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Older Adults Mount Less Durable Humoral Responses to Two Doses of COVID-19 mRNA Vaccine but Strong Initial Responses to a Third Dose

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Background. Third coronavirus disease 2019 (COVID-19) vaccine doses are broadly recommended, but immunogenicity data remain limited, particularly in older adults.

Methods. We measured circulating antibodies against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein receptor-binding domain, ACE2 displacement, and virus neutralization against ancestral and omicron (BA.1) strains from prevaccine up to 1 month following the third dose, in 151 adults aged 24–98 years who received COVID-19 mRNA vaccines.

Results. Following 2 vaccine doses, humoral immunity was weaker, less functional, and less durable in older adults, where a higher number of chronic health conditions was a key correlate of weaker responses and poorer durability. One month after the third dose, antibody concentrations and function exceeded post–second-dose levels, and responses in older adults were comparable in magnitude to those in younger adults at this time. Humoral responses against omicron were universally weaker than against the ancestral strain after both the second and third doses. Nevertheless, after 3 doses, anti-omicron responses in older adults reached equivalence to those in younger adults. One month after 3 vaccine doses, the number of chronic health conditions, but not age, was the strongest consistent correlate of weaker humoral responses.

Conclusions. Results underscore the immune benefits of third COVID-19 vaccine doses, particularly in older adults.

Keywords. COVID-19; vaccine; mRNA; SARS-CoV-2; humoral immunity; older adults; binding antibodies; ACE2 displacement; viral neutralization; omicron.

Older adults are at increased risk of lethal coronavirus disease 2019 (COVID-19) following severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection [1–3]. While 2 COVID-19 mRNA vaccine doses broadly protect against hospitalization and death [4–6], weaker vaccine-induced immunity observed in the elderly and other groups [7–12] led to their prioritization for third doses [13–16]. Vaccine-induced antibodies also decline over time, which can increase the risk of postvaccination infections [17–19], particularly with the more transmissible and immune-evasive omicron variant [20–22].

We and others have shown that older age is associated with weaker antibody responses to COVID-19 mRNA vaccines [10–12]. We previously characterized longitudinal humoral responses up to 3 months after the second vaccine dose in 151 adults 24 to 98 years of age [12]. Here, we examine binding and neutralizing antibody responses up to 6 months after the second dose, and at 1 month after the third dose. We also evaluate binding antibodies, ACE2 displacement, and virus neutralization against omicron (BA.1). Characterization of the immunological benefits of a third dose is critical to promote continued public uptake, particularly in light of recent omicron-driven infection waves.

METHODS

Study Design

We conducted a prospective longitudinal cohort study in British Columbia, Canada, to examine SARS-CoV-2 specific humoral responses following vaccination with Comirnaty

Table 1. Participant Characteristics and Sampling Information

Variable Category	Characteristic	Health Care Workers (n = 81)	Older Adults (n = 56)	COVID-19 Convalescent at Study Entry (n = 14)
Sociodemographic/health	Age, y, median (IQR)	41 (35–51)	78 (73–83)	48 (36–87)
	Female sex, n (%)	61 (75)	38 (68)	10 (71)
	White/Caucasian ethnicity, n (%)	37 (46)	43 (77)	7 (50)
	Chronic health or immunosuppressive conditions, median (IQR)	0 (0–0)	1 (0–2)	0 (0–1)
Vaccine information	Comirnaty, first mRNA vaccine, n (%)	80 (99)	48 (86)	13 (93)
	Comirnaty, second mRNA vaccine, n (%)	79 (98)	46 (82)	13 (93)
	Time between first and second doses, d, median (IQR)	97 (91–102)	76 (45–85)	112 (87–118)
	Comirnaty, third mRNA vaccine, n (%)[a]	32/61 (52)	19/47 (40)	3/6 (50)
	Time between second and third dose, d, median (IQR)	210 (200–241)	169 (160–231)	189 (170–194)
Specimen collection	Specimens collected prevaccine, n (%)	80 (99)	49 (88)	13 (93)
	Specimens collected 1 mo after first dose, n (%)	79 (98)	49 (88)	13 (93)
	Day of specimen collection 1 mo after first dose, median (IQR)	28 (27–30)	30 (28–32)	31 (28–32)
	Specimens collected 1 mo after second dose, n (%)	81 (100)	55 (98)	14 (100)
	Day of specimen collection 1 mo after second dose, median (IQR)	29 (29–32)	29 (29–31)	32 (30–36)
	Specimens collected 3 mo after second dose, n (%)	79 (98)	53 (95)	13 (93)
	Day of specimen collection 3 mo after second dose, median (IQR)	90 (90–91)	90 (89–92)	90 (87–91)
	Specimens collected 6 mo after second dose, n (%)	78 (96)	40 (71)	10 (71)
	Day of specimen collection 6 mo after second dose, median (IQR)	181 (179–182)	176 (167–182)	180 (179–181)
	Specimens collected 1 mo after third dose, n (%)	61 (75)	47 (84)	6 (38)
	Day of specimen collection 1 mo after third dose, median (IQR)	30 (29–31)	32 (29–33)	30 (29–30)
COVID-19 postvaccination	Anti-N seroconversion during study follow-up, n (%)	6 (7.4)	2 (3.6)	…

Abbreviations: COVID-19, coronavirus disease 2019; IQR, interquartile range; N, nucleocapsid.

[a]Denominators are the number of specimens collected 1 month after third dose.

(BNT162b2 -BioNTech/Pfizer) or Spikevax (mRNA-1273-Moderna). Our cohort (total n = 151) included 81 health care workers (HCW) and 56 older adults (including 18 residents of long-term care or assisted living facilities) who were COVID-19 naive at study entry, and 14 individuals (including 8 HCW and 6 older adults) with anti-SARS-CoV-2 nucleocapsid (N) antibodies at study entry (COVID-19 convalescent group) [12]. Serum and plasma were collected prior to vaccination; 1 month after the first dose; 1, 3, and 6 months after the second dose; and 1 month following the third dose (see Table 1 for exact collection timings) [12].

Ethics Approval

Written informed consent was obtained from all participants or their authorized decision makers. This study was approved by the University of British Columbia/Providence Health Care and Simon Fraser University Research Ethics Boards.

Data Sources

Sociodemographic, health, and vaccine information was collected by self-report and confirmed through medical records where available. Chronic health conditions were defined as hypertension, diabetes, asthma, obesity (body mass index ≥30), chronic diseases of lung, liver, kidney, heart, or blood, cancer, and immunosuppression due to chronic conditions or medication, to generate a score ranging from 0 to 11 per participant [12].

Binding Antibody Assays

We measured total binding antibodies against SARS-CoV-2 N and spike (S) receptor binding domain (RBD) in serum using the Roche Elecsys Anti-SARS-CoV-2 and Anti-SARS-CoV-2 S assays, respectively, on a Cobas e601 module analyzer (Roche Diagnostics). Following SARS-CoV-2 infection, both assays should be positive, whereas postvaccination only S should be positive, allowing identification of convalescent individuals. Both tests are electrochemiluminescence sandwich immunoassays, and report results in arbitrary units (AU)/mL, calibrated against an external standard. For the S assay, the manufacturer indicates that AU values can be considered equivalent to World Health Organization-defined international binding antibody units [23]. For the S assay, sera were tested undiluted, with samples above the upper limit of quantification (ULOQ) retested at 1:100 dilution, allowing a 0.4–25 000 U/mL measurement range. We also quantified plasma immunoglobulin G (IgG) binding antibodies against RBD using the V-plex SARS-CoV-2 (IgG) Panel 22 ELISA kit (Meso Scale Diagnostics), which features the ancestral (Wuhan) and omicron (BA.1) RBD antigens, on a Meso QuickPlex SQ120 instrument. Plasma was diluted 1:10 000 as directed, with results reported in AU/mL.

ACE2 Competition Assay

We assessed the ability of plasma antibodies to block the RBD-ACE2 receptor interaction by competition enzyme-linked immunosorbent assay (ELISA; Panel 22 V-plex SARS-CoV-2 [ACE2]; Meso Scale Diagnostics). ACE2 competition assays represent a higher-throughput method to estimate potential virus neutralizing activity [24]. Plasma was diluted 1:20 as directed and results reported as percent ACE2 displacement.

Live Virus Neutralization

Neutralizing activity in plasma was examined using a live SARS-CoV-2 infectivity assay as previously described [12] with isolate USA-WA1/2020 (BEI Resources) and a local omicron isolate (BA.1 strain; GISAID accession No. EPI_ISL_9805779) on VeroE6-TMPRSS2 (JCRB-1819) target cells. Viral stock was diluted to 50 $TCID_{50}$ (50% tissue culture infectious dose)/200 μL in the presence of serial 2-fold dilutions of plasma (from 1/20 to 1/2560), incubated for 1 hour, and added to target cells in 96-well plates in triplicate. Viral cytopathic effects were recorded 3 days postinfection. Neutralizing activity is reported as the highest reciprocal plasma dilution able to prevent cytopathic effects in all 3 wells. Samples exhibiting partial or no neutralization at 1/20 dilution were coded as below the limit of quantification (BLOQ) in this assay.

Statistical Analysis

Comparisons of binary variables were performed using Fisher exact test. Comparisons of continuous variables were performed using the Mann-Whitney U test (for unpaired data) or Wilcoxon test (for paired data). Multiple linear regression was used to investigate the relationship between sociodemographic, health, and vaccine-related variables, and humoral outcomes. Variables included age (per year), sex at birth (female as reference group), ethnicity (non-white as reference), number of chronic health conditions (per additional), mRNA vaccine received (Comirnaty as reference), interval between doses (per day), sampling date following the most recent dose (per day), and convalescent status (COVID-19 naive as reference). Binding antibody and viral neutralization-related dependent variables were log-transformed prior to model input. Independent variables were examined for multi collinearity by calculating the bivariate correlation between all pairs of variables. Here, coefficients less than −0.75 or greater than 0.75 would have been considered highly collinear; however, no variables in any model met this threshold. No interaction terms were considered. Serum antibody half-lives were calculated by fitting exponential curves to antibody concentrations at 1, 3, and 6 months after the second dose. All tests were 2-tailed, with $P < .05$ considered statistically significant. Analyses were conducted using Microsoft Excel and Prism version 9.2.0 (GraphPad).

RESULTS

Participant Characteristics

HCW, older adults, and COVID-19 convalescent individuals were a median of 41, 79, and 48 years old, respectively, and predominantly female (Table 1). Older adults were predominantly (77%) of white ethnicity (compared to 46% of HCW). Older adults also had a higher number of chronic conditions (a median of 1, interquartile range [IQR] 0–2, range 0–5 in this group vs a median of 1, IQR 0–0, range 0–3 in HCW). All participants received 2 COVID-19 mRNA vaccine doses between December 2020 and July 2021, where the dose interval was up to 112 days as per national guidelines to delay second doses due to initially limited vaccine supply. Overall, more than 90% of first and second doses were Comirnaty. At the time of writing, 114 participants had received a third dose between October and December 2021, on average 7 months following their second dose. For Spikevax third doses (53% overall), those aged ≥70 years received a full dose, whereas those <70 years received a half-dose, as per national guidelines.

An additional 6 (7.4%) HCW and 2 (3.6%) older adults developed anti-N antibodies during follow-up (Table 1). Three of these postvaccination infections, all in HCW, occurred between December 2021 and Jan 2022 and were likely omicron BA.1 [25]. In analyses that span the whole study, these participants are retained in their original "COVID-19–naive at study entry" group, but identified in the figures at their first postinfection visit. In analyses of third-dose responses, they are classified as "prior COVID-19."

After 2-Dose Vaccination, Lower Binding Antibodies Are Associated With Older Age and Chronic Conditions But Older Adults Mount Strong Third-Dose Responses

We measured total anti-RBD binding antibody concentrations in serum before and after immunization (Figure 1A). As reported previously [12], antibody concentrations in older adults were significantly lower than those in HCW 1 month after the first dose (a median of 2.00 [IQR, 1.75–2.25] $\log_{10}$ U/mL in HCW vs a median of 1.50 [IQR, 1.05–1.99] in older adults), as well as 1 month after the second dose (a median of 4.02 [IQR, 3.88–4.25] in HCW vs a median of 3.74 [IQR, 3.49–3.91] in older adults) (Mann-Whitney; both $P < .0001$). Three months after the second dose, antibody concentrations had declined by approximately 0.4 $\log_{10}$ on average, to a median of 3.63 (IQR, 3.44–3.83) in HCW versus a median 3.32 (IQR, 3.04–3.56) in older adults (Mann-Whitney $P < .0001$ for comparison between groups). Six months after the second dose, antibody concentrations had declined by a further approximately 0.3 $\log_{10}$ on average, to a median of 3.30 (IQR, 3.09–3.47) in HCW versus a median 2.96 (IQR, 2.68–3.20) in older adults ($P < .0001$). Thus, following 2-dose COVID-19 mRNA vaccination, antibody concentrations remained consistently and significantly lower in older compared to younger adults.
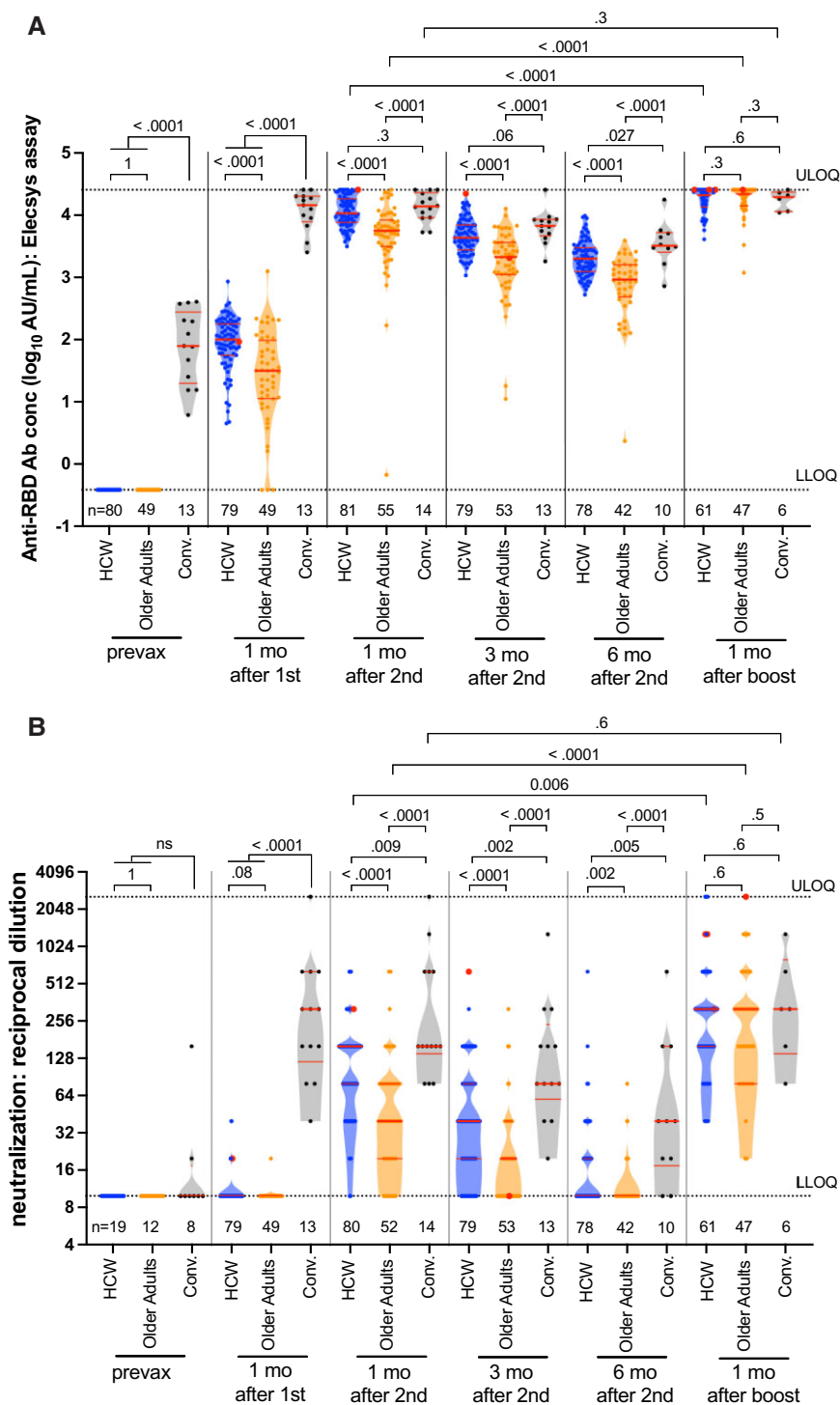
Figure 1. Longitudinal antibody binding and neutralization responses to spike RBD following 1, 2, and 3 COVID-19 vaccine doses. *A*, Binding antibody responses to the SARS-CoV-2 spike RBD in serum, in HCW (blue circles) and older adults (orange circles) who were COVID-19 naive at study entry, as well as COVID-19 convalescent individuals (black circles) at 6 time points: prior to vaccination (pre-vax); 1 month following the first dose; 1, 3, and 6 months following the second dose; and 1 month following the third vaccine dose. Individuals with postvaccination infections are indicated by red dots at their first N-seropositive time point. Participant numbers are provided at the bottom of the plot. A thick horizontal red bar represents the median; thinner horizontal red bars represent the interquartile range. *P* values were computed using the Mann-Whitney *U* test (for comparisons between groups) or the Wilcoxon matched pairs test (for comparisons across time points within a group) and are uncorrected for multiple comparisons. *B*, As in (*A*) but for virus neutralization activity, defined as the lowest reciprocal plasma dilution at which neutralization was observed in all wells of a triplicate assay. Plasma samples showing neutralization in fewer than 3 wells at a 1/20 dilution were coded as LLOQ. The highest dilution tested was 1/2560, which corresponds to the ULOQ. Note that only a subset of prevaccine plasma samples was assayed for this activity. Abbreviations: conc, concentration; Conv, convalescent; COVID-19, coronavirus disease 2019; HCW, health care worker; LLOQ, lower limit of quantification; N, nucleocapsid; prevax, prevaccination; RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; ULOQ, upper limit of quantification.

By contrast, antibody concentrations in COVID-19 convalescent individuals remained consistently higher than in COVID-19 naive individuals after 2 doses. Six months after the second dose, for example, convalescent individuals showed median responses of 3.50 (IQR, 3.40–3.71) $\log_{10}$ U/mL (compared to a median of 3.30 [IQR, 3.09–3.47], $P = .027$ in HCW; and a median of 2.96 [IQR, 2.68–3.20], $P < .0001$ in older adults).

Multivariable analyses of antibody concentrations after 2 doses, that adjusted for sex, ethnicity, number of chronic health conditions, first-dose vaccine brand, dosing interval, and specimen collection date, confirmed that older age remained independently associated with lower antibody concentrations at 1 and 3 months after the second dose (Supplementary Table 1). One month after the second dose, for example, each decade of older age was associated with an approximately 0.06 $\log_{10}$ lower antibody concentration ($P = .0067$). A higher number of chronic conditions was also independently associated with lower antibody concentrations at both these time points. Six months after the second dose, a higher number of chronic conditions remained the strongest independent correlate of lower responses, with each condition associated with a 0.14 $\log_{10}$ lower antibody concentration ($P = .0001$). A longer dose interval was associated with higher antibody concentrations at all time points after the second dose (all $P < .05$), consistent with previous reports [26–28]. COVID-19 convalescent status was also associated with maintaining 0.26 $\log_{10}$ higher antibody concentrations at 3 and 6 months after the second dose (both $P < .05$), consistent with superior durability of hybrid (combined infection and vaccine-induced) immunity [29–31].

In both HCW and older adults, the third dose boosted antibody concentrations at least approximately 0.3–0.4 $\log_{10}$ higher than peak values observed after 2 doses (Wilcoxon paired test $P < .0001$ for both groups). Binding antibodies in HCW rose to a median of 4.31 (IQR, 4.13 to ULOQ) while those in older adults rose to a median of 4.33 (IQR, 4.14 to ULOQ) ($P = .33$), indicating that older and younger adults mounted comparable initial binding antibody responses to third doses. In multivariable analyses of third-dose responses, a higher number of chronic conditions was the sole significant correlate of lower antibody concentrations ($P = .0078$), while having received a Spikevax third dose was associated with higher antibody concentrations ($P = .0091$) (Supplementary Table 2).

After 2-Dose Vaccination, Weaker Virus Neutralizing Activity Is Associated With Age and Chronic Conditions But Older Adults Mount Strong Third-Dose Responses

We next quantified the ability of plasma to prevent target cell infection by the ancestral (USA-WA1/2020) SARS-CoV-2 strain in a live virus neutralization assay (Figure 1B). Neutralizing activity is reported as the highest reciprocal plasma dilution capable of preventing viral cytopathic effects in all triplicate assay wells. As previously reported [12], 1 vaccine dose largely failed to induce neutralization in COVID-19 naive individuals, although 2 doses induced this activity in most participants, albeit at consistently lower levels in older compared to younger adults. One month after the second dose, for example, the median reciprocal dilution to achieve neutralization was 160 (IQR, 80–160) in HCW versus 40 (IQR, 20–80) in older adults ($P < .0001$). Three months after the second dose, neutralizing activity had declined by more than 2-fold on average, to a median reciprocal dilution of 40 (IQR, 20–80) in HCW versus a median of 20 (IQR, BLOQ to 40) in older adults ($P < .0001$). Six months after the second dose, neutralizing activity had declined to BLOQ in 58% of HCW and 83% of older adults (Mann-Whitney $P = .0048$ for comparison between groups). COVID-19 convalescent individuals, by contrast, maintained significantly higher neutralizing activity compared to naive individuals at all time points following 2-dose vaccination. Multivariable analyses confirmed that older age remained significantly associated with weaker neutralizing activity at 1 and 3 months after 2-dose vaccination, while COVID-19 convalescent status was associated with superior neutralization at all time points following 2-dose vaccination (all $P \leq .0002$; Supplementary Table 1).

A third vaccine dose boosted neutralizing activity in both HCW and older adults to levels that were 2- and 8-fold higher than peak post–second-dose values, respectively (Wilcoxon paired test $P \leq .006$ for both groups; Figure 1B). Specifically, the median reciprocal dilution in HCW and older adults rose to 320 (IQR, 160–320) and 320 (IQR, 80–320), respectively ($P = .6$), indicating that older adults mounted comparable initial neutralizing responses to younger adults after the third dose. A multivariable analysis identified prior COVID-19 as the strongest independent predictor of higher neutralization after the third dose ($P = .0044$; Supplementary Table 2).

Chronic Conditions Are Associated With Faster Binding Antibody Decline After 2 Vaccine Doses

We next assessed antibody decline after 2-dose vaccination (Figure 2A). Assuming exponential decay and restricting the analysis to participants with a complete longitudinal data series with no values above the ULOQ, we estimated antibody concentration half-lives to be a median of 59 (IQR, 52–75) days in HCW versus a median of 52 (IQR, 45–65) days in older adults ($P = .016$; Figure 2B). This suggests that, in addition to overall weaker responses to 2-dose vaccination compared to younger adults, antibody concentrations in older adults also decline more rapidly. In multivariable analyses, however, a higher number of chronic conditions emerged as the sole independent correlate of antibody decline, with each additional condition associated with a 5-day shorter half-life ($P = .017$; Table 2). COVID-19 convalescent status was associated with a 14-day longer antibody half-life after adjustment for other factors ($P = .056$), consistent with more durable hybrid immunity [29–31].
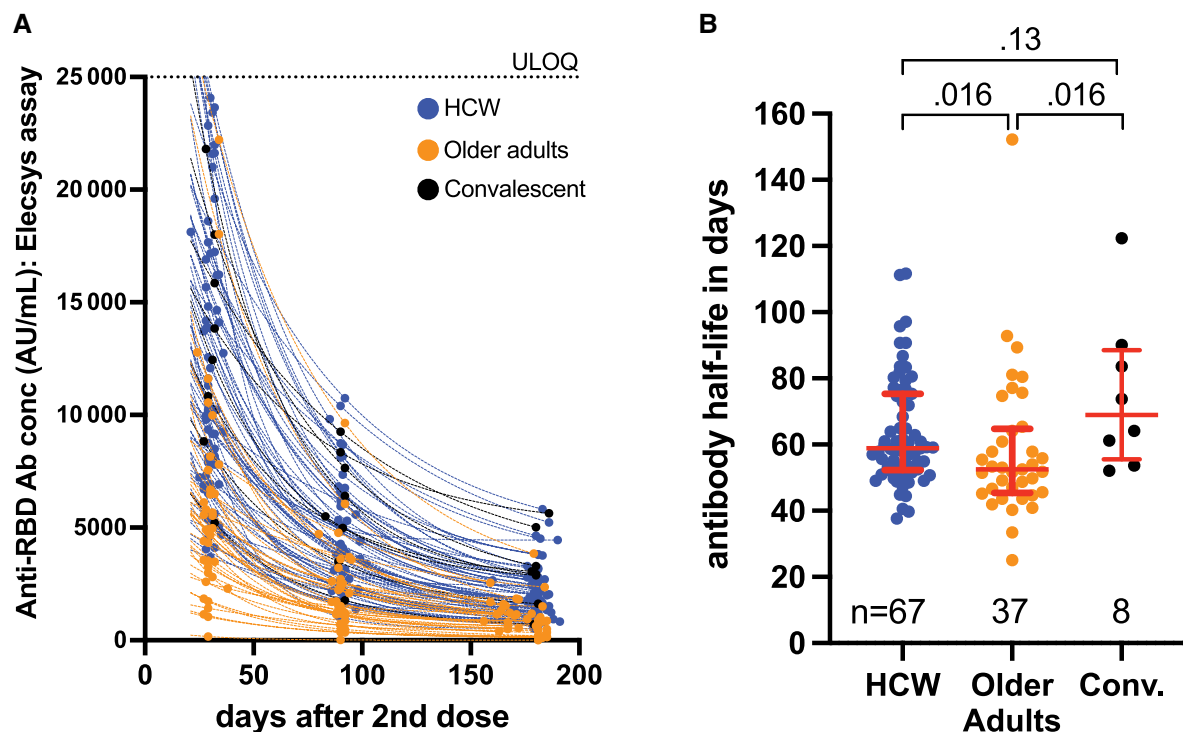
Figure 2. Decay rates of serum binding antibody responses to spike RBD following 2 COVID-19 vaccine doses. *A*, Temporal declines in serum binding antibody responses to spike RBD following 2 vaccine doses in HCW (blue) and older adults (orange) who were COVID-19 naive at study entry, as well as COVID-19 convalescent participants (black). Only participants with a complete longitudinal data series with no values above the ULOQ are shown. *B*, Binding antibody half-lives following 2 COVID-19 vaccine doses, calculated by fitting an exponential curve to each participant's data shown in (*A*). Participant numbers are indicated at the bottom of the plot. Red bars and whiskers represent the median and interquartile range. *P* values were computed using the Mann-Whitney *U* test and are uncorrected for multiple comparisons. Abbreviations: Ab, antibody; conc, concentration; Conv, convalescent; COVID-19, coronavirus disease 2019; CW, health care worker; RBD, receptor-binding domain; ULOQ, upper limit of quantification.

Omicron-Specific Humoral Responses

Given the rise of omicron, we compared peak antibody responses against this strain in plasma at 1 month after the second and third vaccine doses. Here, all participants with prior

Table 2. Multivariable Analysis of the Relationship Between Sociodemographic, Health, and Vaccine-Related Variables and Serum Antibody Half-Life Following 2-Dose COVID-19 mRNA Vaccination

Outcome Measure	Variable	Estimate	95% CI	P Value
Antibody half-life after 2 vaccine doses	Age, per y	.058	−.17 to .29	.61
	Male sex	5.31	−2.57 to 13.18	.18
	White ethnicity	3.11	−4.67 to 10.88	.43
	No. chronic conditions, per additional	−4.62	−8.39 to −.85	.017
	Spikevax as first dose	3.37	−13.26 to 20.00	.69
	Dose interval, per d	.00014	−.16 to .16	.99
	COVID-19 convalescent[a]	13.78	−.37 to 27.93	.056

Abbreviations: CI, confidence interval; COVID-19, coronavirus disease 2019.

[a]Participants with positive anti-N serology at study entry.

COVID-19, regardless of infection timing, were included in the convalescent category. Overall, omicron-specific anti-RBD IgG binding antibodies, measured using the Meso Scale Diagnostics V-Plex assay, were on average 0.4 to 0.5 $\log_{10}$ U/mL lower than those against the wild type (WT; ancestral Wuhan strain) RBD after 2 and 3 doses (all within-group comparisons $P \leq .0002$; Figure 3A). Nevertheless, the third dose universally boosted omicron-specific anti-RBD IgG concentrations to an average of 0.5 $\log_{10}$ higher than levels induced by 2 doses (all within-group comparisons $P < .05$). Consistent with total anti-RBD binding antibody concentrations quantified using the Roche assay (Figure 1A), anti-RBD IgG concentrations against WT were significantly higher in HCW compared to older adults after 2 doses ($P < .0001$) but reached equivalence after 3 doses ($P = .4$) (Figure 3A). The latter result further confirms that initial third-dose binding antibody responses were comparable in older and younger adults (as higher dilutions used in the Meso Scale assay allow quantification over a larger dynamic range than the Roche assay).

Omicron-specific anti-RBD IgG concentrations followed a similar pattern, with HCW showing marginally higher levels compared to older adults after 2 doses ($P = .09$), but equivalent levels after 3 doses ($P = .49$). A multivariable analysis of
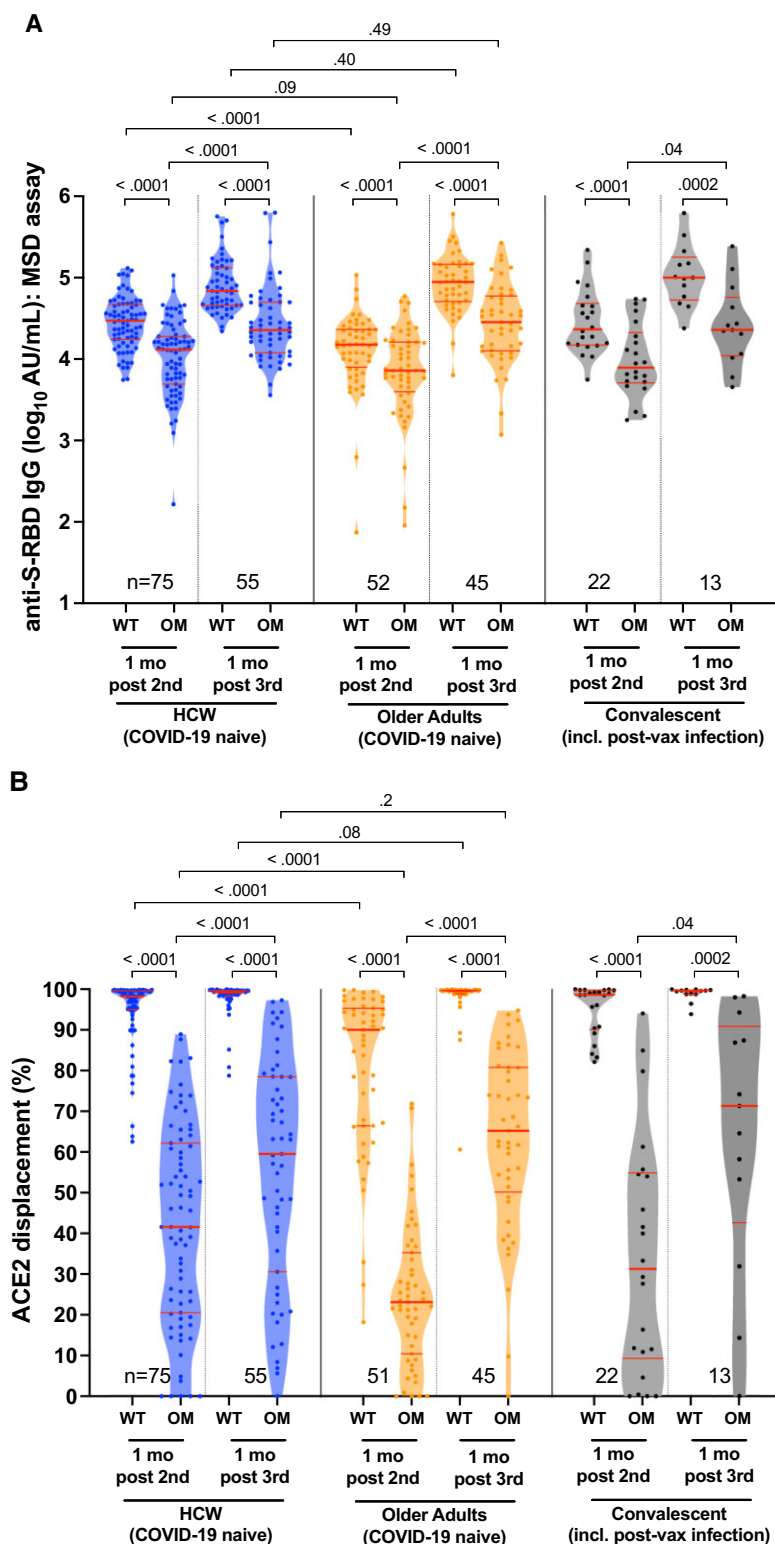
Figure 3. Anti-omicron IgG binding and ACE2 displacement activities 1 month after the second and third COVID-19 vaccine doses. *A*, Binding IgG responses in plasma to the WT (ancestral Wuhan strain) and omicron S-RBD, measured using the MSD V-Plex assay, in HCW (blue circles) and older adults (orange circles) who remained COVID-19 naive throughout the study, as well as individuals with prior COVID-19 regardless of infection timing (COVID-19 convalescent; black circles) at 1 month after the second and third COVID-19 vaccine doses. Participant numbers are shown at the bottom of the plot. A thick horizontal red bar represents the median; thinner horizontal red bars represent the interquartile range. *P* values were computed using the Wilcoxon matched pairs test (for all within-group comparisons) or the Mann-Whitney *U* test (for between-group comparisons) and are uncorrected for multiple comparisons. *B*, As in (*A*) but for ACE2 displacement activity, measured using the V-plex SARS-CoV-2 (ACE2) assay, where results are reported in terms of % ACE2 displacement. Abbreviations: COVID-19, coronavirus disease 2019; HCW, health care worker; SD, Meso Scale Diagnostics; OM, omicron; postvax, postvaccination; S-RBD, spike receptor-binding domain; WT, wild type.

Table 3. Multivariable Analyses of the Relationship Between Sociodemographic, Health, and Vaccine-Related Variables and Omicron-Specific Humoral Immunogenicity Measures Following 3-Dose COVID-19 mRNA Vaccination

Humoral Measure	Variable	1 mo After 3rd Dose		
		Estimate	95% CI	P Value
Anti-omicron RBD IgG, log10[a]	Age, per y	.0035	−.0027 to .0097	.26
	Male sex	−.14	−.34 to .054	.15
	White ethnicity	−.018	−.21 to .17	.85
	No. chronic conditions, per additional	−.12	−.20 to −.041	.0033
	Spikevax as third dose, vs Comirnaty	.15	−.039 to .34	.12
	Interval between 1st and 2nd dose, per d	−.0066	−.012 to −.0011	.020
	Interval between 2nd and 3rd dose, per d	.00043	−.0034 to .0042	.83
	Days since 3rd vaccine dose	−.0086	−.038 to .021	.56
	Prior COVID-19[b]	.1	−.16 to .37	.43
Anti-omicron ACE2 % displacement[a]	Age, per y	.29	−.046 to .63	.090
	Male sex	−12.38	−23.16 to −1.60	.025
	White ethnicity	−2.36	−12.74 to 8.01	.65
	No. chronic conditions, per additional	−6.41	−10.80 to −2.03	.0046
	Spikevax as third dose, vs Comirnaty	1.69	−8.58 to 11.97	.74
	Interval between 1st and 2nd dose, per d	−.41	−.71 to −.11	.0079
	Interval between 2nd and 3rd dose, per d	−.038	−.25 to .17	.72
	Days since 3rd vaccine dose	−1.82	−3.43 to −.21	.027
	Prior COVID-19[b]	12.28	−2.11 to 26.67	.094

Abbreviations: CI, confidence interval; COVID-19, coronavirus disease 2019; RBD, receptor-binding domain.

[a]Measured using the Meso Scale Diagnostics V-plex assay system.

[b]Includes all participants with positive anti-nucleocapsid serology at any time during the study (ie, both pre- and postvaccine COVID-19 cases).

omicron-specific anti-RBD IgG concentrations after the third dose identified a higher number of chronic conditions as the strongest correlate of poorer responses, with each additional condition associated with a 0.12 $\log_{10}$ lower response ($P = .0033$; Table 3). A longer interval between the first and second vaccine doses was marginally associated with a lower third dose response ($P = .02$).

We also assessed the ability of plasma to block the interaction between WT and omicron RBD and the cellular ACE2 receptor using a competition assay. This activity was significantly weaker against omicron compared to WT after both 2 and 3 doses in all groups (all within-group comparisons $P \leq .0002$; Figure 3B), although the discrepancy was most pronounced for older adults after 2 doses (where median anti-WT activity was 90% compared to 23% against omicron). The third dose universally boosted ACE2 competition activity against omicron (all within-group comparisons $P < .05$), with, for example, median activity in older adults rising to 66% (from 23%). Consistent with results for anti-RBD IgG antibodies, plasma ability to block the WT-RBD/ACE2 interaction was significantly higher in HCW compared to older adults after 2 doses ($P < .0001$), but reached equivalence after 3 doses (in fact, activities in older adults were slightly higher at this time point; $P = .08$). Ability to block the omicron-RBD/ACE2 interaction followed a similar pattern, with HCW exhibiting significantly higher activity compared to older adults after 2 doses ($P < .0001$), but equivalent levels after 3 doses ($P = .2$). In multivariable analyses, a

higher number of chronic conditions was the strongest correlate of poorer omicron-specific ACE2 competition activity after 3 vaccine doses, with each additional condition associated with an approximately 6% reduction in this activity ($P = .0046$; Table 3). Male sex, a longer interval between the first and second doses, and days elapsed since the third dose also correlated with weaker post–third-dose responses (all $P < .05$).

Finally, we compared plasma neutralization of WT and omicron strains using a live virus assay in a subset of 20 HCW and 21 older adults who remained COVID-19 negative throughout the study (Figure 4). Neutralizing activity against omicron was significantly weaker than that against WT following 2 and 3 doses in both groups (all $P < .0001$). The third dose nevertheless boosted omicron-specific neutralization in both groups, where the increase in older adults was particularly pronounced (from a median of BLOQ after the second dose to a median reciprocal dilution of 40 after the third; $P < .0001$). Consistent with anti-RBD IgG and ACE2 competition results, omicron-specific neutralization was significantly lower in older adults compared to HCW after 2 vaccine doses ($P = .0003$) but reached equivalence after 3 doses ($P = .79$).

DISCUSSION

At 1, 3, and 6 months following 2-dose COVID-19 mRNA vaccination, antibody binding and neutralizing activity were significantly weaker in older compared to younger adults.
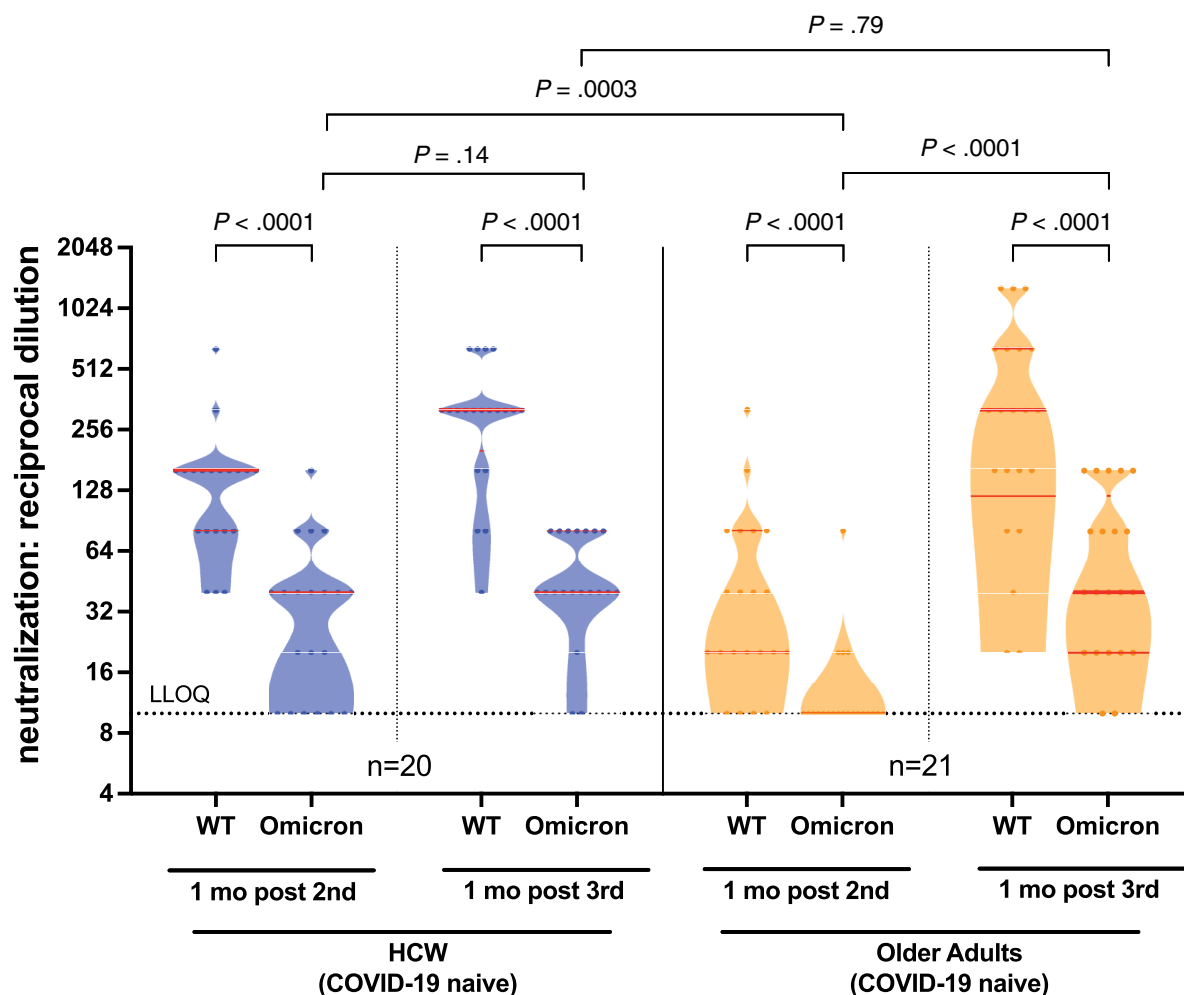
Figure 4. Anti-omicron neutralization activities 1 month after the second and third COVID-19 vaccine doses. Neutralization activities, reported as the highest reciprocal plasma dilution at which neutralization was observed in all wells of a triplicate assay, against the WT (ancestral WA1/2020 strain) and omicron virus isolates, in a subset of HCW (blue circles) and older adults (orange circles) who remained COVID-19 naive throughout the study. Participant numbers are shown at the bottom of the plot. A thick horizontal red bar represents the median; thinner horizontal red bars represent the interquartile range. *P* values were computed using the Wilcoxon matched pairs test (for within-group comparisons) or the Mann-Whitney *U* test (for between-group comparisons) and are uncorrected for multiple comparisons. Abbreviations: COVID-19, coronavirus disease 2019; HCW, health care worker; LLOQ, lower limit of quantification; WT, wild type.

Antibody concentrations were also less durable in older adults, although responses declined substantially in all groups over time (eg, by 6 months after the second dose, neutralization had declined to BLOQ in almost 60% of HCW and >80% of older adults). In multivariable analyses, a higher number of chronic conditions remained consistently and independently associated with weaker and less durable binding antibody responses, while a longer interval between first and second doses was consistently associated with higher binding antibody responses after the second dose, as previously reported [26–28]. These findings support public health decisions to provide third doses on or before the 6-month mark, with older adults receiving priority.

One month after a third COVID-19 vaccine dose, antibody binding and neutralization reached levels that were significantly higher than peak levels after the second dose, where the magnitude of boosting in older adults was particularly prominent. Indeed, 1 month after the third dose, antibody binding, ACE2 competition activity, and live virus neutralization in older adults reached equivalence to younger adults. Consistent with recent evidence [20, 22, 32–38], omicron-specific antibody responses were universally weaker than those specific to the ancestral strain after both 2 and 3 vaccine doses; nevertheless, anti-omicron responses in older adults also reached equivalence to those observed in younger adults 1 month after the third dose. Notably, the number of chronic conditions persisted as an independent correlate of weaker omicron-specific responses, even after 3 doses. Given ongoing transmission of omicron variants, these results clearly underscore the benefits of a third dose, and support public health decisions to provide them to adults of all ages [39].

Like others [29–31], our findings indicate that individuals who have contracted COVID-19 still benefit from vaccination. Compared to naive participants, convalescent individuals displayed slower antibody decline, and multivariable analyses demonstrated that binding and neutralization activities were higher in this group at 6 months after the second dose.

Our study has several limitations. As the precise immune correlates of protection for SARS-CoV-2 transmission and disease severity remain incompletely characterized [40], particularly in light of omicron, we cannot directly interpret our results in terms of individual-level protection. Nevertheless, stronger responses are likely to confer increased protection, and the average $>0.3–0.4 \log_{10}$ increase in binding antibodies and 2- to 8-fold average boost in neutralization conferred by third doses again underscores the benefit of this dose. We did not investigate T-cell responses, which may play critical roles in protection against severe COVID-19, particularly in the context of variants [41–48]. Our study was not powered to investigate differences between the 2 mRNA vaccines [49, 50], nor in full versus half-doses of Spikevax when administered as third doses. Post–third-dose response durability assessments are also needed.

In conclusion, while the observation of strong initial binding and neutralizing antibody responses to third COVID-19 vaccine doses in older adults, including to omicron, are encouraging, it will be important to closely monitor third-dose response durability in this population.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

References

1. Fisman DN, Bogoch I, Lapointe-Shaw L, McCready J, Tuite AR. Risk factors associated with mortality among residents with coronavirus disease 2019 (COVID-19) in long-term care facilities in Ontario, Canada. JAMA Netw Open **2020**; 3:e2015957.
2. Chinnadurai R, Ogedengbe O, Agarwal P, et al. Older age and frailty are the chief predictors of mortality in COVID-19 patients admitted to an acute medical unit in a secondary care setting—a cohort study. BMC Geriatr **2020**; 20:409.
3. Banerjee A, Pasea L, Harris S, et al. Estimating excess 1-year mortality associated with the COVID-19 pandemic according to underlying conditions and age: a population-based cohort study. Lancet **2020**; 395:1715–25.
4. Baden LR, El Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med **2021**; 384:403–16.
5. Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA COVID-19 vaccine. N Engl J Med **2020**; 383:2603–15.

6. Dagan N, Barda N, Kepten E, et al. BNT162b2 mRNA COVID-19 vaccine in a nationwide mass vaccination setting. N Engl J Med **2021**; 384:1412–23.

7. Grupper A, Rabinowich L, Schwartz D, et al. Reduced humoral response to mRNA SARS-CoV-2 BNT162b2 vaccine in kidney transplant recipients without prior exposure to the virus. Am J Transplant **2021**; 21:2719–26.

8. Massarweh A, Eliakim-Raz N, Stemmer A, et al. Evaluation of seropositivity following BNT162b2 messenger RNA vaccination for SARS-CoV-2 in patients undergoing treatment for cancer. JAMA Oncol **2021**; 7:1133–40.

9. Monin L, Laing AG, Munoz-Ruiz M, et al. Safety and immunogenicity of one versus two doses of the COVID-19 vaccine BNT162b2 for patients with cancer: interim analysis of a prospective observational study. Lancet Oncol **2021**; 22:765–78.

10. Collier DA, Ferreira I, Kotagiri P, et al. Age-related immune response heterogeneity to SARS-CoV-2 vaccine BNT162b2. Nature **2021**; 596:417–22.

11. Muller L, Andree M, Moskorz W, et al. Age-dependent immune response to the Biontech/Pfizer BNT162b2 coronavirus disease 2019 vaccination. Clin Infect Dis **2021**; 73: 2065–72.

12. Brockman MA, Mwimanzi F, Lapointe HR, et al. Reduced magnitude and durability of humoral immune responses to COVID-19 mRNA vaccines among older adults. J Infect Dis **2022**; 225:1129–40.

13. Barda N, Dagan N, Cohen C, et al. Effectiveness of a third dose of the BNT162b2 mRNA COVID-19 vaccine for preventing severe outcomes in Israel: an observational study. Lancet **2021**; 398:2093–100.

14. Bar-On YM, Goldberg Y, Mandel M, et al. Protection of BNT162b2 vaccine booster against Covid-19 in Israel. N Engl J Med **2021**; 385:1393–400.

15. Falsey AR, Frenck RW Jr, Walsh EE, et al. SARS-CoV-2 neutralization with BNT162b2 vaccine dose 3. N Engl J Med **2021**; 385:1627–9.

16. Gilboa M, Mandelboim M, Indenbaum V, et al. Early immunogenicity and safety of the third dose of BNT162b2 messenger RNA coronavirus disease 2019 vaccine among adults older than 60 years: real-world experience. J Infect Dis **2022**; 225:785–92.

17. Mizrahi B, Lotan R, Kalkstein N, et al. Correlation of SARS-CoV-2-breakthrough infections to time-from-vaccine. Nat Commun **2021**; 12:6379.

18. Goldberg Y, Mandel M, Bar-On YM, et al. Waning immunity after the BNT162b2 vaccine in Israel. N Engl J Med **2021**; 385:e85.

19. Levin EG, Lustig Y, Cohen C, et al. Waning immune humoral response to BNT162b2 COVID-19 vaccine over 6 months. N Engl J Med **2021**; 385:e84.

20. Collie S, Champion J, Moultrie H, Bekker LG, Gray G. Effectiveness of BNT162b2 vaccine against omicron variant in South Africa. N Engl J Med **2022**; 386:494–6.

21. Song JS, Lee J, Kim M, et al. Serial intervals and household transmission of SARS-CoV-2 omicron variant, South Korea, 2021. Emerg Infect Dis **2022**; 28:756–9.

22. Cele S, Jackson L, Khoury DS, et al. Omicron extensively but incompletely escapes Pfizer BNT162b2 neutralization. Nature **2022**; 602:654–6.

23. Bentley E, Mee ET, Routley S, et al. World Health Organization. Expert Committee on Biological Standardization. Collaborative study for the establishment of a WHO international standard for SARS-CoV-2 RNA. 2020. WHO/BS/2020.2402. Geneva, Switzerland: WHO.

24. Tan CW, Chia WN, Qin X, et al. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein-protein interaction. Nat Biotechnol **2020**; 38:1073–8.

25. British Columbia Centre for Disease Control. BC Covid-19 data. Variants. http://www.bccdc.ca/health-info/diseases-conditions/covid-19/data#variants. Accessed 28 April 2022.

26. Amirthalingam G, Bernal JL, Andrews NJ, et al. Serological responses and vaccine effectiveness for extended COVID-19 vaccine schedules in England. Nat Commun **2021**; 12:7217.

27. Parry H, Bruton R, Stephens C, et al. Differential immunogenicity of BNT162b2 or ChAdOx1 vaccines after extended-interval homologous dual vaccination in older people. Immun Ageing **2021**; 18:34.

28. Payne RP, Longet S, Austin JA, et al. Immunogenicity of standard and extended dosing intervals of BNT162b2 mRNA vaccine. Cell **2021**; 184:5699–714.e11.

29. Cho A, Muecksch F, Schaefer-Babajew D, et al. Anti-SARS-CoV-2 receptor-binding domain antibody evolution after mRNA vaccination. Nature **2021**; 600: 517–22.

30. Stamatatos L, Czartoski J, Wan YH, et al. mRNA vaccination boosts cross-variant neutralizing antibodies elicited by SARS-CoV-2 infection. Science **2021**; 372:1413–8.

31. Wang Z, Muecksch F, Schaefer-Babajew D, et al. Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. Nature **2021**; 595:426–31.

32. Schmidt F, Muecksch F, Weisblum Y, et al. Plasma neutralization of the SARS-CoV-2 omicron variant. N Engl J Med **2022**; 386:599–601.

33. Garcia-Beltran WF, St Denis KJ, Hoelzemer A, et al. mRNA-based COVID-19 vaccine boosters induce neutralizing immunity against SARS-CoV-2 omicron variant. Cell **2022**; 185:457–66.e4.

34. Muik A, Lui BG, Wallisch AK, et al. Neutralization of SARS-CoV-2 omicron by BNT162b2 mRNA vaccine-elicited human sera. Science 2022; 375:678–80.

35. Debes AK, Xiao S, Egbert ER, et al. Comparison of total and neutralizing SARS-CoV-2 spike antibodies against omicron and other variants in paired samples after two or three doses of mRNA vaccine. medRxiv, doi: 10.1101/2022.01.26.22269819, 31 January 2022, preprint: not peer reviewed.

36. Ai J, Zhang H, Zhang Y, et al. Omicron variant showed lower neutralizing sensitivity than other SARS-CoV-2 variants to immune sera elicited by vaccines after boost. Emerg Microbes Infect 2022; 11:337–43.

37. Muecksch F, Wang Z, Cho A, et al. Increased memory B cell potency and breadth after a SARS-CoV-2 mRNA boost. Nature. 2022. doi:10.1038/s41586-022-04778-y. Online ahead of print 21 April 2022.

38. Pajon R, Doria-Rose NA, Shen X, et al. SARS-CoV-2 omicron variant neutralization after mRNA-1273 booster vaccination. N Engl J Med 2022; 386:1088–91.

39. National Advisory Committee on Immunization (NACI). An Advisory Committee Statement (ACS) National Advisory Committee on Immunization (NACI): guidance on booster COVID-19 vaccine doses in Canada—update December 3, 2021. Canada: Public Health Agency of Canada, 2021.

40. Feng S, Phillips DJ, White T, et al. Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. Nat Med 2021; 27:2032–40.

41. Peng Y, Felce SL, Dong D, et al. An immunodominant NP105-113-B*07:02 cytotoxic T cell response controls viral replication and is associated with less severe COVID-19 disease. Nat Immunol 2022; 23:50–61.

42. Swadling L, Diniz MO, Schmidt NM, et al. Pre-existing polymerase-specific T cells expand in abortive seronegative SARS-CoV-2. Nature 2022; 601:110–7.

43. Grifoni A, Sidney J, Vita R, et al. SARS-CoV-2 human T cell epitopes: adaptive immune response against COVID-19. Cell Host Microbe 2021; 29:1076–92.

44. Dan JM, Mateus J, Kato Y, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science 2021; 371:eabf4063.

45. Riou C, Keeton R, Moyo-Gwete T, et al. Escape from recognition of SARS-CoV-2 variant spike epitopes but overall preservation of T cell immunity. Sci Transl Med 2022; 14:eabj6824.

46. Redd AD, Nardin A, Kared H, et al. Minimal crossover between mutations associated with omicron variant of SARS-CoV-2 and CD8+ T-cell epitopes identified in COVID-19 convalescent individuals. mBio 2022; 13:e0361721.

47. Keeton R, Tincho MB, Ngomti A, et al. T cell responses to SARS-CoV-2 spike cross-recognize omicron. Nature 2022; 603:488–92.

48. Liu J, Chandrashekar A, Sellers D, et al. Vaccines elicit highly conserved cellular immunity to SARS-CoV-2 omicron. Nature 2022; 603:493–6.

49. 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; 326:1533–5.

50. Nanduri S, Pilishvili T, Derado G, et al. Effectiveness of Pfizer-BioNTech and Moderna vaccines in preventing SARS-CoV-2 infection among nursing home residents before and during widespread circulation of the SARS-CoV-2 B.1.617.2 (delta) variant—National Healthcare Safety Network, March 1-August 1, 2021. MMWR Morb Mortal Wkly Rep 2021; 70:1163–6.