Clinical Infectious Diseases

MAJOR ARTICLE

Poor Antibody Response to BioNTech/Pfizer Coronavirus Disease 2019 Vaccination in Severe Acute Respiratory Syndrome Coronavirus 2–Naive Residents of Nursing Homes

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Background. Residents of nursing homes (NHs) are at high risk of coronavirus disease 2019 (COVID-19)–related disease and death and may respond poorly to vaccination because of old age and frequent comorbid conditions.

Methods. Seventy-eight residents and 106 staff members, naive to infection or previously infected with severe acute respiratory syndrome coronavirus (SARS-CoV-2), were recruited in NHs in Belgium before immunization with 2 doses of 30 µg BNT162b2 messenger RNA (mRNA) vaccine at days 0 and 21. Binding antibodies (Abs) to SARS-CoV-2 receptor-binding domain (RBD), spike domains S1 and S2, RBD Ab avidity, and neutralizing Abs against SARS-CoV-2 wild type and B.1.351 were assessed at days 0, 21, 28, and 49.

Results. SARS-CoV-2–naive residents had lower Ab responses to BNT162b2 mRNA vaccination than naive staff. These poor responses involved lower levels of immunoglobulin (Ig) G to all spike domains, lower avidity of RBD IgG, and lower levels of Abs neutralizing the vaccine strain. No naive residents had detectable neutralizing Abs to the B.1.351 variant. In contrast, SARS-CoV-2–infected residents had high responses to mRNA vaccination, with Ab levels comparable to those in infected staff. Cluster analysis revealed that poor vaccine responders included not only naive residents but also naive staff, emphasizing the heterogeneity of responses to mRNA vaccination in the general population.

Conclusions. The poor Ab responses to mRNA vaccination observed in infection-naive NH residents and in some naive staff members suggest suboptimal protection against breakthrough infection, especially with variants of concern. These data support the administration of a third dose of mRNA vaccine to further improve protection of NH residents against COVID-19.

Clinical Trials Registration. NCT04527614.

Keywords. COVID-19; mRNA vaccination; antibody response; nursing homes; immunosenescence.

Nursing home (NH) residents are at a disproportionately high risk of coronavirus disease 2019 (COVID-19)–related disease and death, representing about 5% of all cases while accounting for >30% of all COVID-19–related deaths in the United States [1, 2]. Most vaccination campaigns have therefore prioritized NHs, achieving high coverage rates especially among residents [3, 4]. As a result, new cases and deaths have declined steeply in such facilities, outpacing decreases in national rates [5–7].

The success of COVID-19 messenger RNA (mRNA) vaccination in NHs is consistent with data from phase 2 studies indicating their potent immunogenicity in younger and older adults [8, 9]. However, more recent observational studies found lower antibody (Ab) responses to BNT162b2 vaccination in older adults [10–13]. Moreover, chronic comorbid conditions, such as diabetes and cardiovascular disease, were associated with lower vaccine responses [11, 14]. This raises the concern that NH residents, who are often frail and have comorbid conditions, might respond more poorly to COVID-19 vaccination. Supporting this concern, a retrospective observational cohort study from Denmark found lower vaccine effectiveness in NH residents (64%) than in healthcare workers (90%) 1 week after the second BNT162b2 mRNA vaccine dose [15].

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Decreased vaccine effectiveness in NH residents may be particularly problematic in the face of emerging severe acute respiratory syndrome coronavirus (SARS-CoV-2) variants that are less susceptible to vaccine-induced neutralizing Abs [16–20]. Breakthrough infections with SARS-CoV-2 variants after complete mRNA vaccination have been reported in healthy adults, and, more recently, severe COVID-19 and death after breakthrough infections in NH residents have been reported in several countries [21–25]. Breakthrough infections with the SARS-CoV-2 Delta variant are also rising in Israel, with hospitalization most common among individuals ≥60 years [26, 27]. The concern of severe breakthrough infection with SARS-CoV-2 variants may be lower in NH residents who survived natural infection. Indeed, COVID-19 mRNA vaccination induces higher Ab responses in previously infected adults than in infection-naive adults and boosts levels of neutralizing Abs cross-reacting with variants of concern [28–33]. The level of cross-reactive immunity induced by mRNA vaccination in infection-naive and previously infected NH residents remains poorly documented.

Taken together, available data raise concern regarding COVID-19 mRNA vaccine–induced immunity in infection-naive and frail NH residents, especially in the context of emerging SARS-CoV-2 variants. We therefore established a longitudinal cohort of SARS-CoV-2–naive or previously infected NH residents and staff who received 2 doses of the BNT162b2 mRNA vaccine and assessed the magnitude and quality of Ab responses to SARS-CoV-2 Wuhan (wild-type [WT]) strain and B.1.351 Beta variant, first identified in South Africa, as a prototype variant of concern.

MATERIAL AND METHODS

Study Design and Approvals

The current study is nested in a prospective cohort study, PICOV (Prior Infection with SARS-CoV-2) [34]. The objective was to measure immune responses to SARS-CoV-2 mRNA vaccination in infection-naive and previously infected NH residents and staff. The study was approved by the Ethics Committee of Hôpital Erasme, Brussels, Belgium (reference B4062020000134) and by the Federal Agency for Medicines and Health Products (reference 2021-000401-24) and is registered at ClinicalTrials.gov (NCT04527614).

Recruitment and Clinical Sample Collection

SARS-CoV-2 infection–naive and previously infected residents and staff from 2 Belgian NHs were recruited. Those with a documented positive reverse-transcription quantitative polymerase chain reaction or clinical serological result at baseline were considered previously infected with SARS-CoV-2. Clinical serology consisted of a semiquantitative anti–receptor-binding domain (RBD) immunoglobulin (Ig) enzyme-linked immunosorbent assay (ELISA), detecting IgA/IgG/IgM (SARS-CoV-2 total Ig ELISA; Bejing Wantai Biological Pharmacy Enterprise) and using a manufacturer-defined cutoff for positivity. Exclusion criteria for NH residents included previous diagnosis of dementia, Mini-Mental State Examination score ≤18/30, and life expectancy <6 months. As described elsewhere, the Clinical Frailty Scale and the Quality of Life index were used to assess residents at baseline [34].

All participants were immunized with two 30-µg doses of BNT162b2 mRNA (Comirnaty; BioNTech/Pfizer), 21 days apart. Blood samples were collected on the day of the primary dose (baseline or day 0), the day of the boost (day 21), and 1 and 4 weeks after the boost (days 28 and 49, respectively). Serum samples were separated by blood centrifugation at 1000*g* for 10 minutes and stored at −20°C for downstream Ab analyses.

SARS-CoV-2–Specific Binding Abs

Levels of serum Abs were assessed using a multiplexed immunoassay (Multi-SARS-CoV-2 Immunoassay), developed in collaboration with InfYnity Biomarkers. This technology was described earlier for *Trypanosoma cruzi* serology and is analogous to Mesoscale Discovery technology [35, 36]. In this microarray, SARS-CoV-2 antigens, selected for their individual performance, were printed in duplicate in 96-well polystyrene microplates, using a sciFLEXARRAYER printing system (Scienion). Individual SARS-CoV-2 antigens included spike 1 domain (S1; encompassing amino acids 16–685 of S), spike 2 domain (S2, encompassing amino acids 686–1213 of S), and RBD (GenBank YP009724390.1). Three spots of positive controls designed to check for the presence of human IgG, and enzyme conjugates were printed on the array using a precise orientation pattern. Positioning onto the microplate surface is defined in x-y coordinates to allow recognition of specific reacting Abs. Serially diluted serum samples were tested against the World Health Organization international standard (National Institute for Biological Standards and Control 20/136; https://www.nibsc.org/science_and_research/idd/cfar/covid-19_reagents.aspx) or an in-house reference calibrated against this standard, and positive and negative control serum samples were included on each plate.

Test samples, calibrators, and controls were incubated in microarray plates for 1 hour at room temperature (RT) and washed with phosphate-buffered saline (PBS) with 0.05% Tween 20. Next, plates were incubated (1 hour at RT) with horseradish peroxidase–conjugated goat anti-human IgG and washed with PBS-Tween before addition of a precipitating 3,3′,5,5′-tetramethylbenzidine solution for 20 minutes (RT; dark). The solution was then removed, and plates were dried at 37°C for 10 minutes. Microplates were imaged and analyzed using a microplate reader (SciReader CL; Scienion). The average pixel intensity for each spot was calculated for each antigen/dilution and reported as mean pixel intensity. This was converted

to binding Ab units per milliliter by interpolation from a 4-parameter logistic standard curve, using GraphPad Prism software (version 9.0.0; GraphPad) with export to Microsoft Excel Professional Plus 2016.

The dynamic range for each antigen measurement was defined using serial dilutions of positive serum samples. Only antigen measurements within the dynamic range were considered and multiplied by the dilution factor. Results are reported as binding Ab units per milliliter. Receiver operating characteristic analyses using an independent population for validation generated cutoff concentrations of 15, 20, and 20 binding Ab units/mL for RBD, S1, and S2 Abs, respectively (Supplementary Methods). Assay performance data and comparisons with commercially available immunoassays are presented in Supplementary Methods.

Neutralizing Abs Against SARS-CoV-2

Serial dilutions of heat-inactivated serum (1/50–1/25 600 in Eagle's minimal essential medium supplemented with 2-mmol/L L-glutamine, penicillin (100 U/mL)–streptomycin (100 µg/mL), and 2% fetal bovine serum) were incubated for 1 hour (37°C; 7% carbon dioxide) with 3× the 100% tissue culture infective dose of (1) a WT Wuhan strain (2019-nCoV-Italy-INMI1; reference 008V-03893) and (2) the B.1.351 variant of SARS-CoV-2, in parallel. Sample-virus mixtures and virus/cell controls were added to Vero cells (18 000 cells per well) in a 96-well plate and incubated for 5 days (37°C; 7% carbon dioxide). The cytopathic effect caused by viral growth was scored microscopically. The Reed-Muench method was used to calculate the neutralizing Ab titer that reduced the number of infected wells by 50%, which was used as a proxy for the neutralizing Ab concentration in the sample [37, 38].

SARS-CoV-2 RBD-Specific Ab Avidity

Biolayer interferometry measurements were performed with an Octet HTX instrument (FortéBio) using AR2G biosensors. Data analyses were performed using FortéBio Data Analysis 9.0 software. Kinetic assays were performed at 25˚C–30˚C at a sample plate agitation speed of 1000 rpm. Sensors were first activated by immersion in a solution containing 20-mmol/L 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride and 10-mmol/L n-hydroxysulfosuccinimide. Next, 0.05 mg/mL RBD antigen in 10-mmol/L sodium acetate (pH 6.0) was loaded for 600 seconds. After antigen loading, biosensors were immersed in a solution of 1-mol/L ethanolamine (pH 8.5) to prevent nonspecific interactions. Antigen-loaded AR2G sensors were first dipped in PBS to establish a baseline time curve and then immersed for 10 minutes in wells containing purified serum IgG at 3 dilutions (3×, 5×, and 8×). After IgG association, dissociation was monitored for 600 seconds in PBS. Negative controls included ligand without IgG and IgG without ligand. Kinetic parameters were determined by global fitting of the association and dissociation phases of the binding curves, according to a 1:1 binding model.

Statistical Analyses

Analyses were performed using R software (version 4.0.3). Categorical data were presented as frequencies and percentages, and continuous data as means (with standard deviations) and geometric means (with 95% confidence intervals). The Kruskal-Wallis test and post hoc Mann-Whitney *U* test alongside multiple testing correction with the false discovery rate were used for time-wise group comparisons. The Mann-Whitney test was used to compare WT and B.1.351 variant neutralizing Abs at day 49. Spearman's rank correlation coefficients (rho, ρ) were determined for associations between WT and the B.1.351 variant neutralizing Abs, SARS-CoV-2 binding Abs, and Ab avidity.

Uniform Manifold Approximation and Projection (UMAP) analysis was performed using the R package umap for dimensionality reduction of the following outcomes at day 49: anti-RBD/S1/S2 IgG, anti-RBD IgG avidity, and WT 50% neutralization titer. To achieve normality, avidity was $\log_{10}$-transformed, and neutralization $\log_2$-transformed. The optimal number of clusters was tested using the k-means method (range, 1-10) and visually identified with an "elbow" in a plot of variance versus number of clusters. Density-based spatial clustering of applications with noise (DBSCAN; dbscan package) was used to identify clusters within the UMAP reduced dimensions.

RESULTS

The study included 53 SARS-CoV-2 infection–naive and 25 previously infected NH residents as well as 40 infection-naive and 66 previously infected staff members. In previously infected participants, SARS-CoV-2 infection occurred 151–316 days before vaccination. Complete cohort and demographic information is provided in Table 1. Although residents with the poorest health status were excluded, most enrolled residents were frail, and many had multiple comorbid conditions requiring medication.

Levels of Ab binding to SARS-CoV-2 RBD, S1, and S2 were measured in longitudinal serum samples using a multiplex immunoassay. Detailed numerical data are presented in Supplementary Table 1. At baseline, infection-naive staff and residents had undetectable levels of SARS-CoV-2–specific IgG, and higher spike protein– and nucleoprotein-specific Ab levels were detected in previously infected participants (Figure 1A and Supplementary Figures 1 and 2). Primary vaccination induced a significant increase in SARS-CoV-2 Ab levels in both naive and previously infected staff and residents, and these levels were further boosted after the second vaccination at day 21 (Figure 1A).

Levels of vaccine-induced Abs to RBD and S1 were about 7-fold lower in infection-naive residents than in naive staff after

Table 1. Demographic Characteristics of Infection-Naive and Infected Participants, by Study Group

Characteristic	Participants, No. (%)[a]					*P* Value[b]
	Naive Staff (n = 40)	Naive Residents (n = 53)	Infected Staff (n = 66)	Infected Residents (n = 25)	Total (N = 184)	
Age, y						
Mean (SD)	46.8 (10.2)	86.1 (9.0)	46.6 (10.5)	85.0 (8.0)	63.2 (21.6)	<.001
Range	23.0–64.0	53.0–102.0	22.0–68.0	65.0–95.0	22.0–102.0	
Sex						
Female	29 (72.5)	37 (69.8)	56 (84.8)	16 (64.0)	138 (75.0)	.12
Male	11 (27.5)	16 (30.2)	10 (15.2)	9 (36.0)	46 (25.0)	
Race/ethnicity						
White	38 (95.0)	53 (100.0)	59 (89.4)	25 (100.0)	175 (95.1)	.03
Other	2 (5.0)	0 (0.0)	7 (10.6)	0 (0.0)	9 (4.9)	
BMI[c]						
Mean (SD)	27.0 (5.5)	23.3 (5.1)	27.1 (4.7)	22.6 (4.3)	25.4 (5.3)	<.001
Range	18.5–37.8	16.7–36.3	18.3–44.2	14.6–30.5	14.6–44.2	
Self-reported smoking status						
Former smoker	2 (5.0)	4 (7.5)	5 (7.6)	5 (20.0)	16 (8.7)	.03
Nonsmoker	29 (72.5)	47 (88.7)	50 (75.8)	19 (76.0)	145 (78.8)	
Current smoker	9 (22.5)	2 (3.8)	11 (16.7)	1 (4.0)	23 (12.5)	
Daily exercise						
<30 min	6 (15.0)	27 (50.9)	7 (10.6)	12 (48.0)	52 (28.3)	<.001
30–60 min	8 (20.0)	24 (45.3)	19 (28.8)	7 (28.0)	58 (31.5)	
≥60 min	24 (60.0)	2 (3.8)	38 (57.6)	5 (20.0)	69 (37.5)	
None	2 (5.0)	0 (0.0)	2 (3.0)	1 (4.0)	5 (2.7)	
Self-reported health status						
Very good	14 (35.0)	4 (7.5)	20 (30.3)	3 (12.0)	41 (22.3)	<.001
Good	22 (55.0)	33 (62.3)	39 (59.1)	10 (40.0)	104 (56.5)	
Reasonable	4 (10.0)	16 (30.2)	6 (9.1)	11 (44.0)	37 (20.1)	
Bad	0 (0.0)	0 (0.0)	1 (1.5)	1 (4.0)	2 (1.1)	
Quality of Life index						
Mean (SD)	0.9 (0.1)	0.7 (0.2)	0.9 (0.1)	0.8 (0.2)	0.9 (0.2)	<.001
Range	0.7–1.0	0.2–1.0	0.4–1.0	0.4–1.0	0.2–1.0	
Medication use[d]						
Cardiovascular disease	6 (15.0)	48 (90.6)	3 (4.5)	24 (96.0)	81 (44.0)	<.001
Hypertension	6 (15.0)	41 (77.4)	9 (13.6)	24 (96.0)	80 (43.5)	<.001
Pain	0 (0.0)	42 (79.2)	0 (0.0)	15 (60.0)	57 (31.0)	<.001
Diabetes mellitus	1 (2.5)	10 (18.9)	0 (0.0)	4 (16.0)	15 (8.2)	<.001
Psychosis	2 (5.0)	23 (43.4)	0 (0.0)	8 (32.0)	33 (17.9)	<.001
Depression	0 (0.0)	18 (34.0)	0 (0.0)	7 (28.0)	25 (13.6)	<.001
Pulmonary disease	0 (0.0)	9 (17.0)	0 (0.0)	1 (4.0)	10 (5.4)	<.001
Allergy	1 (2.5)	5 (9.4)	1 (1.5)	4 (16.0)	11 (6.0)	.03
Neurological disease	0 (0.0)	7 (13.2)	0 (0.0)	2 (8.0)	9 (4.9)	.003
Immunological disorder	0 (0.0)	0 (0.0)	1 (1.5)	0 (0.0)	1 (0.5)	.62

Table 1. Continued

Characteristic	Participants, No. (%)[a]					
	Naive Staff (n = 40)	Naive Residents (n = 53)	Infected Staff (n = 66)	Infected Residents (n = 25)	Total (N = 184)	*P* Value[b]
MMSE score[e]						
Mean (SD)	…	25.4 (3.2)	…	25.9 (3.0)	25.6 (3.1)	.98
Range	…	18.0–30.0	…	18.0–30.0	18.0–30.0	
CFS[e]						
Very fit	…	0 (0.0)	…	1 (4.0)	1 (1.3)	.40
Fit	…	8 (15.1)	…	1 (4.0)	9 (11.5)	.09
Managing well	…	18 (34.0)	…	9 (36.0)	27 (34.6)	.87
Very mild frailty	…	7 (13.2)	…	3 (12.0)	10 (12.8)	.55
Mild frailty	…	10 (18.9)	…	4 (16.0)	14 (17.9)	.55
Moderate frailty	…	4 (7.5)	…	4 (16.0)	8 (10.3)	.23
Severe frailty	…	6 (11.3)	…	3 (12.0)	9 (11.5)	.39

Abbreviations: BMI, body mass index; CFS, Clinical Frailty Scale; MMSE, Mini-Mental State Examination; SD, standard deviation.

[a]Data represent no. (%) of participants, unless otherwise specified.

[b]Analysis of variance was used to compare numeric variables, and χ^2 tests to compare categorical variables.

[c]BMI calculated as weight in kilograms divided by height in meters squared, with BMI data available for 40, 51, 66, 25, and 184 participants, respectively.

[d]Medication used as a treatment for the listed conditions.

[e]The MMSE and CFS were used only in residents (n = 78).

primary vaccination, and 2-fold lower after booster vaccination (Figure 1B). Between days 28 and 49, levels of vaccine-induced Abs decreased in naive staff and increased in naive residents, indicating a delayed peak Ab response in naive residents (Figure 1A). Compared with naive participants, vaccine-induced Ab levels were markedly higher in both residents and staff previously infected with SARS-CoV-2 (Figure 1B and Supplementary Figure 2). Notably, Ab levels were already similar in previously infected residents and staff after a single dose of vaccine (Figure 1B). Between days 28 and 49, RBD-specific Ab levels increased in previously infected residents and staff, whereas S2-specific Ab levels decreased during this period, suggesting dynamic changes in Ab repertoire after booster vaccination (Figure 1A).

The avidity of RBD-specific Abs was measured in samples containing sufficiently high levels of RBD Abs to be characterized. Rapid avidity maturation was observed after primary and booster vaccination of infection-naive staff, with peak avidity detected at day 28, followed by a decrease between days 28 and 49 (Figure 2A). Slower IgG avidity maturation was observed in naive residents. At day 49, naive residents had lower Ab avidity than naive staff (Figure 2B). Before vaccination, the avidity of Abs induced by natural infection of staff and residents was lower than that induced by vaccination of naive participants (Figure 2A). Rapid and intense avidity maturation was observed in previously infected staff and residents after a single dose of vaccine (Figure 2A). Slower and less marked maturation was observed after booster vaccination in both groups. At day 49, Ab avidity was higher in previously infected participants than in naive participants and was comparable between previously infected staff and residents (Figure 2B).

The lower levels and avidity of vaccine-induced Abs observed in infection-naive residents as compared with naive staff suggested lower neutralizing Ab capacity. To explore this possibility, titers of neutralizing Abs against WT Wuhan strain and B.1.351 variant were measured. Rapid neutralizing Ab responses were induced by vaccination of naive staff (Figure 2C). Neutralizing Ab levels peaked at day 28 and decreased between days 28 and 49. Slower and less intense neutralizing Ab responses were observed in naive residents. At day 49, naive residents had markedly lower levels of neutralizing Ab than naive staff (Figure 2D). Neutralizing Abs were detected before vaccination in 38 of 66 previously infected staff (58%) and 16 of 25 previously infected residents (64%).

In both groups, levels of neutralizing Abs markedly increased after primary vaccination and peaked at day 28, after booster vaccination (Figure 2C). At day 49, previously infected participants had higher levels of neutralizing Abs, and these levels were comparable in previously infected staff and residents (Figure 2D). Compared with the WT strain, levels of Abs neutralizing the B.1.351 variant were reduced 5–10-fold across study groups (Figure 2E). At day 49, only 4 of 40 infection-naive staff (10%) and none of the naive residents had detectable B.1.351

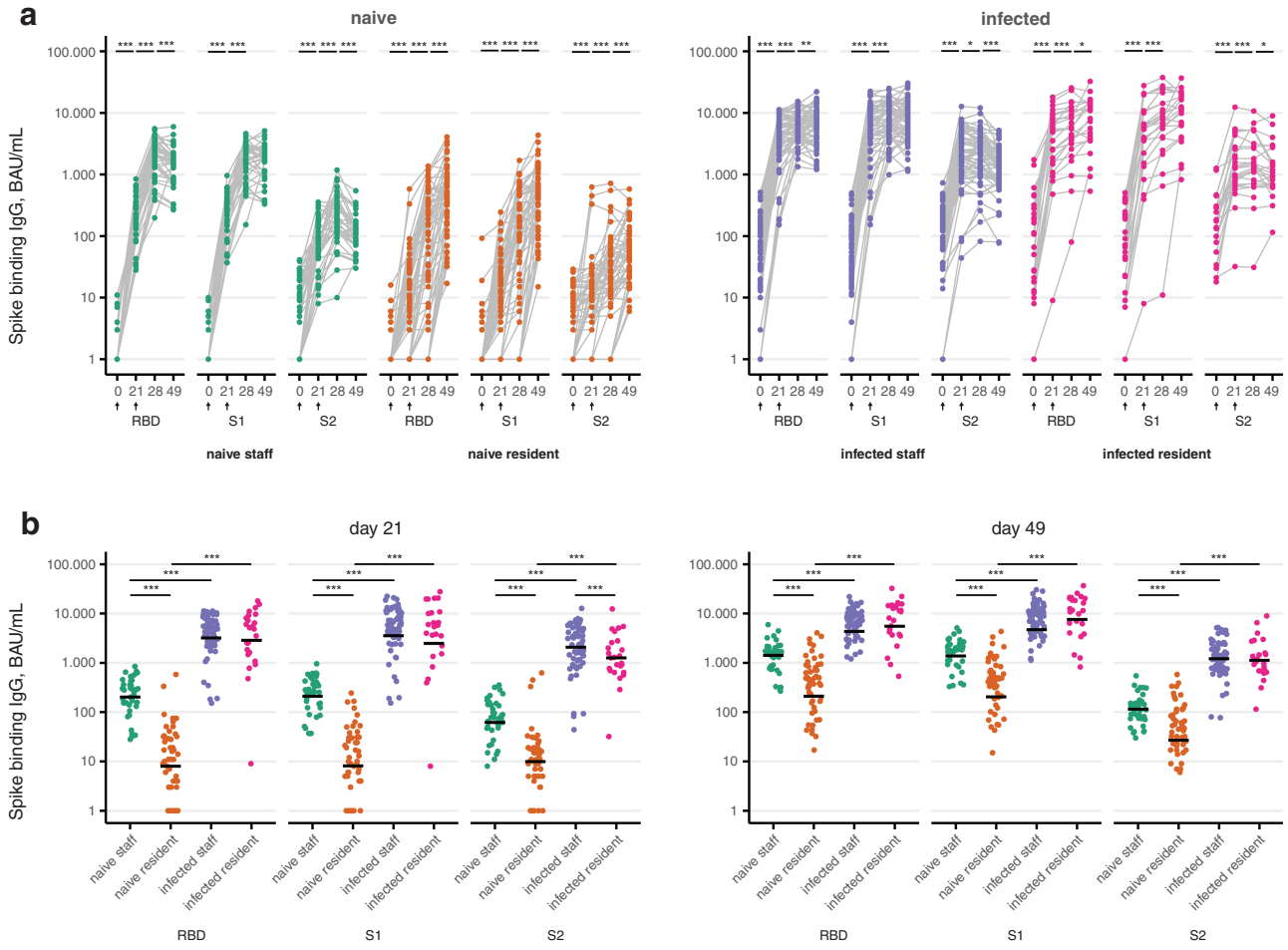

Figure 1. Severe acute respiratory syndrome coronavirus (SARS-CoV-2) spike-specific binding antibody (Ab) responses to BNT162b2 messenger RNA vaccination in residents and staff of nursing homes. SARS-CoV-2–naive and previously infected nursing home residents and staff received two 30-µg doses of BNT162b2 vaccine on days 0 and 21 (*arrows*). The level of spike-specific binding Abs was measured using a multiplex assay before vaccination and at days 21, 28, and 49 after the first dose and is shown as binding Ab units (BAU) per milliliter. Each data point represents a serum sample, and black bars indicate geometric mean titers. Cutoff concentrations are 15, 20, and 20 BAU/mL for anti–receptor-binding domain (RBD) immunoglobulin (Ig) G, anti–spike 1 (S1) IgG, and anti–spike 2 (S2) IgG, respectively. The statistical significance of differences between time points (*A*) and study groups (*B*) was determined using Kruskal-Wallis test by ranks and using Mann-Whitney *U* post hoc test and Benjamini-Hochberg correction for multiple testing. $*P < .05$; $**P < .01$; $***P < .001$. (Comparisons between groups where differences were not significant are not shown.).

neutralizing Abs, whereas neutralizing Abs were detected in 61 of 66 previously infected staff (92%) and 21 of 25 previously infected residents (84%).

The consistent differences in Ab responses observed between the 4 study groups suggested a coordinated response to mRNA vaccination across the measured immunological parameters. Indeed, titers of neutralizing Abs against the WT strain were strongly correlated with RBD, S1, and S2 binding Ab levels, RBD IgG avidity, and levels of neutralizing Abs to the B.1.351 variant (Figure 2F).

To further explore interindividual variability of this coordinated response, a cluster analysis was performed to reduce the complete data set to 2 dimensions and identify groups of participants with similar profiles of Ab responses. Five clusters of study participants with distinct Ab levels, avidity, and neutralizing activity at day 49 were identified (Figure 3A–3D). These clusters were not correlated with age of the study participants (Figure 3E). Separate cluster analyses of infection-naive and previously infected individuals indicated additional clustering within these study groups (Supplementary Figure 3). Cluster 5 exclusively contained previously infected participants with high Ab responses, and those with the highest responses were previously infected residents. In contrast, cluster 1, including the lowest Ab responses, was a mix of mostly naive residents and naive staff, indicating that both populations contain low responders to mRNA vaccination. Clusters 2 and 3 included intermediate Ab responses and were a mix of naive residents, naive staff, and some previously infected staff and residents. The cluster analysis therefore revealed a group of poor Ab responders that included not only naive residents but also naive staff.

DISCUSSION

Reports on lower Ab responses to COVID-19 mRNA vaccination in older people and people with chronic comorbid

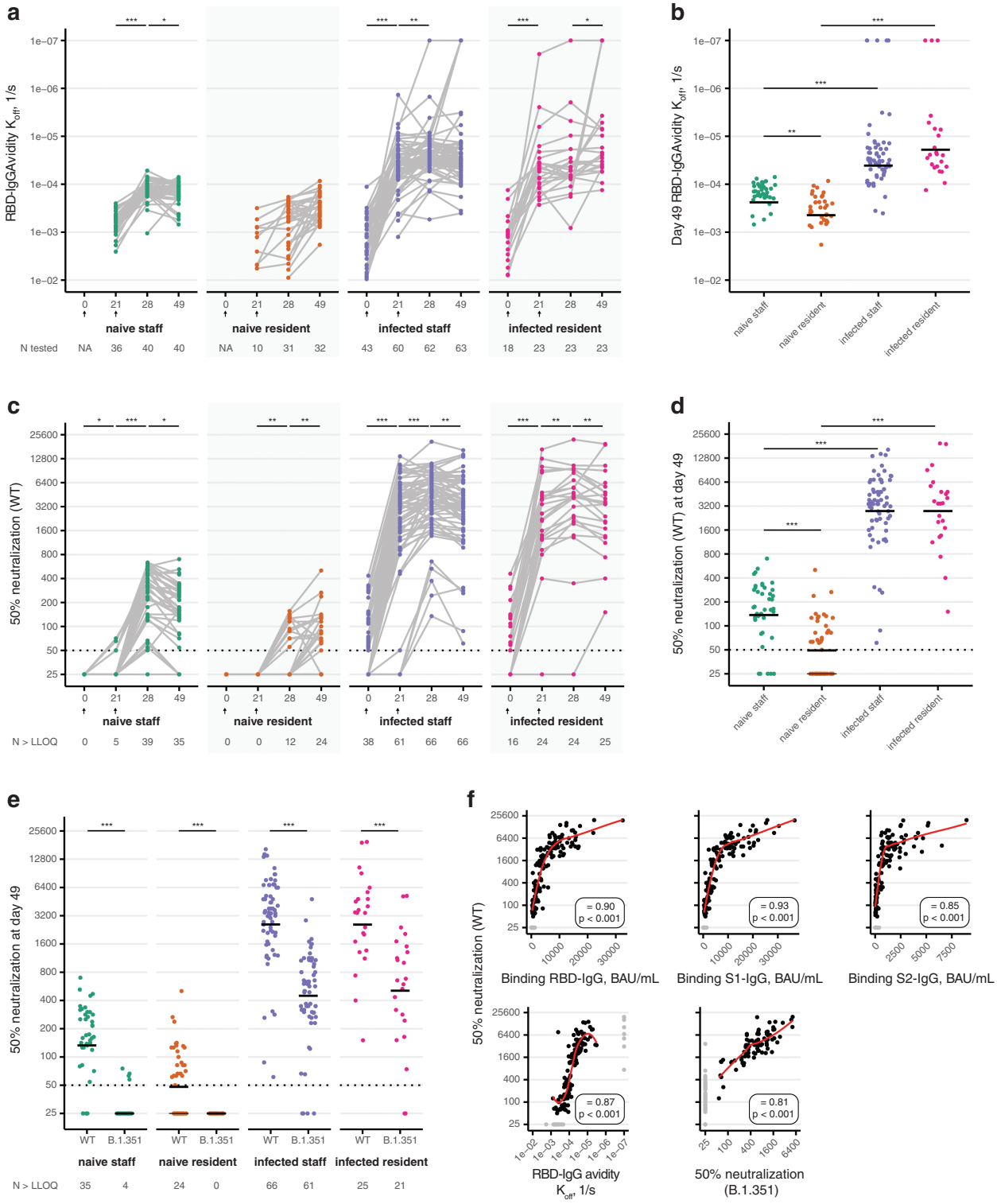

Figure 2. Low receptor-binding domain (RBD) immunoglobulin (Ig) G avidity and neutralizing antibody (Ab) levels in severe acute respiratory syndrome coronavirus (SARS-CoV-2)–naive residents of nursing homes. RBD IgG avidity and neutralizing Ab responses to messenger RNA vaccination were measured at days 0, 21, 28, and 49 in SARS-CoV-2–naive and previously infected residents and staff of nursing homes. *A, B.* Avidity of RBD-specific IgG. "N tested" (in *A*) indicates the number of participants with sufficiently high Ab concentrations for avidity testing. Abbreviations: 1e-07 (etc), 1×10^{-7} (etc); k_{off}, dissociation constant; NA, not applicable. *C–E,* 50% neutralizing Ab titers of SARS-CoV-2 wild type (WT) and B.1.351 variant (lower limit of quantification [LLOQ], 1/50). "N > LLOQ" (in *C*) indicates the number of participants with quantifiable neutralizing Abs. Black bars represent geometric mean titers. The statistical significance of differences between time points and study groups was determined using the Kruskal-Wallis test by ranks and the Mann-Whitney *U* post hoc test and Benjamini-Hochberg correction for multiple testing; for differences between WT and the B.1.351 variant, the Mann-Whitney test was used. $*P < .05$; $**P < .01$; $***P < .001$. (Comparisons between groups where differences were not significant are not shown.) *F,* Spearman rank correlation coefficients (ρ values) comparing titers of neutralizing Abs to the WT strain and the other Ab response parameters. Data below or above limits of quantification were excluded (*gray dots*). Abbreviation: BAU, binding Ab units.

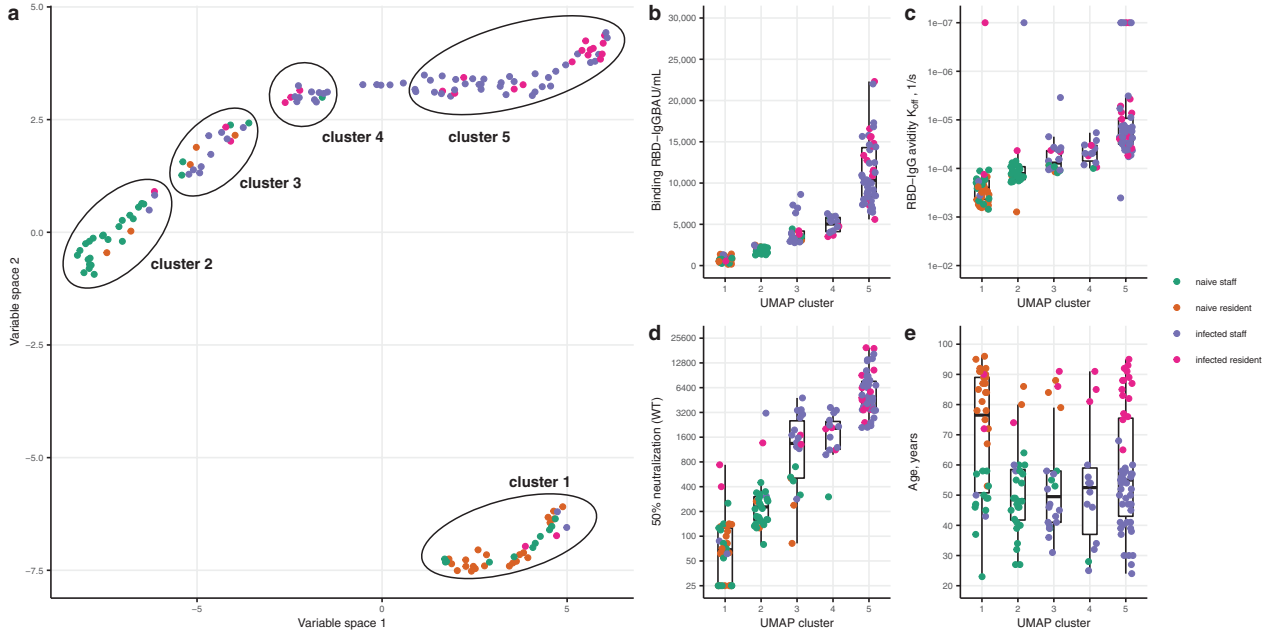

Figure 3. Low vaccine responders include severe acute respiratory syndrome coronavirus (SARS-CoV-2)–naive nursing home residents and staff. *A,* Cluster (Uniform Manifold Approximation and Projection [UMAP]) analysis of all study participants with available receptor-binding domain (RBD)/spike 1 (S1)/spike 2 (S2) binding immunoglobulin (Ig) G antibody (Ab) concentrations, RBD-IgG avidity, and SARS-CoV-2 wild-type (WT) neutralization at day 49. The positions of individual participants in variable spaces 1 and 2 indicate similarities or differences in Ab responses. DBSCAN (density-based spatial clustering of applications with noise) was used to identify clusters. *B–D,* Clusters 1 to 5 are plotted against the RBD binding IgG, RBD IgG avidity, and WT neutralizing titers, respectively. Abbreviations: 1e-07 (etc), 1×10^{-7} (etc); BAU, binding Ab units; k_{off}, dissociation constant. *E,* Ages of participants included in clusters of Ab responses. Black bars represent geometric mean titers.

conditions raise concern about the susceptibility of NH residents to severe breakthrough infections, especially with SARS-CoV-2 variants of concern [10–14, 39, 40]. In the current study, SARS-CoV-2 infection–naive NH residents had lower Ab responses to BNT162b2 mRNA vaccination than naive staff, in line with data reported by Canaday et al [41]. These defective responses included lower levels of IgG to all domains of the vaccine antigen, lower avidity of RBD IgG, and lower levels of neutralizing Abs. Worryingly, none of the naive residents had detectable neutralizing Abs to the B.1.351 variant.

Although an immune correlate of protection against COVID-19 has not been established yet, levels of virus-specific binding and neutralizing Abs have been shown to correlate with vaccine efficacy in phase 3 studies across different vaccination platforms [40, 42, 43]. In addition, data from preclinical studies in nonhuman primates indicate that mRNA vaccine-induced neutralizing Abs can mediate protection against COVID-19 [44–46]. Although T-cell immunity probably contributes to protection induced by mRNA vaccines, the poor Ab responses observed in NH residents are likely associated with lower vaccine-induced protection, especially against variants of concern. This notion is supported by the high proportion of older individuals among patients hospitalized for breakthrough infection with SARS-CoV-2 Delta variant in Israel and supports the administration of a third dose of mRNA vaccine for improved protection of NH residents [27, 47].

Both age and health status differentiate NH residents and staff. In this cohort, Ab responses were not strongly correlated with age, suggesting a more important role of health status, including frailty and comorbid conditions. This observation is consistent with the robust Ab responses to mRNA vaccination observed in older people living outside NHs with preserved health status [48]. In both residents and staff, previous SARS-CoV-2 infection was a major determinant of Ab response, with markedly higher Ab levels and quality in previously infected than in infection-naive participants. NH residents previously infected with SARS-CoV-2 had remarkably high Ab responses to mRNA vaccination and included the highest responders of the cohort.

Higher levels of vaccine-induced binding Abs in previously infected than in infection-naive NH residents were also recently reported by Van Praet et al [49]. Although these potent vaccine responses could partly involve a survival bias, they probably also involve the induction of "hybrid immunity" observed after mRNA vaccination of healthy adults previously infected with SARS-CoV-2 [50]. Unravelling the mechanisms underlying the induction of hybrid immunity may open new avenues for the development of improved vaccines circumventing the immunosenescence of elderly populations. In contrast with naive residents, NH residents previously infected with SARS-CoV-2 may be at particularly low risk of breakthrough infection after mRNA vaccination.

Another important finding in this study is that poor vaccine responders were not limited to infection-naive residents but also included healthy naive staff. This observation emphasizes the heterogeneity of Ab responses to mRNA vaccination in the general population [51–53]. As mRNA vaccination has only recently been implemented in large populations, the immunological basis of this heterogeneity is currently unknown. Systems immunology, involving high-dimensional analyses of the immune system, is emerging as a promising approach to identify determinants of vaccine responsiveness and has the potential to guide the development of next-generation mRNA vaccines against COVID-19 and other target pathogens [54, 55].

Identifying vulnerable populations who may benefit less from current mRNA vaccination regimens is essential for control of the COVID-19 pandemic. The data from the current study support the administration of a third dose of mRNA vaccine to improve protection of NH residents against COVID-19.

Supplementary Data

Supplementary materials are available at *Clinical 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.

Notes

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References

1. Werner RM, Hoffman AK, Coe NB. Long-term care policy after Covid-19—solving the nursing home crisis. N Engl J Med **2020**; 383:903–5.
2. State COVID-19 data and policy actions. KFF. **2021**. Available at: https://www.kff.org/coronavirus-covid-19/issue-brief/state-covid-19-data-and-policy-actions/. Accessed 1 September 2021.
3. Dooling K, McClung N, Chamberland M, et al. The Advisory Committee on Immunization Practices' interim recommendation for allocating initial supplies of COVID-19 vaccine—United States, 2020. MMWR Morb Mortal Wkly Rep **2020**; 69:1857–9.
4. Gharpure R, Guo A, Bishnoi CK, et al. Early COVID-19 first-dose vaccination coverage among residents and staff members of skilled nursing facilities participating in the Pharmacy Partnership for Long-Term Care Program—United States, December 2020–January 2021. MMWR Morb Mortal Wkly Rep **2021**; 70:178–82.
5. Conlen M, Mervosh S, Ivory D. Nursing homes, once hotspots, far outpace U.S. in Covid declines. New York Times. 25 February **2021**. Available at: https://www.nytimes.com/interactive/2021/02/25/us/nursing-home-covid-vaccine.html. Accessed 27 April 2021.
6. Britton A. Effectiveness of the Pfizer-BioNTech COVID-19 vaccine among residents of two skilled nursing facilities experiencing COVID-19 outbreaks—Connecticut, December 2020–February 2021. MMWR Morb Mortal Wkly Rep **2021**; 70:396–401.
7. Kuehn BM. Israel's real-life evidence that vaccine can prevent severe COVID-19. JAMA **2021**; 325:1603–3.
8. Walsh EE, Frenck RW, Falsey AR, et al. Safety and immunogenicity of two RNA-based Covid-19 vaccine candidates. N Engl J Med **2020**; 383:2439–50.
9. Anderson EJ, Rouphael NG, Widge AT, et al. Safety and immunogenicity of SARS-CoV-2 mRNA-1273 vaccine in older adults. N Engl J Med **2020**; 383:2427–38.
10. Prendecki M, Clarke C, Brown J, et al. Effect of previous SARS-CoV-2 infection on humoral and T-cell responses to single-dose BNT162b2 vaccine. Lancet **2021**; 397:1178–81.
11. Müller L, Andrée 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. 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.
13. Schwarz T, Tober-Lau P, Hillus D, et al. Delayed antibody and T-cell response to BNT162b2 vaccination in the elderly, Germany. Emerg Infect Dis J **2021**; 27:2174–8.
14. Yelin I, Katz R, Herzel E, et al. Associations of the BNT162b2 COVID-19 vaccine effectiveness with patient age and comorbidities. medRxiv [Preprint: not peer reviewed]. 17 March 2021. Available from: https://www.medrxiv.org/content/10.1101/2021.03.16.21253686v1.
15. Moustsen-Helms IR, Emborg HD, Nielsen J, et al. Vaccine effectiveness after 1st and 2nd dose of the BNT162b2 mRNA Covid-19 vaccine in long-term care facility residents and healthcare workers—a Danish cohort study. medRxiv [Preprint: not peer reviewed]. 9 March **2021**. Available from: https://www.medrxiv.org/content/10.1101/2021.03.08.21252200v1.
16. Chen RE, Zhang X, Case JB, et al. Resistance of SARS-CoV-2 variants to neutralization by monoclonal and serum-derived polyclonal antibodies. Nat Med **2021**; 27:717–26.
17. Wang P, Nair MS, Liu L, et al. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. Nature **2021**; 593:130–5.
18. Zhou D, Dejnirattisai W, Supasa P, et al. Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera. Cell **2021**; 184:2348–61.e6.
19. Planas D, Veyer D, Baidaliuk A, et al. Reduced sensitivity of SARS-CoV-2 variant Delta to antibody neutralization. Nature **2021**; 596:276–80.
20. Planas D, Veyer D, Baidaliuk A, et al. Reduced sensitivity of SARS-CoV-2 variant Delta to antibody neutralization. Nature **2021**; 596:276–80.
21. Hacisuleyman E, Hale C, Saito Y, et al. Vaccine breakthrough infections with SARS-CoV-2 variants. N Engl J Med **2021**; 384:2212–8.
22. Teran RA, Walblay KA, Shane EL, et al. Postvaccination SARS-CoV-2 infections among skilled nursing facility residents and staff members—Chicago, Illinois, December 2020–March 2021. MMWR Morb Mortal Wkly Rep **2021**; 70:632–8.
23. Stephenson J. COVID-19 vaccinations in nursing home residents and staff give robust protection, though breakthrough infections still possible. JAMA Health Forum **2021**; 2:e211195.
24. Richtel M, Abelson R. Nursing homes confront new Covid outbreaks amid calls for staff vaccination mandates. New York Times. 4 August **2021**. Available at: https://www.nytimes.com/2021/08/04/health/nursing-homes-vaccine-delta-covid.html. Accessed 31 August 2021.
25. Centers for Disease Control and Prevention. COVID data tracker. **2020**. Available at: https://covid.cdc.gov/covid-data-tracker. Accessed 31 August 2021.
26. Mizrahi B, Lotan R, Kalkstein N, et al. Correlation of SARS-CoV-2 breakthrough infections to time-from-vaccine; preliminary study. medRxiv [Preprint: not peer reviewed]. 31 July 2021. Available from: https://www.medrxiv.org/content/10.1101/2021.07.29.21261317v1.
27. Wadman M. A grim warning from Israel: vaccination blunts, but does not defeat Delta. 16 August **2021**. Available at: https://www.science.org/content/article/grim-warning-israel-vaccination-blunts-does-not-defeat-delta. Accessed 31 August 2021.

28. Manisty C, Otter AD, Treibel TA, et al. Antibody response to first BNT162b2 dose in previously SARS-CoV-2-infected individuals. Lancet Lond Engl **2021**; 397:1057–8.
29. Krammer F, Srivastava K, Alshammary H, et al. Antibody responses in seropositive persons after a single dose of SARS-CoV-2 mRNA vaccine. N Engl J Med **2021**; 384:1372–4.
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. Samanovic MI, Cornelius AR, Gray-Gaillard SL, et al. Robust immune responses after one dose of BNT162b2 mRNA vaccine dose in SARS-CoV-2 experienced individuals. **2021**: 2021.02.07.21251311. Available at: https://www.medrxiv.org/content/10.1101/2021.02.07.21251311v3. Accessed 14 December 2021.
32. Lustig Y, Nemet I, Kliker L, et al. Neutralizing response against variants after SARS-CoV-2 infection and one dose of BNT162b2. N Engl J Med **2021**; 384:2453–4.
33. Ebinger JE, Fert-Bober J, Printsev I, et al. Antibody responses to the BNT162b2 mRNA vaccine in individuals previously infected with SARS-CoV-2. Nat Med **2021**; 27:981–4.
34. Goossens ME, Neven KY, Pannus P, et al. The Prior Infection with SARS-CoV-2 study (PICOV) in nursing home residents and staff—study protocol description and presentation of preliminary findings on symptoms. Arch Public Health Arch Belg Sante Publique **2021**; 79:195.
35. Granjon E, Dichtel-Danjoy ML, Saba E, Sabino E, Campos de Oliveira L, Zrein M. Development of a novel multiplex immunoassay multi-cruzi for the serological confirmation of chagas disease. PLoS Negl Trop Dis **2016**; 10:e0004596.
36. Medina LJ, Chassaing E, Ballering G, et al. Prediction of parasitological cure in children infected with *Trypanosoma cruzi* using a novel multiplex serological approach: an observational, retrospective cohort study. Lancet Infect Dis **2021**; 21:1141–50.
37. Mariën J, Ceulemans A, Michiels J, et al. Evaluating SARS-CoV-2 spike and nucleocapsid proteins as targets for antibody detection in severe and mild COVID-19 cases using a Luminex bead-based assay. J Virol Methods **2021**; 288:114025.
38. Selhorst P, van Ierssel SH, Michiels J, et al. Symptomatic Severe Acute Respiratory Syndrome Coronavirus 2 Reinfection of a Healthcare Worker in a Belgian Nosocomial Outbreak Despite Primary Neutralizing Antibody Response. Clin Infect Dis **2021**; 73:e2985–91.
39. Tober-Lau P, Schwarz T, Hillus D, et al. Outbreak of SARS-CoV-2 B.1.1.7 lineage after vaccination in long-term care facility, Germany. Emerg Infect Dis J **2021**; 27:2169–73.
40. Bergwerk M, Gonen T, Lustig Y, et al. Covid-19 breakthrough infections in vaccinated health care workers. N Engl J Med **2021**; 385:1474–84.
41. Canaday DH, Carias L, Oyebanji OA, et al. Reduced BNT162b2 messenger RNA vaccine response in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–naive nursing home residents. Clin Infect Dis **2021**; 73:2112–5.
42. Earle KA, Ambrosino DM, Fiore-Gartland A, et al. Evidence for antibody as a protective correlate for COVID-19 vaccines. Vaccine **2021**; 39:4423–8.
43. Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. Nat Med **2021**; 27:1205–11.
44. Vogel AB, Kanevsky I, Che Y, et al. BNT162b vaccines protect rhesus macaques from SARS-CoV-2. Nature **2021**; 592:283–9.
45. Corbett KS, Flynn B, Foulds KE, et al. Evaluation of the mRNA-1273 Vaccine against SARS-CoV-2 in nonhuman primates. N Engl J Med **2020**; 383:1544–55.
46. McMahan K, Yu J, Mercado NB, et al. Correlates of protection against SARS-CoV-2 in rhesus macaques. Nature **2021**; 590:630–4.
47. 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.
48. Parry HM, Tut G, Faustini S, et al. BNT162b2 vaccination in people over 80 years of age induces strong humoral immune responses with cross neutralisation of P.1 Brazilian variant. Rochester, NY: Social Science Research Network, **2021**. Available at: https://papers.ssrn.com/abstract=3816840. Accessed 8 April 2021.
49. Van Praet JT, Vandecasteele S, De Roo A, De Vriese AS, Reynders M. Humoral and cellular immunogenicity of the BNT162b2 messenger RNA coronavirus disease 2019 vaccine in nursing home residents. Clin Infect Dis **2021**; 73:2145–7.
50. Crotty S. Hybrid immunity. COVID-19 vaccine responses provide insights into how the immune system perceives threats. Science **2021**; 372:1392–3.
51. Keehner J, Horton LE, Pfeffer MA, et al. SARS-CoV-2 infection after vaccination in health care workers in California. N Engl J Med **2021**; 384:1774–5.
52. Kustin T, Harel N, Finkel U, et al. Evidence for increased breakthrough rates of SARS-CoV-2 variants of concern in BNT162b2 mRNA vaccinated individuals. Nat Med **2021**; 27:1379–84.
53. Centers for Disease Control and Prevention. COVID-19 breakthrough case investigations and reporting. **2021**. Available at: https://www.cdc.gov/vaccines/covid-19/health-departments/breakthrough-cases.html. Accessed 5 May 2021.
54. Tsang JS, Dobaño C, VanDamme P, et al. Improving vaccine-induced immunity: can baseline predict outcome?. Trends Immunol **2020**; 41:457–65.
55. Kotliarov Y, Sparks R, Martins AJ, et al. Broad immune activation underlies shared set point signatures for vaccine responsiveness in healthy individuals and disease activity in patients with lupus. Nat Med **2020**; 26:618–29.