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MAJOR ARTICLE

Impact of Age and Severe Acute Respiratory Syndrome Coronavirus 2 Breakthrough Infection on Humoral Immune Responses After Three Doses of Coronavirus Disease 2019 mRNA Vaccine

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Background. Longer-term immune response data after 3 doses of coronavirus disease 2019 (COVID-19) mRNA vaccine remain limited, particularly among older adults and after Omicron breakthrough infection.

Methods. We quantified wild-type- and Omicron-specific serum immunoglobulin (Ig)G levels, angiotensin-converting enzyme 2 displacement activities, and live virus neutralization up to 6 months after third dose in 116 adults aged 24–98 years who remained COVID-19 naive or experienced their first severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection during this time.

Results. Among the 78 participants who remained COVID-19 naive throughout follow up, wild-type- and Omicron-BA.1-specific IgG concentrations were comparable between younger and older adults, although BA.1-specific responses were consistently significantly lower than wild-type-specific responses in both groups. Wild-type- and BA.1-specific IgG concentrations declined at similar rates in COVID-19-naive younger and older adults, with median half-lives ranging from 69 to 78 days. Antiviral antibody functions declined substantially over time in COVID-19-naive individuals, particularly in older adults: by 6 months, BA.1-specific neutralization was undetectable in 96% of older adults, versus 56% of younger adults. Severe acute respiratory syndrome coronavirus 2 infection, experienced by 38 participants, boosted IgG levels and neutralization above those induced by vaccination alone. Nevertheless, BA.1-specific neutralization remained significantly lower than wild-type, with BA.5-specific neutralization lower still. Higher Omicron BA.1-specific neutralization 1 month after third dose was an independent correlate of lower SARS-CoV-2 infection risk.

Conclusions. Results underscore the immune benefits of the third COVID-19 mRNA vaccine dose in adults of all ages and identify vaccine-induced Omicron-specific neutralization as a correlate of protective immunity. Systemic antibody responses and functions however, particularly Omicron-specific neutralization, decline rapidly in COVID-19-naive individuals, particularly in older adults, supporting the need for additional booster doses.

Keywords. COVID-19; mRNA vaccine; older adults; Omicron; postvaccination infection.

Third coronavirus disease 2019 (COVID-19) mRNA vaccine doses have been provided to clinically vulnerable individuals to complete their initial vaccine series [1–3] and more broadly as "booster doses" to offset the natural decline of systemic antibodies [4, 5] and to augment responses against Omicron variants [6], which are more immune evasive [7–13]. Although third doses enhance protection against severe disease [1, 14, 15], they are not as effective at preventing Omicron infections [16–18]. Few studies have assessed the durability of immune responses post-third vaccine dose across the adult age spectrum or compared vaccine-induced to "hybrid" (combined vaccine and infection-induced [19]) responses elicited in the now substantial number of individuals who experienced their first severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection post-third vaccine dose.

We previously reported that older adults mounted weaker antibody responses than younger adults after 2 mRNA vaccine doses, but that their initial post-third-dose responses were

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equivalent [20]. In this study, we longitudinally examine wild-type- and Omicron (BA.1, BA.2, BA.3, and BA.5)-specific antibody concentrations and antiviral functions up to 6 months post-third dose in 116 individuals ranging from 24 to 98 years old, who remained COVID-19 naive until at least 1 month post-third dose. Although two thirds of the cohort remained COVID-19 naive throughout follow up, one third experienced their first SARS-CoV-2 infection during this time, presumably with Omicron BA.1 or BA.2, based on local molecular epidemiology trends [21]. This allowed us to additionally compare vaccine-induced and "hybrid" responses specific to wild-type and Omicron variants, across age groups.

METHODS

Participants

Our cohort, based in British Columbia (BC), Canada, has been described previously [20]. Here, we studied the 69 healthcare workers (HCWs) and 47 older adults (OAs) who remained COVID-19 naive until at least 1 month post-third COVID-19 vaccine dose (Table 1). Severe acute respiratory syndrome coronavirus 2 infections were identified through self-reported polymerase chain reaction or rapid antigen test results and/or the presence of anti-nucleocapsid antibodies using the Elecsys Anti-SARS-CoV-2 assay (Roche Diagnostics). No participants received monoclonal antibodies for SARS-CoV-2 treatment or prevention.

Patient Consent Statement

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.

Assays

We quantified immunoglobulin (Ig)G-binding antibodies in serum against the SARS-CoV-2 Spike receptor-binding domain (RBD) using the V-plex SARS-CoV-2 (IgG) enzyme-linked immunosorbent assay (ELISA) kit (Panel 22; Meso Scale Diagnostics), which features wild-type ([WT] Wuhan) and Omicron (BA.1) RBD antigens, on a Meso QuickPlex SQ120 instrument. Serum was diluted 1:10 000, with results reported in arbitrary units (AU)/mL. We assessed surrogate virus neutralization activity [22] in serum by competition ELISA (Panel 22; V-plex SARS-CoV-2 [angiotensin-converting enzyme 2 {ACE2}]) to measure blockade of the RBD-ACE2 receptor interaction. Sera were diluted 1:40 and results were reported as the percentage of ACE2 displacement. A subset of specimens was also tested for IgG-binding antibodies and ACE2 displacement using V-plex ELISA kits that featured Wuhan, BA.1, BA.2, and BA.3 Spike antigens (Panel 25; Meso Scale Diagnostics). Virus-neutralizing activity in plasma was examined using live WT (USA-WA1/2020; BEI Resources), Omicron BA.1 (GISAID EPI_ISL_9805779), and Omicron BA.5 (GISAID EPI_ISL_15226696) SARS-CoV-2 strains on VeroE6-TMPRSS2 (JCRB-1819) target cells [20]. Virus stocks were diluted to 50 median tissue culture infectious doses ($TCID_{50}$)/200 µL in the presence of serial 2-fold plasma dilutions (1/20 to 1/2560) and added to target cells in triplicate. Viral cytopathic effect (CPE) was recorded 3 days after infection. Neutralization was reported as the highest reciprocal dilution able to prevent CPE in all 3 wells. Partial or no neutralization at 1/20 dilution was considered below the limit of quantification (BLOQ).

Statistical Analyses

Continuous variables were compared using the Mann-Whitney *U* test (unpaired data) or Wilcoxon test (paired data). Correlations between continuous variables were explored using Spearman's correlation. Univariable and multivariable logistic regression was used to investigate the relationship between sociodemographic, health, vaccine, and immune response variables and SARS-CoV-2 infection risk after 3-dose vaccination. All tests were 2-tailed, with $P < .05$ considered statistically significant. *P* values are not corrected for multiple comparisons.

RESULTS

Participant Characteristics

Healthcare workers and older adults were a median of 40 and 78 years old, respectively (overall range, 24–98 years old), and predominantly female (Table 1). Older adults were predominantly of White ethnicity (74% compared with 46% of HCWs) and had a median of 1 chronic health condition (compared with a median of 0 in HCWs). No participants had an immunocompromising condition. Most participants (97% of HCWs and 81% of OAs) initially received 2 doses of BNT162b2; the remainder received 2 mRNA-1273 doses or a heterologous regimen. Third doses, administered an average of ~7 months after the second, were predominantly mRNA-1273 (where OAs were eligible for 100 µg, whereas younger adults received the standard 50 µg booster; all BNT162b2 doses were 30 µg). During follow up, 43% of HCWs and 17% of OAs experienced their first SARS-CoV-2 infection, the vast majority of which were likely Omicron BA.1 or BA.2, based on local molecular epidemiology trends [21].

Binding Antibody Responses

As reported previously [20, 23], anti-RBD serum IgG concentrations specific to both WT and Omicron BA.1 were significantly lower in COVID-19-naive OAs compared with younger HCWs after 2 vaccine doses, but these were substantially boosted, and reached equivalence, 1 month post-third dose (Figure 1*A*). At this time, for example, WT-specific

Table 1. Participant Characteristics

Characteristic	HCW (n = 69)	Older Adults (n = 47)
Sociodemographic and Health Variables	…	…
Age in years, median [IQR]	40 [34–51]	78 [72–83]
Female sex at birth, n (%)	53 (77%)	34 (72%)
White ethnicity, n (%)	32 (46%)	35 (74%)
Number of chronic health conditions, median [IQR]	0 [0–1]	1 [0–2]
Hypertension, n (%)	9 (13%)	19 (40%)
Diabetes, n (%)	0 (0%)	11 (23%)
Asthma, n (%)	6 (9%)	3 (6%)
Obesity, n (%)	10 (14%)	5 (11%)
Chronic lung disease, n (%)	0 (0%)	5 (11%)
Chronic liver disease, n (%)	0 (0%)	0 (0%)
Chronic kidney disease, n (%)	0 (0%)	5 (11%)
Chronic heart disease, n (%)	0 (0%)	10 (21%)
Chronic blood disease, n (%)	1 (1%)	1 (2%)
Cancer, n (%)	0 (0%)	6 (13%)
Immunosuppression, n (%)	0 (0%)	0 (0%)
At least one of the above, n (%)	17 (25%)	31 (66%)
Vaccine Details	…	…
Initial regimen	…	…
BNT162b2—BNT162b2	67 (97%)	38 (81%)
mRNA-1273—mRNA-1273	1 (1.5%)	7 (15%)
Heterologous mRNA	1 (1.5%)	2 (4%)
Third dose	…	…
BNT162b2	33 (48%)	18 (38%)
mRNA-1273	36 (52%)	29 (62%)
Date range that third doses were received	October 2021–February 2022	October 2021–January 2022
Time between second and third doses in days, median [IQR]	210 [199–232]	198 [173–216]
Specimen Collection	…	…
1 month after third dose, n (%)	68 (99%)	47 (100%)
3 months after third dose, n (%)	68 (99%)	45 (96%)
6 months after third dose, n (%)	57 (83%)	44 (94%)
Postvaccination SARS-CoV-2 infections, n (%)	30 (43%)	8 (17%)

Abbreviations: HCW, healthcare worker; IQR, interquartile range; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

anti-RBD IgG concentrations were a median of 5.21 (interquartile range [IQR], 5.08–5.45) $\log_{10}$ AU/mL in HCWs versus 5.25 (IQR, 4.99–5.43) $\log_{10}$ AU/mL in OAs ($P = .7$). Among the participants who remained COVID-19 naive throughout follow up, WT-specific IgG responses declined to a median 4.95 (IQR, 4.79–5.16) $\log_{10}$ AU/mL in HCWs versus 4.96 (IQR, 4.76–5.17) $\log_{10}$ AU/mL in OAs at 3 months post-third dose (between-group comparison $P = .9$). By 6 months post-third dose, WT-specific IgG responses had declined to a median of 4.64 (IQR, 4.42–4.84) $\log_{10}$ AU/mL in HCWs versus 4.59 (IQR, 4.35–4.87) $\log_{10}$ AU/mL (between-group comparison $P = .7$). Nevertheless, the magnitude of these declines meant that WT-specific IgG concentrations in COVID-19-naive HCWs were significantly below peak post-second dose levels by 6 months post-third dose ($P < .0001$), whereas in OAs they were comparable to post-second-dose responses ($P = .2$). Identical trends were observed for BA.1-specific IgG among COVID-19-naive HCWs and OAs, although these were on average ~0.6 $\log_{10}$ AU/mL lower than WT-specific responses at all time points (all comparisons between WT- and BA.1-specific responses were $P < .0001$; data not shown).

By contrast, individuals who experienced their first SARS-CoV-2 infection between 1 and 6 months post-third dose exhibited markedly higher WT- and BA.1-specific IgG concentrations than their COVID-19-naive counterparts at both subsequent time points (all comparisons $P < .001$) (Figure 1*A*). Stratifying hybrid immune responses by age group also revealed that OAs who contracted SARS-CoV-2 mounted equivalent or higher WT-specific IgG responses ($P = .01$ to .8) and equivalent BA.1-specific IgG responses ($P = .1$ to .9) compared with HCWs at all postinfection time points (Figure 1*B*). In fact, 6 months post-third dose, WT- and BA.1-specific IgG concentrations in hybrid immune participants were significantly higher than the original peak vaccine responses post-third dose: at 6 months, the median WT-specific IgG concentration was 5.50 (IQR, 5.17–5.79) $\log_{10}$ AU/mL in the combined hybrid group, which was ~0.25 $\log_{10}$ AU/mL higher than that observed at 1 month post-third dose ($P = .0005$).

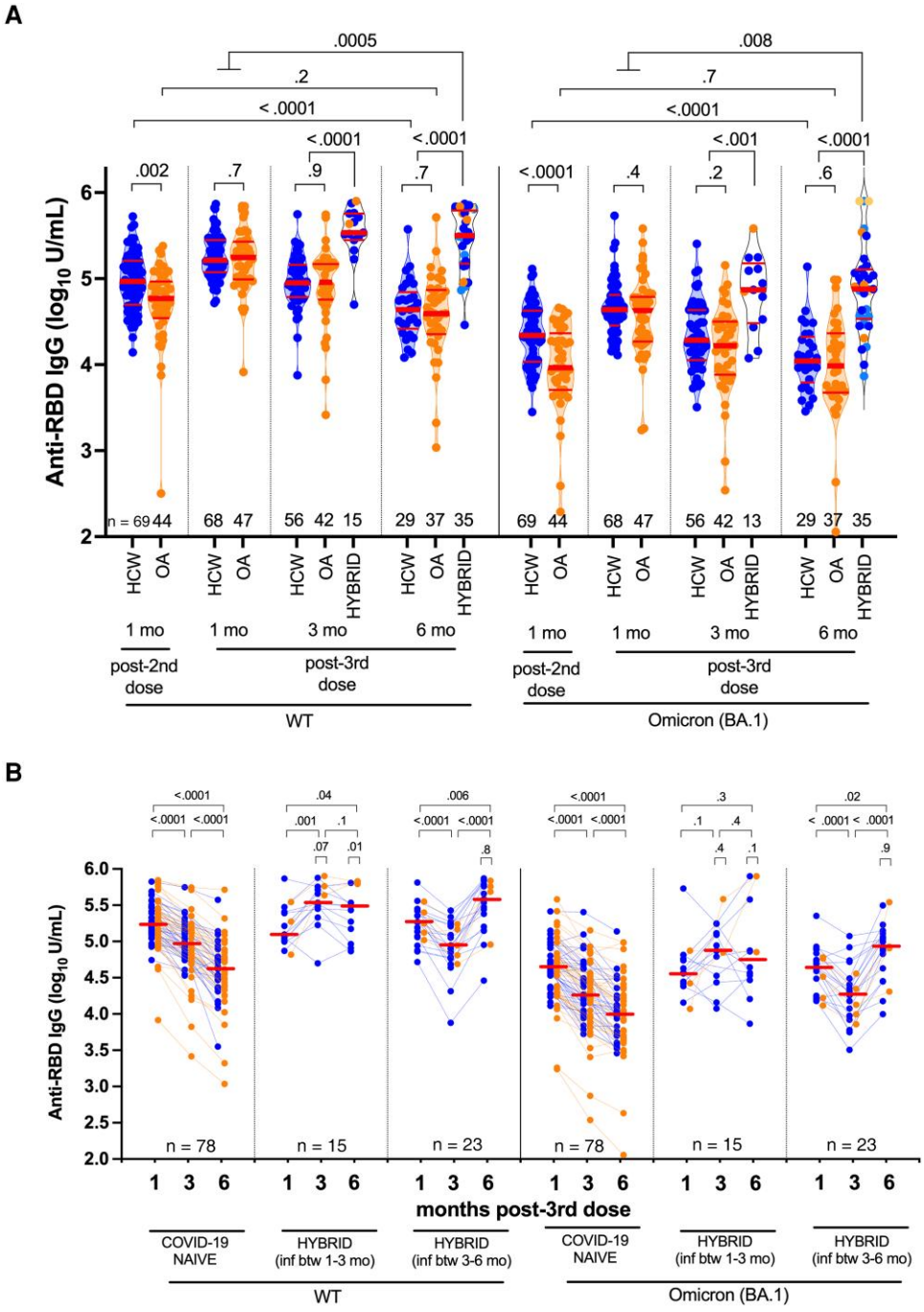

Figure 1. Wild-type (WT)- and Omicron BA.1-specific anti-receptor-binding domain (RBD) immunoglobulin (Ig)G concentrations after 3-dose coronavirus disease 2019 (COVID-19) vaccination. (*A*) Longitudinal serum anti-RBD IgG concentrations specific to WT (left side) and Omicron BA.1 (right side) in COVID-19-naive healthcare workers ([HCW] blue circles) and older adults ([OA] orange circles), expressed as $\log_{10}$ arbitrary units/milliliter obtained directly from the Meso Scale Diagnostics assay output. Any participant who experienced a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) breakthrough infection between 1 and 3 or 3 and 6 months (mo) post-third dose was reclassified into the "hybrid" group at their following study visit, with HCW in blue and OA in orange circles. At 6 months post-third dose, the darker colored symbols in the hybrid group denote recent infections that occurred between 3 and 6 months, whereas the lighter-colored symbols denote the infections that had previously occurred between 1 and 3 months. Thicker red bars indicate the median, thinner red bars indicate the interquartile range. Comparisons between independent groups were performed using the Mann-Whitney *U* test; longitudinal paired comparisons were performed using the Wilcoxon matched-pairs test. *P* values are not corrected for multiple comparisons. (*B*) Same data as (*A*), but where serum anti-RBD IgG concentrations are plotted longitudinally by participant (HCW in blue, OA in orange). Participants are stratified into 3 groups: those who remained COVID-19 naive throughout the study, those who acquired SARS-CoV-2 between 1 and 3 months post-third dose, and those who acquired SARS-CoV-2 between 3 and 6 months post-third dose. Horizontal lines (in red) denote the overall median response at each time point, where HCW and OA are treated as a combined group. *P* values on top of larger brackets compare responses between time points using the Wilcoxon matched-pairs test, where HCW and OA are treated as a combined group. *P* values on top of small brackets compare responses between HCW and OA at each time point after SARS-CoV-2 infection using the Mann-Whitney *U* test. *P* values are not corrected for multiple comparisons.

We also estimated post-third dose half-lives of virus-specific serum IgG concentrations in participants who remained COVID-19 naive. For WT-specific IgG, these were a median 73 (IQR, 53–101) days in HCWs versus 69 (IQR 54–91) days in OAs ($P = .8$) (Supplementary Figure 1*A* and *B*), whereas for Omicron-BA.1-specific IgG these were a median 75 days (IQR, 58–93) in HCWs and a median 78 (IQR, 64–94) in OAs ($P = .5$) (Supplementary Figure 1*C* and *D*).

Angiotensin-Converting Enzyme 2 Displacement Activity

As reported previously [20, 23], ACE2 displacement activities against WT and Omicron-BA.1 were lower in COVID-19-naive OAs compared with HCWs post-second dose but increased to equivalence 1 month post-third dose. At this time, they reached a median of 98.7% (IQR, 96.3–99.3) in HCWs versus 99.3% (IQR, 96.0–99.7) OAs for WT ($P = .12$) and a median of 62.5% (IQR, 46.2–75.5) in HCWs versus 66.2% (IQR, 44.6–79.3) in OAs for BA.1 ($P = .4$) (Figure 2*A*). Among the participants who remained COVID-19 naive throughout follow up, WT-specific ACE2 displacement activities declined similarly across groups, to a median of 98.0% (IQR, 93.7–99.3) in HCWs versus 98.3% (IQR, 91.7–99.5) in OAs at 3 months post-third dose ($P = .9$) and a median 92.8% (IQR, 80.0–99.9) in HCW versus 91.4% (IQR, 72.3–97.1) in OAs at 6 months ($P = .4$). Somewhat in contrast, BA.1-specific ACE2 displacement activity declined slightly faster in COVID-19-naive OAs compared with HCWs, particularly between 3 and 6 months post-third dose. Specifically, BA.1-specific ACE2 displacement activities had declined to a median 48.6% (IQR, 20.5–73.8) in HCWs versus 47.6% (IQR, 14.2–71.7) in OAs at 3 months ($P = .4$) and to a median 43.3% (IQR, 13.4–62.2) in HCWs versus 20.7% (IQR, 5.6–32.3) in OAs at 6 months ($P = .04$). Similar to our observations for IgG levels, by 6 months post-third dose, WT- and BA.1-specific ACE2 displacement activities in COVID-19-naive HCWs had declined to below peak levels elicited after 2-dose vaccination ($P < .0001$ and $P = .02$, respectively), whereas in OAs they had declined to comparable levels as achieved after 2 doses ($P = .6$ and $P = .4$, respectively).

By contrast, individuals who experienced their first SARS-CoV-2 infection between 1 and 6 months post-third vaccine dose showed a strong boost in ACE2 displacement activity post-infection, where this was most apparent for BA.1-specific responses (all comparisons $P < .0001$ for both WT and BA.1) (Figure 2*A*). In fact, 6 months post-third dose, both WT- and BA.1-specific ACE2 displacement activities in the hybrid immune group significantly exceeded those induced by vaccination alone (both comparisons $P < .0001$) (Figure 2*A*). BA.1-specific ACE2 displacement activities for example rose to a median of 95.9% (IQR, 90.0–97.8) in the hybrid group at 6 months, levels that were ~30% higher than those induced by 3-dose vaccination alone. Similar to our observations for IgG levels, OAs who contracted SARS-CoV-2 exhibited equivalent or higher WT-specific ACE2 displacement activities (P=.006 to .9) and equivalent BA.1-specific ACE2 displacement activities (P=.4 to .9) compared with HCWs at all postinfection time points (Figure 2*B*).

Virus Neutralization

As reported previously [20, 23], WT- and Omicron-BA.1-specific live virus neutralization activities were weaker in OAs compared with younger HCWs after 2 vaccine doses, but these activities were enhanced and reached equivalence 1 month post-third dose (Figure 3*A*). At this time, the reciprocal plasma dilutions required to neutralize WT were a median of 320 (IQR, 160–320) in HCWs versus a median of 320 (IQR, 80–640) in OAs ($P = .9$), whereas those required to neutralize BA.1 were a median of 40 (IQR, 20–80) in both groups ($P = .8$). Thereafter, WT neutralization activity declined relatively similarly in both COVID-19 OAs and HCWs, to median reciprocal dilutions of 80 (IQR, 80–160) in HCWs versus 80 (IQR, 40–160) in OAs at 3 months post-third dose ($P = .03$), and a median 40 (IQR, 20–80) in HCWs versus 20 (IQR, 20–80) in OAs at 6 months ($P = .3$). Nevertheless, by 6 months post-third dose, WT-specific neutralization in COVID-19-naive participants was below that observed after 2 vaccine doses ($P < .0001$ in HCWs and $P = .045$ in OAs) (Figure 3*A*). Moreover, BA.1-specific neutralization activity declined faster in COVID-19-naive OAs compared with HCWs post-third dose. By 3 months, BA.1-specific neutralization in HCWs had declined to a median reciprocal dilution of 20 (IQR, 20–40), whereas in 79% of OAs neutralization was BLOQ ($P = .004$). By 6 months, BA.1-specific-neutralization activity had declined to BLOQ in 56% and 96% of COVID-19-naive HCWs and OAs, respectively ($P = .003$), activities that were below that observed after 2 vaccine doses ($P = .02$ for HCWs; $P = .06$ for OAs).

By contrast, individuals who experienced their first SARS-CoV-2 infection after their third vaccine dose showed a strong enhancement in both WT- and BA.1-specific neutralization (all comparisons $P < .0001$) (Figure 3*A*). In fact, by 6 months post-third dose, their WT- and BA.1-specific neutralization activities were significantly higher than those induced by vaccination alone (all $P \leq .002$) (Figure 3*A*): BA.1-specific neutralization for example was 80 (IQR, 80–160), compared to a median 40 (IQR, 20–80) 1 month post-third dose. More importantly, stratifying the hybrid immune responses by age indicated that OAs exhibited equivalent WT- and BA.1-specific neutralization compared with HCWs at all time points ($P = .3$ to .9) (Figure 4*B*).

Correlations Between Humoral Measures

Overall, all humoral immune measures (anti-WT RBD IgG, anti-BA.1 RBD IgG, anti-WT ACE2% displacement, anti-BA.1 ACE2% displacement, WT neutralization, and BA.1-specific neutralization) correlated strongly with one another at all time points evaluated (Spearman's $\rho = .61$ to .92, all $P < .0001$) (Supplementary Figure 2).

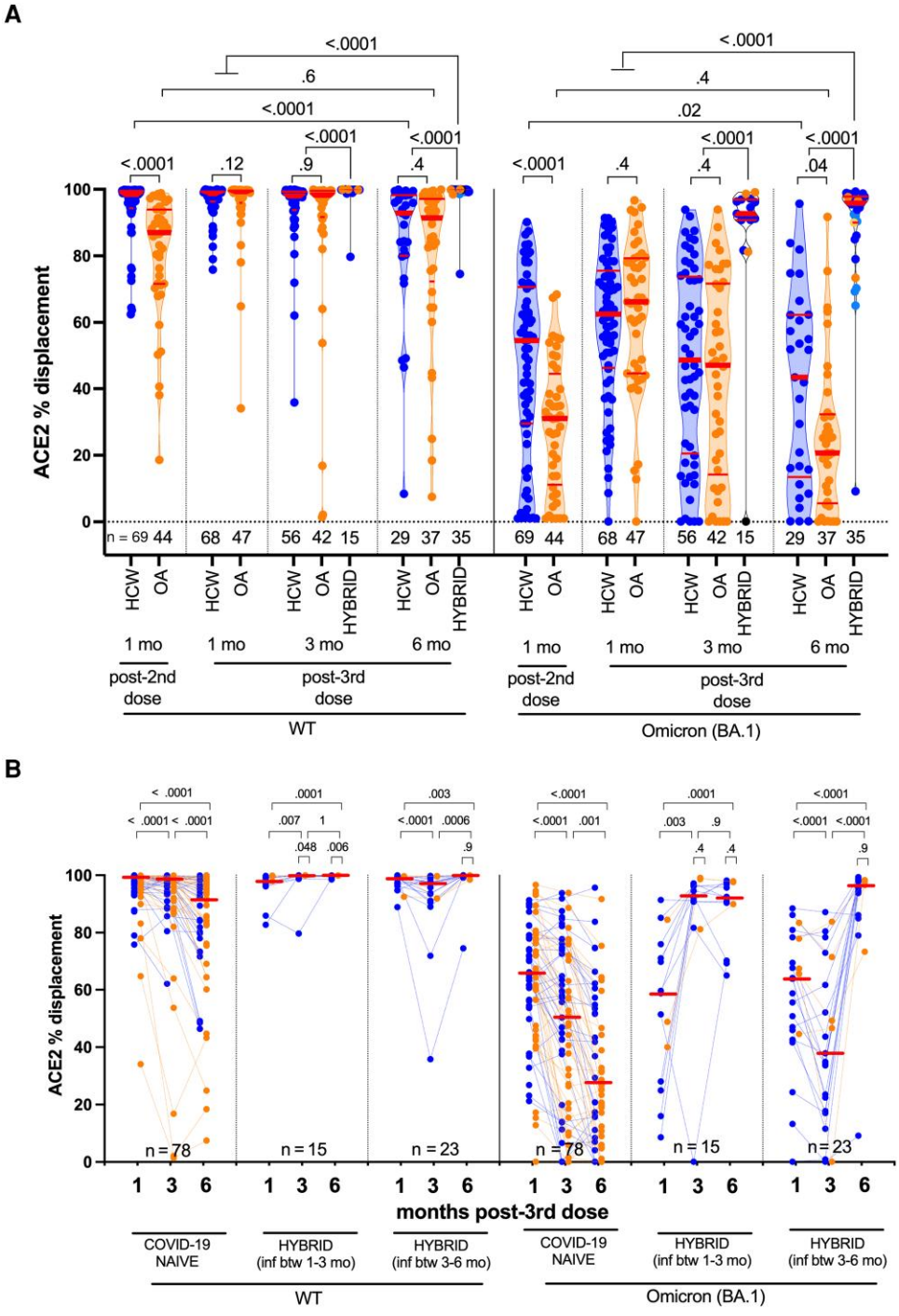

Figure 2. Wild-type (WT)- and Omicron BA.1-specific angiotensin-converting enzyme 2 (ACE2)% displacement function after 3-dose coronavirus disease 2019 (COVID-19) vaccination. (*A*) Same as Figure 1*A*, but for ACE2 displacement activity in serum, a surrogate measure of virus neutralization, where results are reported in terms of %ACE2 displacement. (*B*) Same data as (*A*), but where ACE2% displacement activities are plotted longitudinally by participant (healthcare workers [HCW] in blue, older adults [OA] in orange). The legend is the same as for Figure 1*B*.

Correlates of Severe Acute Respiratory Syndrome Coronavirus 2 Breakthrough Infections Post-Third Dose

We used univariable and multivariable regression to explore the relationship between sociodemographic, health, and vaccine variables, as well as immune response magnitudes 1 month post-third vaccine dose, and the odds of remaining COVID-19 naive during the study period (Supplementary Table 1). Only 2 variables, older age and the magnitude of Omicron-BA.1-specific neutralization post-third dose, independently correlated with the odds of remaining SARS-CoV-2 naive in the following 5 months.

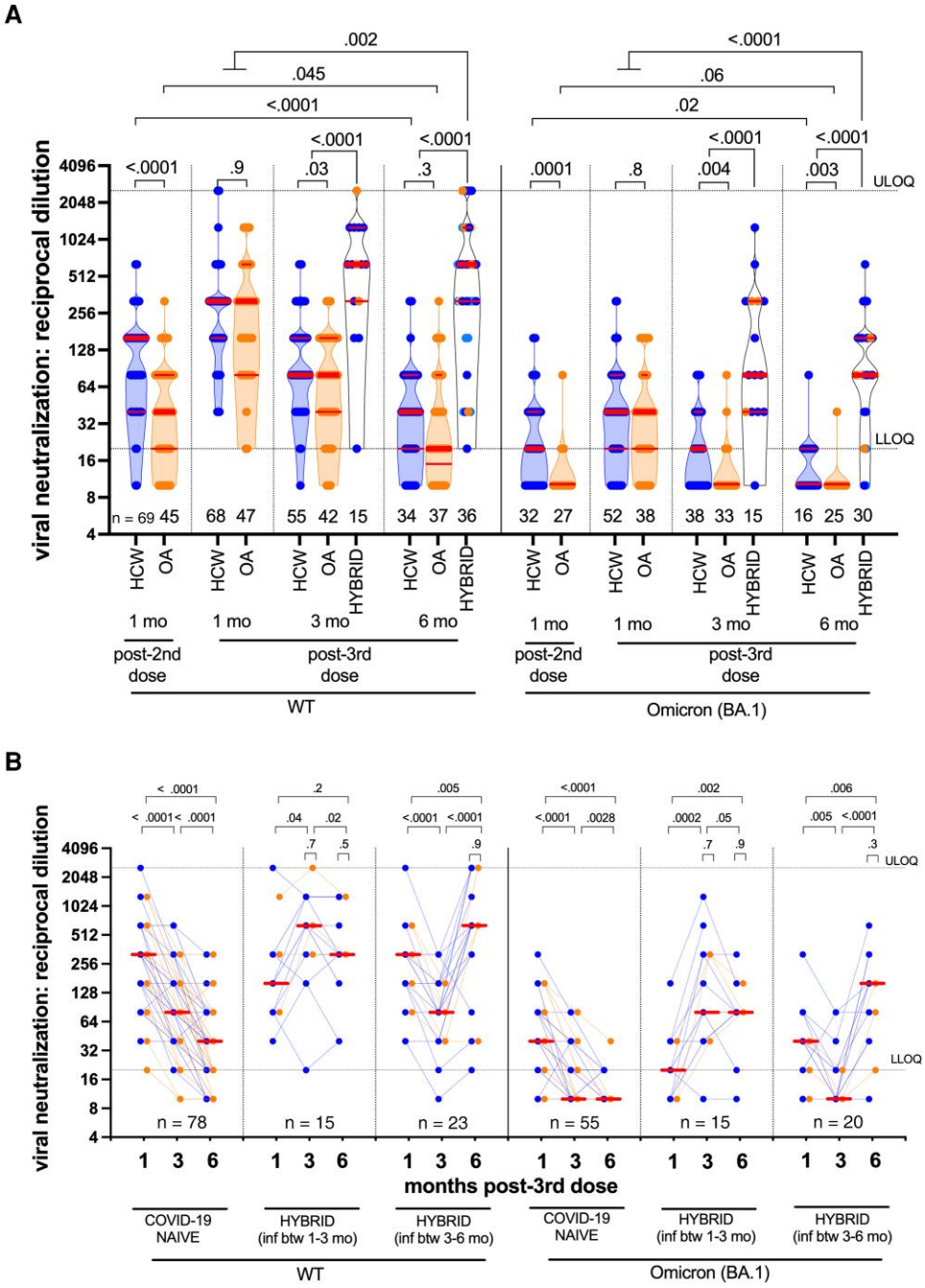

Figure 3. Wild-type (WT)- and Omicron BA.1-specific live virus neutralization activity after 3-dose coronavirus disease 2019 (COVID-19) vaccination. (*A*) Same as Figure 1*A*, but for live virus neutralization activity, defined as the lowest reciprocal plasma dilution at which neutralization was observed in all wells of a triplicate assay. Serial 2-fold dilutions of 1/20 (lower limit of quantification [LLOQ]) to 1/2560 (upper limit of quantification [ULOQ]) were tested. Plasma samples showing neutralization in fewer than 3 wells at a 1/20 dilution are displayed as a reciprocal dilution of "10" and were reported as below limit of quantification (BLOQ) in the text. Omicron-BA.1-specific neutralization was performed on a subset of samples only. (*B*) Same data as (*A*), but where neutralization activities are plotted longitudinally by participant (healthcare workers [HCW] in blue, older adults [OA] in orange). The legend is the same as for Figure 1*B*. Note that many datapoints are superimposed in both panels.

Responses to Newer Omicron Variants, Including BA.5

Although the BA.1 variant drove the first global wave of Omicron infections, this strain has largely been replaced by newer Omicron variants that demonstrate distinct abilities to evade neutralizing antibodies [7, 24–28]. To begin to evaluate vaccine and hybrid immune responses to other Omicron strains, we quantified anti-spike IgG and ACE2 displacement activities specific to WT, BA.1, BA.2, and BA.3 variants in a subset of 25 COVID-19-naive participants at 1 month after their third vaccine dose (Supplementary Figure 3). Compared with

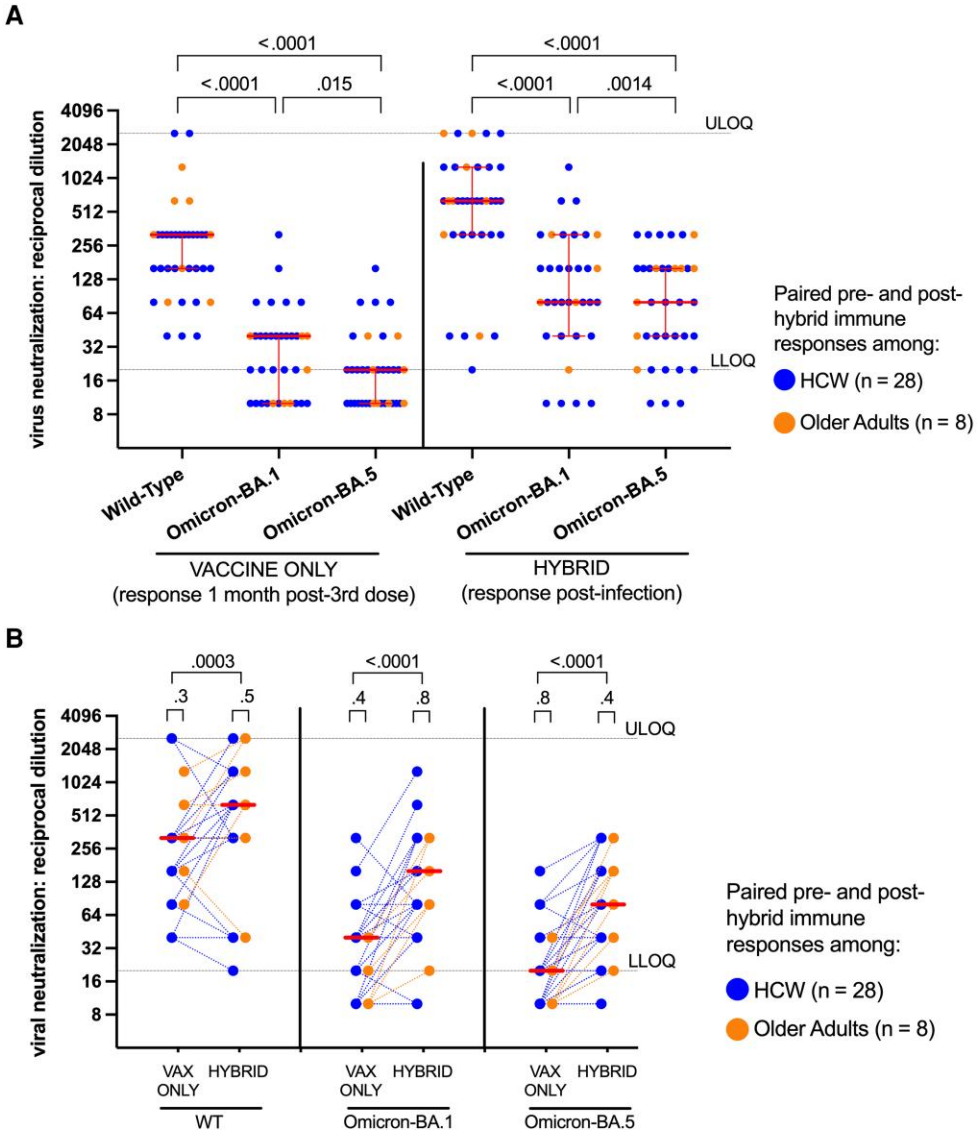

Figure 4. Wild-type (WT)-, Omicron BA.1-, and Omicron BA.5-specific live virus neutralization activity before and after attainment of hybrid immunity. (*A*) The WT-, Omicron BA.1-, and Omicron BA.5-specific neutralization activities in 28 healthcare workers ([HCW] blue circles) and 8 older adults ([OA] orange circles) 1 month after the third vaccine dose ("vaccine only"; or "VAX only") and after subsequent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection ("hybrid"). In this panel, data are plotted to facilitate comparisons between neutralization activities to the 3 SARS-CoV-2 variants, before and after attainment of hybrid immunity. *P* values, computed on combined HCW and OA as a combined group, were computed using the Wilcoxon matched-pairs test. *P* values are not corrected for multiple comparisons. (*B*) Same data as in (*A*), but plotted longitudinally by participant, and stratified by SARS-CoV-2 variant, to highlight the change in neutralization activity before and after attainment of hybrid immunity. Red lines indicate medians of HCW and OA treated as a combined group. *P* values on top of large brackets compare responses before and after hybrid immunity using the Wilcoxon matched-pairs test. *P* values on top of small brackets compare responses between HCW and OA at a given time point using the Mann-Whitney *U* test. Note that a large number of points are superimposed in this plot. LLOQ, lower limit of quantification; ULOQ, upper limit of quantification.

WT-specific responses, anti-spike IgG concentrations were on average ~0.4 $\log_{10}$ AU/mL lower for each of these Omicron variants, whereas ACE2 displacement activities were on average at least 30% lower for these Omicron variants (all $P < .0001$) (Supplementary Figure 3). BA.2-specific anti-spike IgG concentrations were comparable to BA.1 ($P = .2$), whereas BA.2-specific ACE2 displacement activities were marginally lower ($P = .01$). BA.3-specific anti-spike IgG concentrations and ACE2 displacement activities were significantly lower than those against BA.1 (both $P < .0001$), although the magnitude of these differences was modest (eg, median IgG was 5.42 $\log_{10}$ AU/mL for BA.1 vs 5.35 $\log_{10}$ AU/mL for BA.3). The correlation between the present anti-spike and the original anti-RBD measurements for the WT and BA.1 antigens (shown in Figure 1) was strong (Spearman's $\rho \geq .86$, $P < .0001$) (Supplementary Figure 3).

Omicron BA.5 emerged in early 2022 [29], and at the time of writing was the dominant SARS-CoV-2 variant circulating globally [30–33]. To investigate vaccine- and hybrid-induced immune responses against this strain, we performed live virus neutralization assays using a local BA.5 isolate in a subset of 36 participants (28 HCWs and 8 OAs) who experienced SARS-CoV-2 breakthrough infection between 1 and 6 months after receiving 3 vaccine doses (Figure 4). For each individual, vaccine-induced neutralization activity was tested at 1 month post-third dose, and hybrid-induced neutralization activity was tested after SARS-CoV-2 anti-N seroconversion (either 3 months or 6 months post-third vaccine dose). Based on local molecular epidemiology reports [21], SARS-CoV-2 infections among these participants were likely due to BA.1 or BA.2 (not BA.5). Neutralization results for BA.5 were compared to those against WT and BA.1 at the same time points (ie, the data from Figure 4). Consistent with recent reports showing that BA.5 is more immune evasive than prior Omicron variants [25–27], we observed that neutralization activity against BA.5 was significantly lower than activities against WT and BA.1 after 3 vaccine doses ($P < .0001$ and $P = .015$, respectively) (Figure 4*A*). Indeed, the median reciprocal dilution for BA.5 neutralization was 20 (IQR BLOQ-20) at this time point. Neutralization activity against all 3 virus strains was enhanced significantly after infection (all $P \leq .0003$) (Figure 4*B*), but the hybrid immune response against BA.5 (median reciprocal dilution 80; IQR, 40–160) nevertheless remained significantly lower than that against both WT ($P < .0001$) and BA.1 ($P = .0014$) (Figure 4*A*). Overall, no significant difference in BA.5 neutralization activity was found between OAs and younger HCWs at either 1 month post-third vaccine dose nor after acquiring hybrid immunity (Figure 4*B*).

DISCUSSION

A third COVID-19 mRNA vaccine dose significantly enhances antibody responses against both WT and Omicron variants in COVID-19-naive individuals, particularly older adults. Moreover, WT- and Omicron BA.1-specific anti-RBD binding IgG concentrations remained comparable in magnitude and declined at similar rates in older and younger adults in the 6 months post-third dose, although BA.1-specific responses were consistently ~0.6 $\log_{10}$ AU/mL lower than WT-specific ones in all participants. By contrast, antiviral antibody functions, particularly those specific to Omicron, declined substantially in all participants who remained COVID-19 naive over the study period, and especially so in older adults. By 6 months post-third dose, antibody responses in COVID-19-naive participants of all ages had declined to (or in some cases even below) the peak levels elicited by 2 vaccine doses. For example, BA.1-specific neutralization had declined to below the limit of quantification in 56% of younger adults and 96% of older adults by this time; in fact, BA.1-specific neutralization activity had already declined to below the limit of quantification in 79% of COVID-19-naive, older adults by 3 months post-third dose. These observations, along with the finding that ACE2 displacement function declined more rapidly in COVID-19-naive older adults, suggest that Omicron-specific antibody function may be impaired in older age, but where this impairment is only revealed as antibody concentrations decline.

By contrast, both younger and older adults who experienced their first SARS-CoV-2 infection (presumably Omicron BA.1 or BA.2 [21]) after receiving 3 vaccine doses demonstrated superior binding antibody concentrations and functional responses, including against the heterologous Omicron variant BA.5, compared to those induced by 3 vaccine doses alone, although the ability to neutralize BA.5 remained significantly poorer than the ability to neutralize BA.1 even after infection. Nevertheless, and importantly, the magnitude of humoral responses after acquisition of hybrid immunity did not differ significantly between older and younger adults (although the modest number of older adults with hybrid immunity should be acknowledged). These results are consistent with other studies of hybrid immunity [19, 34, 35] and suggest that a post-third dose Omicron infection will prolong immune protection against Omicron strains for at least a short period. The observation that viral infection led to a pronounced enhancement of Omicron-specific responses, including binding antibody concentration and virus neutralization activity, is likely attributable to exposure to Omicron spike. If so, bivalent vaccines that include variant Omicron spike antigens may offer similar advantages, including the ability to elicit superior immune responses against circulating variants compared with existing WT-only vaccines. Although the vast majority of participants displayed a significant boost in humoral responses after SARS-CoV-2 infection as expected, it is notable that a minority did not (see Figures 1*B*, 2*B*, 3*B*, and 4*B*). In some cases, this is likely because their COVID-19 diagnosis occurred less than 2 weeks before their study visit, so there was insufficient time for an immune boost to occur. Antibody concentrations and functional dynamics tended to be concordant; however, in rare cases, we observed a lack of enhancement in neutralization despite increases in binding antibodies, which requires further study.

Our study also identified strong Omicron (BA.1)-specific live virus neutralization post-third dose as an independent predictor of breakthrough infection. To our knowledge, this is a novel observation, although a large study of healthcare workers undertaken in the pre-Omicron era had identified lower peri-infection SARS-CoV-2-neutralizing antibody titers as a correlate of breakthrough infection risk [36]. The observed association between older age and lower breakthrough infection risk is consistent with the significantly lower SARS-CoV-2 seroprevalence among older versus younger adults in BC during the study period, which is likely attributable to decreased exposure

in older adults as a result of enhanced preventive measures in place to protect at-risk groups [37].

This study has some limitations. Spike IgA levels, antibody Fc binding, and T-cell immunity were not assessed. A slightly higher proportion of older adults received an mRNA-1273 third dose compared to younger adults, and older adults would have also received the higher 100 µg mRNA-1273 dose. This may have contributed to the strong post-third dose responses and lower breakthrough infection risk in older adults, because BNT162b2 and mRNA-1273 do not elicit fully equivalent responses [38] and vaccine efficacy is slightly higher for mRNA-1273 [39].

CONCLUSIONS

Third/booster COVID-19 vaccine doses benefit adults of all ages. However, systemic antibody responses and functions decline over time, supporting the use of additional booster doses, particularly in individuals who remain SARS-CoV-2 naive. Additional studies are needed to assess the durability of hybrid immune responses and to evaluate cross-reactivity against emerging SARS-CoV-2 variants, particularly in the context of bivalent vaccines that incorporate Omicron spike antigen.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Author contributions. MGR, ZLB, and MAB led the study. HRL coordinated the study. FM, PKC, YS, FY, LB, SE, and WD collected data under the supervision of MLD, VL, JSGM, DTH, JS, MGR, ZLB, and MAB. FM, HRL, MAB, and ZLB analyzed data. YS curated the specimen repository. LY performed phlebotomy and assisted with logistics. FM, YS, RK, and SD processed specimens and curated data. CJB advised on data analysis. NP provided data. CFL provided specimens. MN isolated SARS-CoV-2. ZLB and MAB wrote the manuscript. All authors contributed to manuscript review and editing.

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Potential conflicts of interest. The authors have no conflicts of interest to declare.

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