subjects had samples collected in the same time frame after their boost. Antibody levels 7-31 days after the primary series and booster were similar, but S-RBD IgG was more durable over time after the boost, regardless of prior infection status. In addition, mRNA-1273 post-boost antibody levels exceeded BNT162b2 out to 5 months.
Conclusion: COVID-19 mRNA vaccine boosters increase antibody durability, suggesting enhanced long-term clinical protection from SARS-CoV-2 infection compared to the two-shot regimen.

Introduction

IgG antibodies targeting the SARS-CoV-2 spike receptor binding domain (S-RBD) play an important role in host defense against the viral culprit of COVID-19. [1,2] Accordingly, the S-RBD is the major antigen that has been targeted by commercially approved COVID-19 vaccines. As vaccine-induced protection against SARS-CoV-2 waned and breakthrough infections increased following the primary series, in fall 2021 the Food and Drug Administration (FDA) authorized third ("booster") doses of two mRNA vaccines, BNT162b2 (Pfizer/BioNTech, Cominarty) and mRNA-1273 (Moderna, Spikevax).[3-5] While the third dose of each of these vaccines has been shown to enhance protection against infection and severe disease, as compared to the primary two-shot series, the durability of protection against SARS-CoV-2 infection over time remains an important question.[6-8] Although antibody levels are an imperfect surrogate of vaccine efficacy, it

is clear that antibodies to S-RBD are an important component of a protective response.[1,2] To date there has been little data showing the dynamics of the antibody response after booster vaccination in comparison to the initial primary series. In addition, there has been a lack of head-to-head studies comparing BNT162b2 and mRNA-1273 after booster vaccination. Here we used a quantitative assay to assess the levels and durability of IgG to S-RBD elicited by booster doses of both mRNA vaccines in an employee cohort. This work builds on prior investigations of the same cohort where we showed that antibodies elicited by BNT162b2 decayed more rapidly after the primary vaccine series as compared to mRNA-1273.[9,10] These studies also revealed that BNT162b2 elicited lower levels of antibodies in older adults (age ≥ 50 years) as compared to younger adults, an effect that was not seen with mRNA-1273. Here we sought to address the following hypotheses about BNT162b2 and mRNA-1273 booster vaccines: i) IgG to S-RBD would reach a higher peak level after the booster vaccination as compared to the primary vaccine series, ii) IgG to S-RBD levels would be more durable after booster vaccination, and iii) that the differences in IgG levels elicited by BNT162b2 and mRNA-1273 observed after the primary series would persist following booster vaccination. We also investigated the effects of prior infection and age on IgG levels following these two vaccines.

Methods

Study Design and Populations

This cohort study was approved by the University of Virginia (UVA) institutional review board (IRB) and all participants provided verbal and written consent. Adults affiliated with UVA were

recruited from December 2020 through August 2021 by flyer and email announcements to participate in a study investigating antibody responses surrounding the initial vaccine series, as previously reported.[9,10] In fall 2021 the study was modified and opened to adults in the greater Charlottesville community. The majority of enrollees in this study were healthcare workers employed by the UVA Health System. The current analysis includes participants who received two primary series doses, and those who received an additional homologous boost dose of the BNT162b2 (30µg) or mRNA-1273 (50µg) vaccines. For inclusion, participants must have had a blood sample collected between 7 and 150 days after the second or third vaccine. There was no exclusion criteria relating to pre-existing co-morbidities. No samples were included in this analysis from subjects who received additional vaccine doses, heterologous booster doses, alternative dosing regimens (i.e. 3 doses of 100µg mRNA-1273), or other COVID-19 vaccines. Blood samples were processed and serum was isolated and banked at -30°C prior to assay. We screened subjects for symptomatic COVID-19 infection at each visit by asking participants to self-report positive COVID-19 antigen or PCR test results, and/or symptoms suggestive of COVID-19 infection.

Antibody Assays

IgG to S-RBD was measured in serum using a quantitative ImmunoCAP-based system with a Phadia 250 (Thermo-Fisher/Phadia), as previously described.[11] In brief, S-RBD (RayBiotech, Peachtree Corners, GA) was biotinylated and conjugated to streptavidin-coated ImmunoCAPs (Thermo-Fisher/Phadia). Background signal was accounted for by subtracting the signal of an unconjugated streptavidin ImmunoCAP, which was run in parallel with each sample. IgG

antibodies to the nucleocapsid protein were measured in a subset of the cohort – specifically subjects who had paired "early" (day 7-31) and "late" (day 90-150) post-booster samples available - using the Abbott SARS-CoV-2 Nucleocapsid Protein IgG assay (Abbott Architect i2000). To optimize sensitivity of this assay for detecting positive cases we used an index threshold of 0.26 as an indicator of previous infection (as compared to the standard index of 1.4), as previously reported.[12] A subject was considered to have been infected before their booster vaccine if anti-nucleocapsid IgG was present in their early sample. A subject was considered to have been infected after their booster vaccine if anti-nucleocapsid IgG was not present in the early sample but was present in the late sample.

Statistical Analysis

Antibody levels were expressed by geometric mean (GM) with 95% confidence intervals. Continuous data were compared using Student's T test, Mann-Whitney U test and ANOVA, as appropriate. Categorical data was compared using Chi-squared test. Regression modeling was performed using log-transformed antibody levels. Only subjects who had paired early and late samples from the primary series or booster series were used in the longitudinal linear regression model. For subjects with multiple samples collected between day 7-31, the sample with the maximum antibody level was used as their early time point in this model, and all draws between day 32 and 150 were included as continuous data. Statistical analysis was performed with GraphPad Prism 8 (GraphPad Software) and R software, version 3.6.2 (R Foundation for Statistical Computing).

Results

Vaccine Cohort

228 subjects provided at least one blood sample between 7 and 150 days after their second dose of BNT162b2 or mRNA-1273 (primary series group), and 117 subjects provided at least one blood sample in the same time window after their third dose of BNT162b2 or mRNA-1273 (booster group). A majority of subjects in the booster group participated in the initial primary series study, and thus have data included in both the primary and booster series analysis [n=106 (91%)].[9] The booster group tended to be older than the primary series group, median age 44 (IQR 34-57) vs. median age 41 (IQR 32-54), but this difference was not significant, (P=0.06) (**Table 1**). Both groups were predominantly female, with women representing 75% of participants in the primary series group and 77% of participants in the booster group. Race/ethnicity were similarly distributed among participants in the primary series group and the booster group. More subjects in the booster group self-reported infection (13%) compared to the primary series group (4%), (P=0.001). There was a similar distribution of BNT162b2 and mRNA-1273 recipients in the primary series group (50% and 50%) and the booster group (51% and 49%). The majority of subjects received their primary series vaccinations in the winter of 2020-2021 and their booster vaccination in fall/winter of 2021-2022 during the Delta and Omicron surges of infections (**Figure 1**).[13] The majority of infections reported in the cohort occurred during the winter of 2021-2022, which coincided with the emergence of Omicron and a dramatic increase in COVID-19 cases in the state of Virginia **(Figure 1)**. [13,14]

IgG to SARS-CoV-2 Spike RBD Following Primary Series and Booster Vaccination

We first compared S-RBD IgG levels elicited by the primary vaccine series and the booster vaccine using all samples collected within three time intervals: 0-7 days pre-vaccine (baseline), 7-31 days post-vaccine (early), and 90-150 days post-vaccine (late). As expected, baseline antibody levels were higher in subjects from the booster group (GM 4.4 µg/mL [95% CI 3.0-6.3µg/mL]) compared to the primary series (GM 0.3 µg/mL [95% CI 0.3-0.5 µg/mL]), P<0.001, as the latter subjects had not been previously vaccinated (**Figure 2A**). Early after vaccination there was not a difference in antibody levels measured after the primary series (GM 55.3 µg/mL [95% CI 49.8-61.3 µg/mL]) or homologous booster vaccination (GM 55.3 µg/mL [95% CI 46.5-65.6 µg/mL]). By contrast, in the late time interval IgG levels following the booster mRNA vaccine were ~3-fold higher (GM 29.1 µg/mL [95% CI 24.3-34.9 µg/mL]) compared to the primary series vaccination (GM 9.5 µg/mL [95% CI 8.1-11.1 µg/mL]), P<0.001 (**Figure 2A**). We then confirmed that there was a difference in trajectories between the booster and primary series by evaluating subjects who had paired early and late samples available. Linear regression indicated a slower IgG decay following booster compared to primary series vaccination, with respective slopes of -0.0024 (95% CI -0.0037 to -0.0012) vs. -0.0074 (95% CI -0.0083 to -0.0065), P<0.001 (**Figure 2B**). A sensitivity analysis restricted to 30 subjects who had paired samples available after both the primary series and booster vaccination also revealed more persistent IgG following the booster vaccination (data not shown).

Effect of COVID-19 infection on booster vaccination IgG levels

Because the booster arm of the study coincided with the wave of infections associated with the omicron variant in the winter of 2021-2022, we sought to determine whether prior or intercurrent COVID-19 infection impacted IgG levels in recipients of booster vaccines. Positive infection status was determined by either self-report or nucleocapsid IgG testing (among the subjects who had paired early and late samples available). In the early time window, S-RBD IgG levels were higher in those who were infected before the booster vaccine dose (GM 96.7 µg/mL [95% CI 60.4-154.8 µg/mL]) as compared to those who were not infected before the booster vaccine dose (GM 50.9 µg/mL [95% CI 40.8-63.5 µg/mL]), P=0.05 (**Figure 3A**). By the late time window, this difference in antibody levels between those who were infected before the booster vaccine dose (GM 40.7 µg/mL [95% CI 16.9-98.2 µg/mL]) and those who were uninfected (GM 28.7 µg/mL [95% CI 20.6-40.0 µg/mL]) was no longer present, P=0.54. However, those who were infected after their booster vaccine (GM 76.5 µg/mL [95% CI 48.9-119.7 µg/mL]) had significantly greater IgG levels than the uninfected subjects in the late time window, P=0.005. Of note, to be considered as uninfected in this analysis required a combination of negative self-report and negative nucleocapsid IgG testing.

Effect of age on booster vaccination IgG levels

We next sought to investigate the effect of age on IgG levels following booster immunization. Building off our previous reports, we stratified subjects into older (≥50 years) and younger (<50 years) age groups.[9,10] To account for the effect of natural infection on IgG levels, this analysis excluded subjects who reported prior COVID-19. Antibody levels were significantly higher in the younger subjects (GM 68.1 µg/mL [95% CI 56.8-81.6 µg/mL]) compared to older subjects

(GM 40.2 µg/mL [95% CI 29.2-55.3 µg/mL]) in the early time window, P=0.01. Interestingly, this difference did not persist in the late time window, P=0.78 (**Figure 3B**).

Comparison of IgG to SARS-CoV-2 Spike RBD Following Booster Vaccination with BNT162b2 (Pfizer/BioNTech) and mRNA-1273 (Moderna)

We then compared S-RBD IgG levels in those who received BNT162b2 and mRNA-1273 booster vaccines, again excluding subjects who reported prior COVID-19. Prior to the boost, recipients of two doses of mRNA-1273 (GM 4.5 µg/mL [95%CI 3.3-6.1]) had higher antibody levels than recipients of two doses of BNT162b2 (GM 2.1 µg/mL [95%CI 1.5-3.0]), P=0.004. In the early post-boost time window, antibody levels were higher in those subjects who received three doses of mRNA-1273 (GM 65.1 µg/mL [95% CI 52.8-80.3 µg/mL]) versus those who received three doses of BNT162b2 (GM 42.1 µg/mL [95% CI 31.2-56.8 µg/mL]), P=0.01. Moreover, during the late time window subjects who received mRNA-1273 retained nearly twice the level of anti-spike RBD IgG (GM 36.8 µg/mL [95% CI 28.6-47.4 µg/mL]) as those who received BNT162b2 (GM 18.6 µg/mL [95% CI 14.2-24.4 µg/mL]), P<0.001 (**Figure 4A**).

We further stratified subjects by both age and vaccine to investigate the combined effects of these variables on IgG magnitude and persistence. Consistent with our prior findings during the primary series, antibody levels in the early time window were significantly lower in older subjects who received BNT162b2 (GM 25.2 µg/mL [95% CI 15.7-40.2 µg/mL]), compared to the younger subjects who received BNT162b2 (GM 70.5 µg/mL [95% CI 55.8-89.1 µg/mL]), P=0.001.[9] For mRNA-1273 there was not an age effect, and older subjects who received mRNA-

1273 had significantly higher antibody levels (GM 62.9 µg/mL [95% CI 43.4-91.1 µg/mL]) compared to age-similar subjects who received BNT162b2, P=0.004. In the late time window, antibody levels were similar among older (GM 16.5 µg/mL [95% CI 9.7-28.2 µg/mL]) and younger (GM 19.8 µg/mL [95% CI 14.3-27.5 µg/mL]) recipients of BNT162b2, and older (GM 31.1 µg/mL [95% CI 22.7-42.5 µg/mL]), and younger (GM 45.6 µg/mL [95% CI 30.0-69.5 µg/mL]) recipients of mRNA-1273, respectively. Taken together, in the late window S-RBD levels were higher in recipients of mRNA-1273 versus BNT162b2 regardless of age (**Figure 4B**). To further explore the effect of age we carried out linear regression comparing S-RBD IgG versus age in the early post-boost window (using peak antibody levels for subjects who had more than one sample available). Antibody levels elicited by BNT162b2 significantly decreased with increasing age (P=0.003), while antibody levels elicited by mRNA-1273 remained stable (P=0.78) **(Figure 4C).**

Discussion

Building on our prior investigations of COVID-19 mRNA vaccine immunogenicity following the primary vaccination series, here we have assessed the magnitude and trajectory of S-RBD IgG antibodies elicited by BNT162b2 and mRNA-1273 up to 5 months after the booster regimen.[9,10] We also investigated the effects of infection and age on antibody durability after the booster vaccine. Our results showed that a third dose of BNT162b2 or mRNA-1273 did not result in a higher peak S-RBD IgG levels compared to a two-dose mRNA vaccine series. This contrasts with some reports, which have found higher peak IgG levels after a third mRNA vaccine compared to the primary series.[15-18] The explanation for the discrepancy is not clear, but the window in which samples were collected could be relevant given that antibody levels are

dynamic and there are differences in trajectory between the two mRNA vaccines. The discrepancy could also relate to variability in assays that have been used to measure IgG to S-RBD. For example, differences in the capacity of the solid-phase could impact assay dynamic ranges.

Our finding that antibody durability after a third mRNA dose was enhanced compared to the primary series is supported by a few reports, but there has been little published data that has compared antibody levels as far as 5 months post-vaccination.[19-21] Although there is no specific antibody level that has been shown to definitively correlate with protective immunity against SARS-CoV-2, antibody magnitude and persistence has been shown to confer greater protection against infection and disease.[22-25] Accordingly, understanding the durability of antibodies after the third vaccination could be relevant to guiding the timing of any additional follow-up vaccinations.

When investigating the relevance of prior COVID-19 infection on antibody levels and durability, we observed that infection prior to the boost was associated with significantly higher antibody levels in the early time window, but the difference did not persist at 3-5 months post-booster. These findings indicate that the enhanced antibody durability observed after booster vaccination was not explained by hybrid immunity and also raises questions about the durability of immune protection resulting from natural infection. Of note, there have been mixed findings reported on the antibody response stemming from hybrid versus vaccine-elicited immunity.[17,26,27] In a separate finding, we observed that subjects who were boosted and who were subsequently infected had the highest levels of antibodies after 4 months. We surmise that vaccine-induced

antibodies likely peaked and began to decline in these subjects before a natural infection further enhanced the IgG levels, though we lacked sufficiently granular sampling to demonstrate this.

Our comparison of the IgG post-boost response between the two FDA-authorized COVID-19 mRNA vaccines revealed that mRNA-1273 had enhanced immunogenicity versus BNT162b2. Antibody levels elicited by mRNA-1273 were higher than BNT162b2 when assessed during the early and the late post-boost time window. To our knowledge this is a novel finding, but it is not unexpected as we and others have previously shown that mRNA-1273 elicits greater levels of S-RBD IgG than BNT162 after the primary vaccination.[9, 10, 28, 29] Moreover, an enhanced antibody response to mRNA-1273 is biologically plausible as it incorporates a greater amount of mRNA than BNT162b2 both during the initial priming series (100 µg in mRNA-1273 vs. 30 µg in BNT162b2) and the boost (50 µg in mRNA-1273 vs. 30 µg in BNT162b2).

Our finding that S-RBD IgG levels were lower in older versus younger subjects early after a third mRNA vaccine was accounted for by lower antibody levels in older subjects who received three doses of the BNT162b2 vaccine. Between 7 and 31 days after a third vaccine, there was no difference in antibody levels between older and younger subjects who received mRNA-1273. In the early time window, older subjects who received BNT162b2 not only had lower antibody levels than their younger counterparts, but they also had lower antibody levels than the older subjects who received mRNA-1273. In contrast to the early findings, by 4 months post-boost we did not observe differences in older vs younger recipients of BNT162b2. However, we found that mRNA-1273 antibody levels were significantly higher than BNT162b2 antibody levels between 3 and 5 months after booster vaccination regardless of age. This data reinforces the finding that

mRNA-1273 elicits more durable antibodies than BNT162b2 and parallels what has previously been reported regarding S-RBD IgG levels after the primary series.[9,10, 28, 29]

There are several limitations to consider. We did not measure binding antibodies to new SARS-CoV-2 variants, such as Omicron and its sub-variants. Moreover, the emergence of these variants could nullify the effects of enhanced antibody durability to the native S-RBD.[8,30,31] Additionally, we did not measure neutralizing antibody titers in this cohort. On the other hand, we and others have previously shown that neutralizing antibodies correlate moderately to strongly with binding IgG levels.[9,16,32] Another limitation is that we had a relatively small sample size for some of the vaccine and age comparisons, particularly after excluding subjects with history of prior COVID-19 infection. We also did not measure nucleocapsid IgG in all of the samples, which could contribute to an underestimate of COVID-19 infection in this cohort.

In conclusion, we found that a booster dose of an mRNA vaccine elicits greater S-RBD IgG durability compared to a primary two-dose regimen, regardless of previous infection status. This finding suggests that a third dose of an mRNA vaccine provides more persistent protection from COVID-19 than a two-dose regimen. Our data also indicate that three doses of mRNA-1273 elicits a stronger antibody response than three doses of BNT162b2, regardless of age. Whether mRNA-1273 confers superior protection against circulating strains compared to BNT162b2 remains an open question. While vaccine-elicited protection against SARS-CoV-2 infection wanes over time, we would highlight that both FDA-authorized mRNA vaccines have shown valuable protection against severe disease and death from COVID-19.[6,16,33,34]

Acknowledgements
We are grateful to the University of Virginia staff and Charlottesville community members who participated in the study, Dr. Jaimin Patel for helping with patient enrollment, Jacob Goldstein-Greenwood for statistical counseling and Panwachit Tongvichit, Karen Dinwiddie, and Judy Hundley with the University of Virginia Clinical Laboratory

Table 1. Demographics and characteristics of COVID-19 vaccine cohort.

Characteristics		Primary Series (n=228)	Booster vaccine (n=117)	P
Age	Median (Range)	41 (19-85)	44 (19-87)	0.06[a]
	<50 yrs, n (%)	152 (67%)	68 (58%)	0.12[b]
	≥50 yrs, n (%)	76 (33%)	49 (42%)	
Sex	Female, n (%)	171 (75%)	90 (77%)	0.69[b]
	Male, n (%)	57 (25%)	27 (23%)	
Race/ethnicity	White, n (%)	179 (79%)	96 (82%)	0.64[b]
	Black, n (%)	17 (8%)	6 (5%)	0.46[b]
	Asian, n (%)	24 (11%)	10 (9%)	0.71[b]
	Other, n (%)	8 (4%)	5 (4%)	0.59[b]
Vaccine Received	BNT162b2, n (%)	114(50%)	60 (51%)	0.82[b]
	mRNA-1273, n (%)	114 (50%)	57 (49%)	
Two or more chronic co-morbidities[d], n (%)		6 (3%)	4 (3%)	0.68 [b]
On immunosuppressing medications[e], n (%)		8 (4%)	4 (3%)	0.97 [b]
Self-report of prior COVID-19 infection, n (%)		8 (4%)	15 (13%)	0.001[b]
Baseline samples, n (%)		41 (18%)	34 (29%)	0.02[b]
S-RBD IgG, GM µg/mL (95%CI)		0.3 (0.3-0.5)	4.4 (3.0-6.3)	<0.001[c]
Early samples (D7-31 post-vaccine), n (%)		169 (74%)	62 (48%)	<0.001[b]
Days, median (IQR)		21 (18-24)	17.5 (13-24)	0.008[a]
S-RBD IgG, GM µg/mL (95%CI)		55.3 (49.8-61.3)	55.3 (46.5-65.6)	0.49[c]
Late samples (D90-150 post-vaccine), n (%)		98 (43%)	98 (76%)	<0.001[b]
Days, median (IQR)		133 (112.5-139)	126 (121-133)	0.98[a]
S-RBD IgG, GM µg/mL (95%CI)		9.5 (8.1-11.1)	29.1 (24.3-34.9)	<0.001[c]
Total # samples per subject, mean (range)		1.79 (1-7)	2.27 (1-8)	0.003[a]

a = Unpaired t-test
b = χ^2 test
c = Mann-Whitney U test
d = Defined as hypertension, heart disease, diabetes, COPD, chronic kidney disease, and/or asthma
e = Defined by self-report of treatment with immunomodulatory or immunosuppressant medication, including oral corticosteroids.

Abbreviations: IQR, Interquartile range; GM, Geometric mean; CI, Confidence interval; S-RBD, Spike receptor binding domain; IgG, Immunoglobulin G

FIGURE LEGEND

Figure 1: Timeline of vaccines, blood draws, and COVID-19 infections in the vaccine cohort. Also shown are VDH-confirmed COVID-19 infections, and genotyped SARS-CoV-2 variants reported by the CDC in HHS region 3.[13,14] VDH, Virginia Department of Health; CDC, Centers for Disease Control and Prevention; HHS, Health and Human Services.

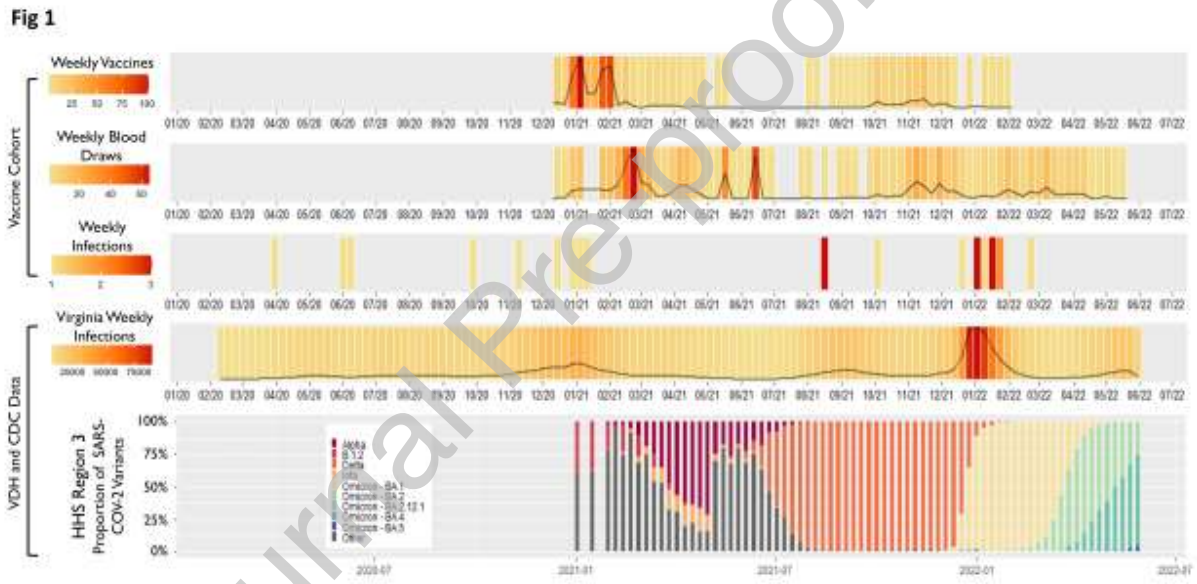


Figure 2. SARS-CoV-2 S-RBD IgG trajectory after two (primary series) and three dose (booster) COVID-19 mRNA vaccine series. (A) IgG levels in the pre-vaccine, early and late windows. (B) Regression analysis of longitudinal paired samples. Bold lines indicate regression slopes and shaded area 95% confidence intervals. S-RBD, spike receptor-binding domain.

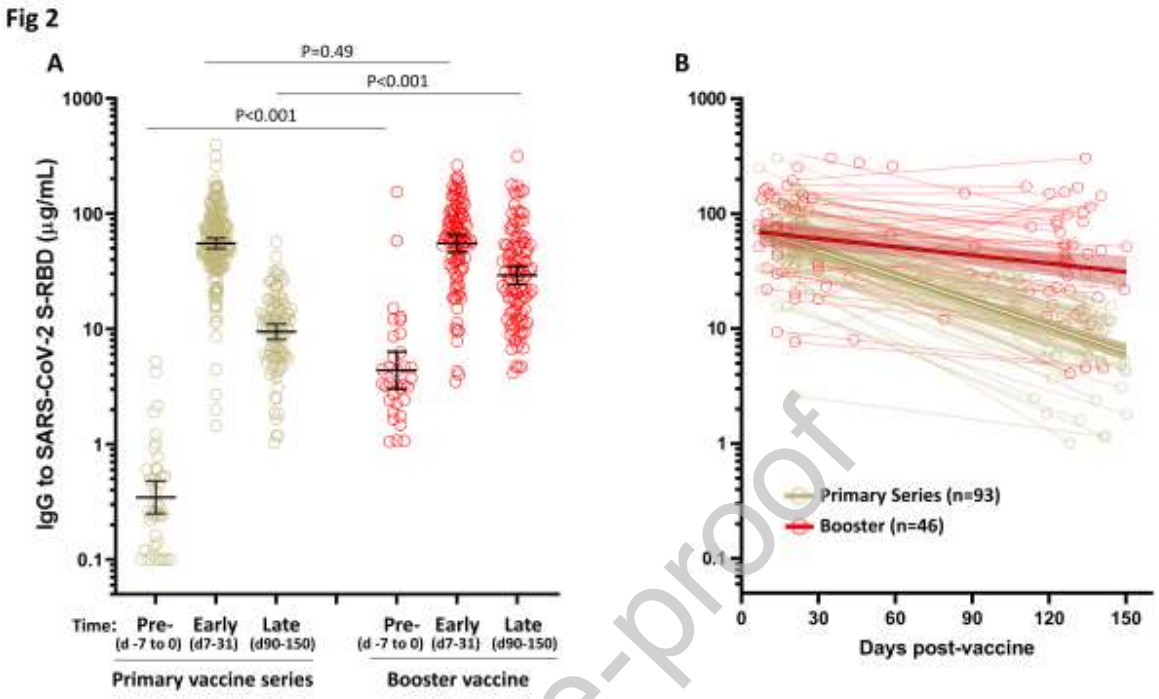


Figure 3. The effects of infection and age on SARS-CoV-2 S-RBD IgG levels. (A) IgG levels in uninfected (Un-) as compared to those infected before (Pre-) or after (Post-) booster vaccination. (B) S-RBD IgG levels stratified by age (excluding participants with prior self-reported COVID-19). S-RBD, spike receptor-binding domain.

Fig 3

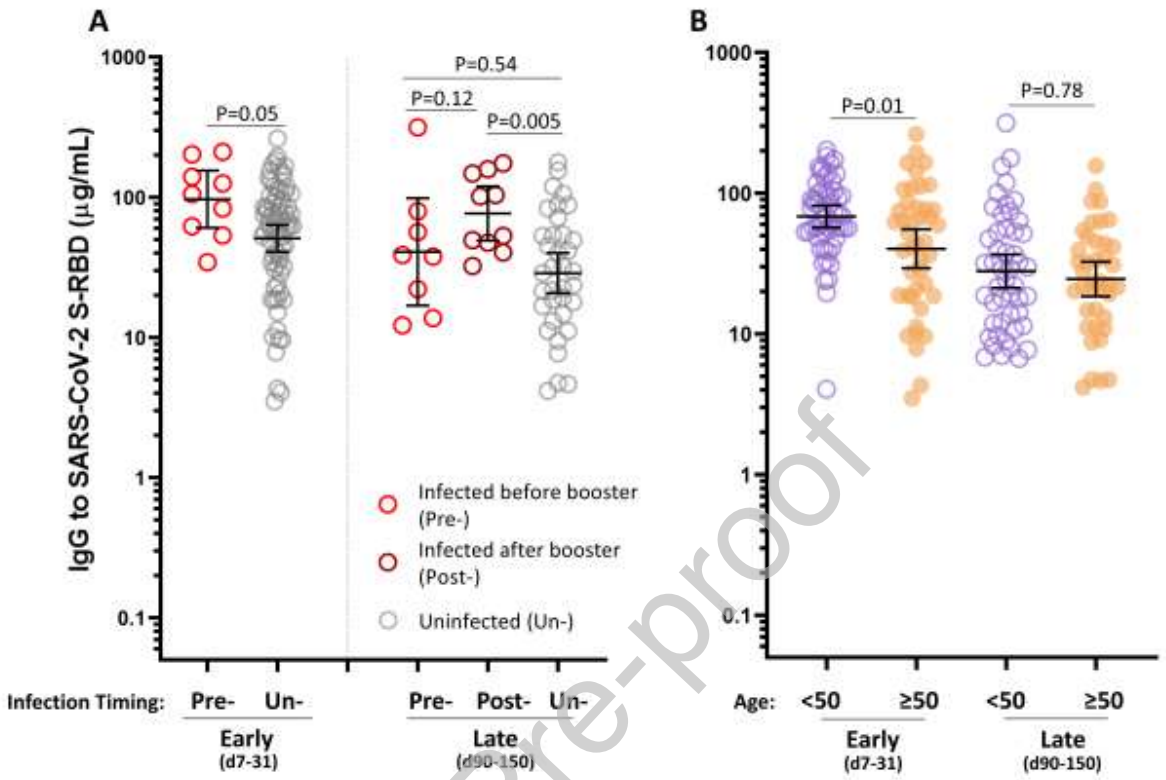
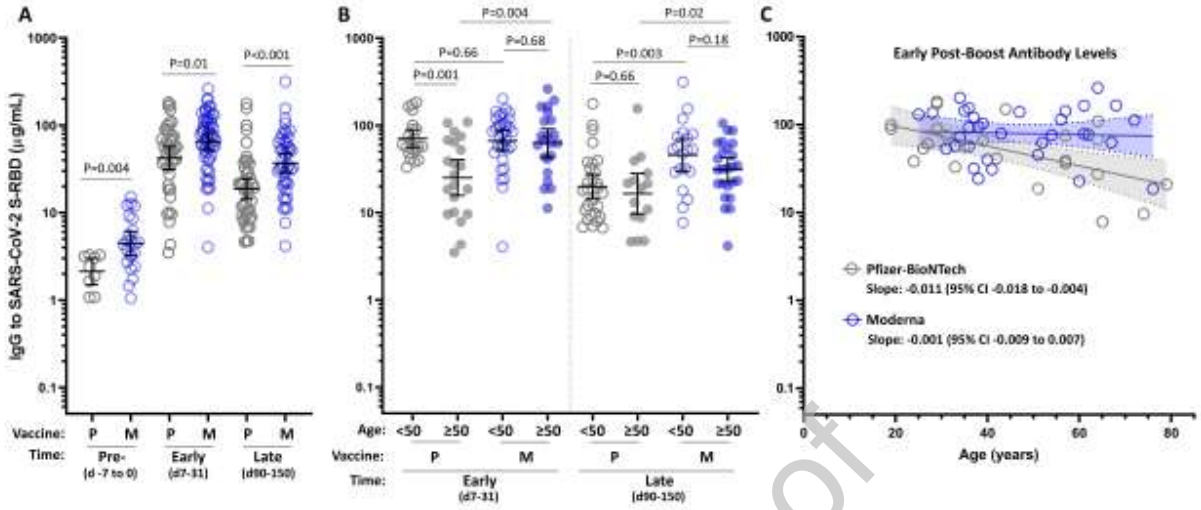


Figure 4. Comparison SARS-CoV-2 S-RBD IgG levels by vaccine, and interaction of vaccine and age. (A) S-RBD IgG stratified by BNT162b2/Pfizer-BioNTech (P) or mRNA-1273/Moderna (M) vaccine. (B) S-RBD IgG stratified by age and vaccine. (C) Linear regression of S-RBD levels in relation to age. S-RBD, spike receptor-binding domain.

Fig 4



REFERENCES

1. Scourfield D, Reed S, Quastel M, Alderson J, Bart VMT, Crespo AT, et al. The role and uses of antibodies in COVID-19 infections: a living review. *Oxf Open Immunol*. 2021;2(1).
2. Molodtsov I, Kegeles E, Mitin A, Mityaeva O, Musatova OE, Panova A, et al. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)–Specific T Cells and Antibodies in Coronavirus Disease 2019 (COVID-19) Protection: A Prospective Study. *Clinical Infectious Diseases*. 2022.
3. Coronavirus (COVID-19) update: FDA expands eligibility for COVID-19 Vaccine Boosters. U.S. Food and Drug Administration. https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-expands-eligibility-covid-19-vaccine-boosters. Published November 19, 2021.
4. Tartof SY, Slezak JM, Fischer H, Hong V, Ackerson BK, Ranasinghe ON, et al. Effectiveness of mrna BNT162B2 COVID-19 vaccine up to 6 months in a large integrated health system in the USA: A retrospective cohort study. *The Lancet*. 2021;398(10309):1407-1416.
5. Menni C, May A, Polidori L, Louca P, Wolf J, Capdevila J, et al. Covid-19 vaccine waning and effectiveness and side-effects of boosters: A Prospective Community study from the Zoe Covid Study. *The Lancet Infectious Diseases*. 2022;22(7):1002-1010.
6. Butt AA, Talisa VB, Yan P, Shaikh OS, Omer SB, Mayr FB. Vaccine effectiveness of 3 versus 2 doses of severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) mrna vaccines in a high-risk national population. *Clinical Infectious Diseases*. 2022.

7. Ferdinands JM, Rao S, Dixon BE, Mitchell PK, DeSilva MB, Irving SA, et al. Waning 2-dose and 3-dose effectiveness of mrna vaccines against COVID-19–Associated Emergency Department and urgent care encounters and hospitalizations among adults during periods of Delta and Omicron variant predominance — vision network, 10 states, August 2021–January 2022. *MMWR Morbidity and Mortality Weekly Report*. 2022;71(7):255-263.
8. Patalon T, Saciuk Y, Peretz A, Perez G, Lurie Y, Maor Y, et al. Waning effectiveness of the third dose of the BNT162b2 mrna COVID-19 vaccine. *Nature Communications*. 2022;13(1).
9. Keshavarz B, Richards NE, Workman LJ, Patel J, Muehling LM, Canderan G, et al. Trajectory of IgG to SARS-COV-2 after vaccination with BNT162B2 or mRNA-1273 in an employee cohort and comparison with natural infection. *Frontiers in Immunology*. 2022;13.
10. Richards NE, Keshavarz B, Workman LJ, Nelson MR, Platts-Mills TA, Wilson JM. Comparison of SARS-COV-2 antibody response by age among recipients of the BNT162B2 vs the mRNA-1273 vaccine. *JAMA Network Open*. 2021;4(9).
11. Keshavarz B, Wiencek JR, Workman LJ, Straesser MD, Muehling LM, Canderan G, et al. Quantitative measurement of IgG to severe acute respiratory syndrome coronavirus-2 proteins using immunocap. *International Archives of Allergy and Immunology*. 2021;182(5):417-424.
12. Mohanraj D, Bicknell K, Bhole M, Webber C, Taylor L, Whitelegg A. Antibody responses to SARS-COV-2 infection—comparative determination of seroprevalence in two high-throughput assays versus a sensitive spike protein Elisa. *Vaccines*. 2021;9(11):1310.
13. Data.virginia.gov. https://data.virginia.gov/Government/VDH-COVID-19-PublicUseDataset-EventDate/9d6i-p8gz/data. Published 2022. Accessed August 26, 2022.
14. Data.cdc.gov. https://data.cdc.gov/Laboratory-Surveillance/SARS-CoV-2-Variant-Proportions/jr58-6ysp. Published 2022. Accessed August 26, 2022.
15. Chu L, Vrbicky K, Montefiori D, Huang W, Nestorova B, Chang Y, et al. Immune response to SARS-COV-2 after a booster of mRNA-1273: An open-label phase 2 trial. *Nature Medicine*. 2022;28(5):1042-1049.
16. Lustig Y, Gonen T, Meltzer L, Gilboa M, Indenbaum V, Cohen C, et al. Superior immunogenicity and effectiveness of the third compared to the second BNT162b2 vaccine dose. *Nature Immunology*. 2022;23(6):940-946.
17. Romero-Ibarguengoitia M, Rivera-Salinas D, Hernández-Ruíz Y, Armendariz-Vazquez AG, Gonzalez-Cantu A, Barco-Flores IA, et al. Effect of the third dose of BNT162b2 vaccine on quantitative SARS-CoV-2 spike 1–2 IgG antibody titers in healthcare personnel. *PLoS One*. 2022;17(3):e0263942.
18. Belik M, Jalkanen P, Lundberg R, Reinholm A, Laine L, Vaisanen E, et al. Comparative analysis of COVID-19 vaccine responses and third booster dose-induced neutralizing antibodies against Delta and Omicron variants. *Nat Commun*. 2022;13(1).

19. Gilboa M, Regev-Yochay G, Mandelboim M, Indenbaum V, Asraf K, Fluss R, et al. Durability of the immune response to a third BNT162b2 dose; five months follow-up. *medRxiv.* 2022.
20. Herzberg J, Fischer B, Becher H, Becker AK, Honarpisheh H, Guraya SY, et al. Short-Term Drop in Antibody Titer after the Third Dose of SARS-CoV-2 BNT162b2 Vaccine in Adults. *Vaccines (Basel)*. 2022;10(5):805.
21. Roeder AJ, Koehler MA, Jasbi P, McKechnie D, Vanderhoof J, Edwards BA, et al. Longitudinal Comparison of Neutralizing Antibody Responses to COVID-19 mRNA Vaccines after Second and Third Doses. Vaccines. 2022; 10(9):1459. https://doi.org/10.3390/vaccines10091459
22. Earle K, Ambrosino D, Fiore-Gartland A, Goldblatt D, Gilbert PB, Siber GR, et al. Evidence for antibody as a protective correlate for COVID-19 vaccines. *Vaccine*. 2021;39(32):4423-4428.
23. Perry J, Osman S, Wright J, Richard-Greenblatt M, Buchan SA, Sadarangani M, et al. Does a humoral correlate of protection exist for SARS-CoV-2? A systematic review. PLoS One. 2022;17(4):e0266852.
24. Montez-Rath M, Garcia P, Han J, Cadden L, Hunsader P, Morgan C, et al. SARS-CoV-2 Infection during the Omicron Surge among Patients Receiving Dialysis: The Role of Circulating Receptor-Binding Domain Antibodies and Vaccine Doses. *Journal of the American Society of Nephrology*. 2022:ASN.2022040504. doi:10.1681/asn.2022040504
25. Feng S, Phillips D, White T, Sayal H, Aley P, Bibi S, et al. Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. Nat Med. 2021;27(11):2032-2040. doi:10.1038/s41591-021-01540-1
26. Kontopoulou K, Nakas C, Papazisis G. Significant Increase in Antibody Titers after the 3rd Booster Dose of the Pfizer–BioNTech mRNA COVID-19 Vaccine in Healthcare Workers in Greece. *Vaccines (Basel)*. 2022;10(6):876.
27. Chen Y, Tong P, Whiteman N, Moghaddam AS, Zarghami N, Zuiani A, et al. Immune recall improves antibody durability and breadth to SARS-COV-2 variants. *Science Immunology*. 2022.
28. Steensels D, Pierlet N, Penders J, Mesotten D, Heylen L. Comparison of SARS-CoV-2 Antibody Response Following Vaccination With BNT162b2 and mRNA-1273. *JAMA*. 2021;326(15):1533.
29. Montoya J, Adams A, Bonetti V, Deng S, Link NA, Pertsch S, et al. Differences in IgG Antibody Responses following BNT162b2 and mRNA-1273 SARS-CoV-2 Vaccines. Microbiol Spectr. 2021;9(3).
30. Accorsi E, Britton A, Fleming-Dutra K, Smith ZR, Shang N, Derado G, et al. Association Between 3 Doses of mRNA COVID-19 Vaccine and Symptomatic Infection Caused by the SARS-CoV-2 Omicron and Delta Variants. *JAMA*. 2022;327(7):639.
31. Qu P, Faraone J, Evans J, Zou X, Zheng YM, Carlin C, et al. Neutralization of the SARS-CoV-2 Omicron BA.4/5 and BA.2.12.1 Subvariants. *New England Journal of Medicine*. 2022;386(26):2526-2528.

32. Maeda K, Amano M, Uemura Y, Amano M, Uemura Y, Tsuchiya K, et al. Correlates of neutralizing/SARS-CoV-2-S1-binding antibody response with adverse effects and immune kinetics in BNT162b2-vaccinated individuals. *Sci Rep*. 2021;11(1).
33. Abu-Raddad L, Chemaitelly H, Bertollini R. Effectiveness of mRNA-1273 and BNT162b2 Vaccines in Qatar. *New England Journal of Medicine*. 2022;386(8):799-800.
34. Tenforde M, Self W, Naioti E, Ginde A, Douin D, Olson S, et al. Sustained Effectiveness of Pfizer-BioNTech and Moderna Vaccines Against COVID-19 Associated Hospitalizations Among Adults —United States, March–July 2021. *MMWR Morb Mortal Wkly Rep*. 2021;70(34):1156-1162.