Poor antibody response to BioNTech/Pfizer COVID-19 vaccination in SARS-CoV-2 naïve residents of nursing homes

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Summary

Poor antibody responses to COVID-19 mRNA vaccination in SARS-CoV-2 infection naïve residents and some naïve staff members of nursing homes suggest suboptimal protection against breakthrough infection, especially with variants of concern, supporting third dose vaccination for residents of nursing homes.

Abstract

Background

Residents of nursing homes (NH) are at high risk of COVID-19 related morbidity and death and may respond poorly to vaccination because of old age and frequent comorbidities.

Methods

Seventy-eight residents and 106 staff members, naïve or previously infected with SARS-CoV-2, were recruited in NH in Belgium before immunization with two doses of 30µg BNT162b2 mRNA vaccine at day 0 and day 21. Binding antibodies (Ab) to SARS-CoV-2 receptor binding domain (RBD), spike domains S1 and S2, RBD Ab avidity, and neutralizing Ab against SARS-CoV-2 wild type and B.1.351 were assessed at days 0, 21, 28, and 49.

Results

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

Conclusions

The poor Ab responses to mRNA vaccination observed in infection naïve residents and in some naïve staff members of NH 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.

Keywords

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

Introduction

Nursing homes (NH) residents are at a disproportionately high risk of COVID-19 related morbidity and mortality, 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 national rates [5–7].

The success of COVID-19 mRNA vaccination in NH 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 comorbidities 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 comorbidities, 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%) as compared to healthcare workers (90%) one week after the second dose of BNT162b2 mRNA vaccination [15].

Decreased vaccine effectiveness in NH residents may be particularly problematic in the face of emerging SARS-CoV-2 variants that are less susceptible to vaccine-induced neutralizing Ab [16–20]. Breakthrough infections with SARS-CoV-2 variants following complete mRNA vaccination have been reported in healthy adults and more recently, severe COVID-19 and death of NH residents have been reported following breakthrough infections in several countries [21–25]. Breakthrough infections with the SARS-CoV-2 Delta variant are also rising in Israel, with hospitalization being 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 as compared to infection-naïve adults and boosts neutralizing Ab cross-reacting with variants of concern [28–33]. The level of cross-reactive immunity induced by mRNA vaccination in naïve and previously infected NH residents remains poorly documented.

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

Material and methods

Study design and approvals

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

Recruitment and clinical sample collection

SARS-CoV-2 infection-naïve and previously infected residents and staff from two Belgian NHs were recruited. Those with a documented positive RT-qPCR or clinical serology result at baseline were considered previously infected with SARS-CoV-2. Clinical serology consisted of a semi-quantitative anti-RBD Ig ELISA, detecting IgA/IgG/IgM (SARS-CoV-2 total Ig ELISA, Bejing Wantai Biological Pharmacy Enterprise Co., China) using manufacturer-defined cut-off for positivity. Exclusion criteria for NH residents included previous diagnosis of dementia, mini-mental state examination (MMSE) score ≤18/30, and life expectancy <6 months. As described previously, scores from the Clinical Frailty Scale (CFS) and Quality of Life (QoL) were determined for residents at baseline [34].

All subjects were immunized with 2x30µg 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 one and four weeks after the boost (respectively day 28 and day 49). Serum was

separated by blood centrifugation at 1000g for 10min and stored at -20°C for downstream Ab analyses.

SARS-CoV-2 Specific Binding Antibodies

Levels of serum Ab were assessed using a multiplexed immunoassay (Multi-SARS-CoV-2 Immunoassay), developed in collaboration with InfYnity Biomarkers (Lyon, France). This technology was described earlier for *Trypanosoma cruzi* serology and is analogous to the MSD-technology (Mesoscale Discovery) [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, Germany). Individual SARS-CoV-2 antigens included Spike 1 domain (S1, encompassing AA16-685 of S), Spike 2 domain (S2, encompassing AA686-1213 of S), and Receptor Binding Domain (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 antibodies. Serially diluted serum samples were tested against the WHO International standard (NIBSC 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 sera were included on each plate. Test samples, calibrators and controls were incubated in microarray plates for 1h at room temperature (RT) and washed with phosphate-buffered saline with 0.05% Tween 20 (PBST). Next, plates were incubated (1h, RT) with horseradish peroxidase-conjugated goat anti-human IgG and washed with PBST before adding a precipitating TMB solution for 20min (RT, dark). Then, TMB was removed and plates were dried at 37°C for 10min. Microplates were imaged and analyzed using a microplate reader (SciReader CL, Scienion, Germany). Average pixel intensity for each spot was calculated for each antigen/dilution and reported as Mean Pixel Intensity (MPI). MPI was converted to Binding Antibody Units per milliliter (BAU/ml) by interpolating from a four-parameter logistic (4PL) standard curve using GraphPad Prism version 9.0.0 (GraphPad Software, San Diego, California USA) and exported to Microsoft Excel. The dynamic range of each antigen measurement was defined using serial dilutions of positive sera. Only antigen measurements within the dynamic range were considered and multiplied by the dilution factor. Results are reported as BAU/ml. ROC-analyses using an independent population for validation generated cutoff concentrations of 15 BAU/ml, 20 BAU/ml and 20 BAU/ml for

RBD, S1 and S2 Ab, respectively (**Supplementary methods**). Assay performance data and comparison with commercially available immunoassays are presented in Supplementary methods.

SARS-CoV-2 Neutralizing Antibodies

Serial dilutions of heat-inactivated serum (1/50-1/25600 in EMEM supplemented with 2mM L-glutamine, 100U/ml - 100µg/ml of Penicillin-Streptomycin and 2% fetal bovine serum) were incubated during 1h (37°C, 7% CO_2) with 3xTCID100 of (i) a wild type (WT) Wuhan strain (2019-nCoV-Italy-INMI1, reference 008V-03893) and (ii) 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/well) in a 96-well plate and incubated for five days (37°C, 7% CO_2). 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% (NT50), which was used as a proxy for the neutralizing Ab concentration in the sample [37,38].

SARS-CoV-2 RBD-Specific antibody avidity

Bio-layer interferometry measurements were performed with an Octet HTX instrument (Fortébio) using AR2G biosensors. Data analyses were performed using FortéBio Data Analysis 9.0. Kinetic assays were performed at 25-30˚C at a sample plate agitation speed of 1000rpm. Sensors were first activated by immersion in a solution containing 20mM EDC and 10mM s-NHS. Then, 0.05mg/ml RBD antigen in 10mM sodium acetate pH6.0 was loaded for 600sec. After antigen loading, biosensors were immersed in a solution of 1M ethanolamine pH8.5 to prevent non-specific interactions. Antigen loaded AR2G sensors were first dipped in PBS to establish a baseline time curve, and then immersed for 10min in wells containing purified serum IgG at three different dilutions (3-5-8x). Following IgG association, dissociation was monitored for 600sec 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 in R (version 4.0.3). Categorical data were presented as frequencies and percentages, continuous data as means (SD) and geometric means (95% CI). The Kruskall-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 Ab at day 49. Spearman's rank correlation coefficients (rho, ρ) were determined for associations between WT and B.1.351 variant neutralizing Ab, SARS-CoV-2 binding Ab, and Ab avidity.

A 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 NT50. To achieve normality, avidity was $\log_{10}$ and neutralization $\log_2$ transformed. The optimal number of clusters was tested via the k-means (range 1:10) and visually identified with an "elbow" in a plot of variance versus number of clusters. DBSCAN ("dbscan" package) identified clusters within the UMAP reduced dimensions.

Results

The study included 53 SARS-CoV-2 infection-naïve and 25 previously infected NH residents as well as 40 infection-naïve and 66 previously infected staff members. In previously infected subjects, SARS-CoV-2 infection occurred between 151 and 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 suffered multiple co-morbidities 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 **Tab.S1**. At baseline, naïve 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 subjects (**Fig.1a, Fig.S1, Fig.S2**). Primary vaccination induced a significant increase in SARS-CoV-2 Ab in both naïve and previously infected staff and residents, and Ab levels were further boosted following secondary

vaccination at day 21 (**Fig.1a**). Levels of vaccine-induced Ab to RBD and S1 were about seven-fold lower in naïve residents as compared to naïve staff following primary vaccination and two-fold lower after booster vaccination (**Fig.1b**). Between day 28 and day 49, levels of vaccine-induced Ab decreased in naïve staff and increased in naïve residents, indicating a delayed peak antibody response in naïve residents **(Fig.1a)**. Compared to naïve subjects, vaccine-induced Ab levels were markedly higher in both residents and staff previously infected with SARS-CoV-2 (**Fig.1b and Fig.S2**). Notably, Ab levels were similar in previously infected residents and staff already after a single dose of vaccine (**Fig.1b**). Between day 28 and day 49, RBD-specific Ab levels increased in previously infected residents and staff whereas S2-specific Ab decreased during this time-period, suggesting dynamic changes in Ab repertoire following booster vaccination **(Fig.1a)**.

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

The lower levels and avidity of vaccine-induced Ab observed in naïve residents as compared to naïve staff suggested lower neutralizing Ab capacity. To explore this possibility, titers of neutralizing Ab against WT Wuhan strain and B.1.351 variant were measured. Rapid neutralizing Ab responses were induced by vaccination of naïve staff (**Fig.2c**). Neutralizing Ab levels peaked at day 28 and decreased between day 28 and day 49. Slower and less intense neutralizing Ab responses were observed in naïve residents. At day 49, naïve residents had markedly lower levels of neutralizing Ab than naïve staff (**Fig.2d**). Neutralizing Ab were detected before vaccination in 38/66 (58%) previously infected

staff and 16/25 (64%) previously infected residents. In both groups, levels of neutralizing Ab markedly increased following primary vaccination and peaked at day 28, following booster vaccination (**Fig.2c**). At day 49, previously infected subjects had higher levels of neutralizing antibodies and these levels were comparable in previously infected staff and residents (**Fig.2d**). Compared to the wild-type strain, levels of Ab neutralizing the B.1.351 variant were reduced five to ten-fold across study groups (**Fig.2e**). At day 49, only 4/40 (10%) staff and none of the naïve residents had detectable B.1.351 neutralizing Ab, whereas neutralizing Ab were detected in 61/66 (92%) previously infected staff and 21/25 (84%) previously infected residents.

The consistent differences in Ab responses observed between the four study groups suggested a coordinated response to mRNA vaccination across the measured immunological parameters. Indeed, titers of neutralizing Ab against the wild-type strain strongly correlated with RBD, S1 and S2 binding Ab, RBD IgG avidity, and neutralizing Ab to the B.1.351 variant (**Fig.2f**).

To further explore inter-individual variability of this coordinated response, a cluster analysis was performed to reduce the complete dataset to two dimensions and identify groups of subjects who have similar profiles of Ab responses. Five clusters of study participants with distinct Ab levels, avidity, and neutralizing activity at day 49 were identified (**Fig.3a-d**). These clusters were not correlated with age of the study participants (**Fig.3e**). Separate cluster analyses of naïve and previously infected individuals indicated additional clustering within these study groups (**Fig.S3**). Cluster 5 exclusively contained previously infected subjects with high Ab responses and individuals with the highest responses were previously infected residents. In contrast, cluster 1, including the lowest Ab responses, was composed of a mix of mostly naïve residents and naïve staff, indicating that both populations contain low responders to mRNA vaccination. Clusters 2 and 3 included intermediate Ab responses and were composed of a mix of naïve residents, naïve staff and some previously infected staff and residents. The cluster analysis therefore revealed a group of poor Ab responders that not only included naïve residents but also naïve staff.

Discussion

Reports on lower Ab responses to COVID-19 mRNA vaccination in older people and people with chronic comorbidities 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 this study, SARS-CoV-2 infection-naïve NH residents had lower Ab responses to BNT162b2 mRNA vaccination as compared to naïve 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 Ab. Worryingly, none of the naïve residents had detectable neutralizing Ab 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 Ab have been shown to correlate with vaccine efficacy in phase 3 studies across different vaccination platforms [40,42,43]. In addition, data from pre-clinical studies in non-human primates indicate that mRNA vaccine-induced neutralizing Ab 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 comorbidities. This observation is consistent with the robust Ab responses to mRNA vaccination observed in older people living outside NH 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 as compared to naïve subjects. 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 Ab in previously infected as compared to naïve 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 following 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 immunosenescence of elderly populations. In contrast with naïve residents, NH residents previously infected with SARS-CoV-2 may be at particularly low risk of breakthrough infection following mRNA vaccination.

Another important finding of this study is that poor vaccine responders were not limited to naïve residents, but also included healthy naïve 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 the control of the COVID-19 pandemic. These data support the administration of a third dose of mRNA vaccine to further improve protection of NH residents against COVID-19.

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Potential conflicts of interest

The authors declare no conflicts of interest. M.Z. is an employee of InfYnity Biomarkers and was not involved in the production of the study results.

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Table 1. Demographic Characteristics of the Participants, According to Study Group.

	naive staff (N=40)	naive resident (N=53)	infected staff (N=66)	infected resident (N=25)	Total (N=184)	p value
Age, years						<0.001
Mean (SD)	46.8 (10.2)	86.1 (9.0)	46.6 (10.5)	85.0 (8.0)	63.2 (21.6)	
Range	23.0 - 64.0	53.0 - 102.0	22.0 - 68.0	65.0 - 95.0	22.0 - 102.0	
Gender						0.12
Female	29 (72.5%)	37 (69.8%)	56 (84.8%)	16 (64.0%)	138 (75.0%)	
Male	11 (27.5%)	16 (30.2%)	10 (15.2%)	9 (36.0%)	46 (25.0%)	
Ethnicity						0.034
Caucasian	38 (95.0%)	53 (100.0%)	59 (89.4%)	25 (100.0%)	175 (95.1%)	
Other	2 (5.0%)	0 (0.0%)	7 (10.6%)	0 (0.0%)	9 (4.9%)	
BMI, kg/m² *						<0.001
Mean (SD)	27.0 (5.5)	23.3 (5.1)	27.1 (4.7)	22.6 (4.3)	25.4 (5.3)	
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						0.027
Ex-smoker	2 (5.0%)	4 (7.5%)	5 (7.6%)	5 (20.0%)	16 (8.7%)	
Non-smoker	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						< 0.001
less than 30 minutes	6 (15.0%)	27 (50.9%)	7 (10.6%)	12 (48.0%)	52 (28.3%)	
30 to 60 minutes	8 (20.0%)	24 (45.3%)	19 (28.8%)	7 (28.0%)	58 (31.5%)	
at least 60 minutes	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						< 0.001
Very good health	14 (35.0%)	4 (7.5%)	20 (30.3%)	3 (12.0%)	41 (22.3%)	
Good health	22 (55.0%)	33 (62.3%)	39 (59.1%)	10 (40.0%)	104 (56.5%)	
Reasonable health	4 (10.0%)	16 (30.2%)	6 (9.1%)	11 (44.0%)	37 (20.1%)	
Bad health	0 (0.0%)	0 (0.0%)	1 (1.5%)	1 (4.0%)	2 (1.1%)	
Quality of Life index						< 0.001
Mean (SD)	0.9 (0.1)	0.7 (0.2)	0.9 (0.1)	0.8 (0.2)	0.9 (0.2)	
Range	0.7 - 1.0	0.2 - 1.0	0.4 - 1.0	0.4 - 1.0	0.2 - 1.0	
Medication use‡						
Cardiovascular disease	6 (15.0%)	48 (90.6%)	3 (4.5%)	24 (96.0%)	81 (44.0%)	< 0.001
Hypertension	6 (15.0%)	41 (77.4%)	9 (13.6%)	24 (96.0%)	80 (43.5%)	< 0.001
Pain	0 (0.0%)	42 (79.2%)	0 (0.0%)	15 (60.0%)	57 (31.0%)	< 0.001
Diabetes Mellitus	1 (2.5%)	10 (18.9%)	0 (0.0%)	4 (16.0%)	15 (8.2%)	< 0.001
Psychosis	2 (5.0%)	23 (43.4%)	0 (0.0%)	8 (32.0%)	33 (17.9%)	< 0.001
Depression	0 (0.0%)	18 (34.0%)	0 (0.0%)	7 (28.0%)	25 (13.6%)	< 0.001
Pulmonary disease	0 (0.0%)	9 (17.0%)	0 (0.0%)	1 (4.0%)	10 (5.4%)	< 0.001
Allergy	1 (2.5%)	5 (9.4%)	1 (1.5%)	4 (16.0%)	11 (6.0%)	0.032
Neurological disease	0 (0.0%)	7 (13.2%)	0 (0.0%)	2 (8.0%)	9 (4.9%)	0.003
Immunological disorder	0 (0.0%)	0 (0.0%)	1 (1.5%)	0 (0.0%)	1 (0.5%)	0.62
MMSE score†						0.98
Mean (SD)	.	25.4 (3.2)	.	25.9 (3.0)	25.6 (3.1)	
Range	.	18.0 - 30.0	.	18.0 - 30.0	18.0 - 30.0	
Frailty scale†						
Very fit	.	0 (0.0%)	.	1 (4.0%)	1 (1.3%)	0.40
Fit		8 (15.1%)	.	1 (4.0%)	9 (11.5%)	0.09
Managing well	.	18 (34.0%)	.	9 (36.0%)	27 (34.6%)	0.87
Very mild frailty	.	7 (13.2%)	.	3 (12.0%)	10 (12.8%)	0.55
Mild frailty	.	10 (18.9%)	.	4 (16.0%)	14 (17.9%)	0.55
Moderate frailty	.	4 (7.5%)	.	4 (16.0%)	8 (10.3%)	0.23
Severe frailty	.	6 (11.3%)	.	3 (12.0%)	9 (11.5%)	0.39

Data are mean (SD) or n (%). Range denotes the lowest to the highest numerical observation. ANOVA was used for statistical comparisons of numeric variables and chi-squared statistics was used for categorical variables.

**** Data available for 40, 51, 66, 25, and 184 subjects.***

‡ Medication used as a treatment for the listed conditions.

† Mini-mental State Examination (MMSE) and Frailty scale was only asked to residents (N = 78).

Figure legends

Figure 1. SARS-CoV-2 Spike Specific Binding Antibody Responses to BNT162b2 mRNA Vaccination in Residents and Staff of Nursing Homes.

SARS-CoV-2 naïve and previously infected nursing home residents and staff received two doses of 30µg BNT162b2 vaccine on day 0 and day 21 (arrows). The level of spike-specific binding Ab 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 per ml (BAU/ml). Each data point represents a serum sample. Black bars indicate geometric mean titres. Cut-off concentrations are 15 BAU/ml, 20 BAU/ml and 20 BAU/ml for anti-RBD IgG, anti-S1 IgG and anti-S2 IgG, respectively. Statistical significance of differences between time points (**panel A**) and study groups (**panel B**) was determined by the Kruskall-Wallis test by ranks, using the Mann-Whitney U post-hoc test and Benjamini-Hochberg correction for multiple testing (*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$). Not significant comparisons between groups are not indicated in the figure.

Figure 2. Low RBD IgG Avidity and Neutralizing Antibody levels in SARS-CoV-2 Naïve Residents of Nursing Homes.

RBD IgG avidity and neutralizing Ab responses to mRNA vaccination were measured at days 0, 21, 28 and 49 in SARS-CoV-2 naïve and previously infected residents and staff of nursing homes. **Panels A and B.** Avidity of RBD-specific IgG (K_{off} in 1/s). 'N tested' indicates the number of participants with sufficiently high antibody concentrations for avidity testing (panel A). **Panels C/D/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' indicates the number of participants with quantifiable neutralizing Ab (panel C). Black bars indicate geometric mean titers. Statistical significance of differences between time

points and study groups was determined by the Kruskall-Wallis test by ranks, using the Mann-Whitney U post-hoc test and Benjamini-Hochberg correction for multiple testing (*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$). For differences between WT and the B.1.351 variant the Mann-Whitney test was used. Not significant comparisons between groups are not indicated in the figure. **Panel F.** Spearman's rank correlation coefficients (rho, ρ) between titers of neutralizing Ab to WT strain and the other Ab response parameters. Data below or above limits of quantification were excluded (gray dots).

Figure 3. Low Vaccine Responders Include both SARS-CoV-2 Naïve Nursing Home Residents and Staff.

Panel A. Cluster (UMAP) analysis of all study participants with available RBD/S1/S2 binding IgG Ab concentrations, RBD-IgG avidity and SARS-CoV-2 wild type neutralization at day 49. The position of individual participants in variable space 1 and 2 indicates similarities or differences in Ab responses. DBSCAN was used to identify clusters. **Panels B/C/D.** Clusters 1 to 5 are plotted against the RBD binding IgG, RBD IgG avidity and WT neutralizing titers, respectively. **Panel E.** Age of participants included in clusters of antibody responses. Black bars indicate geometric mean titres.

Figure 1

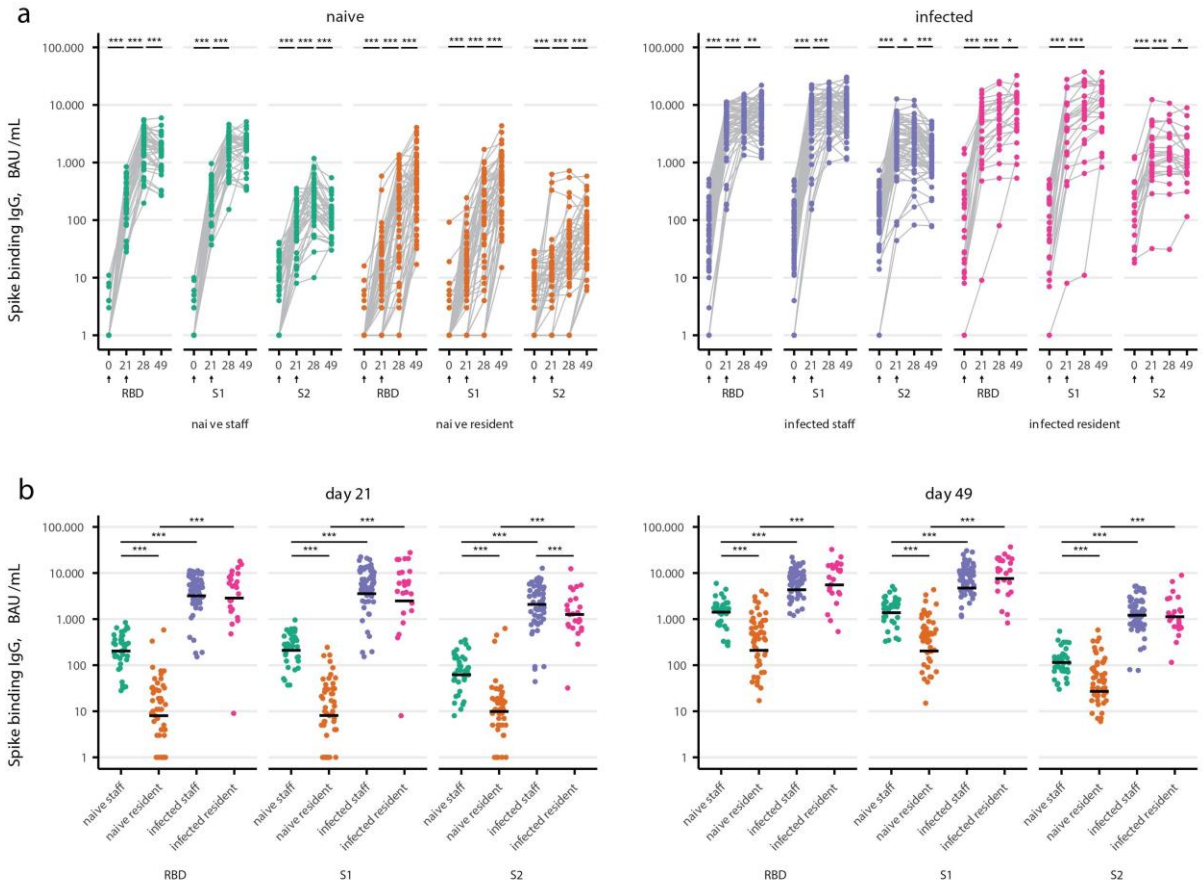

Figure 2

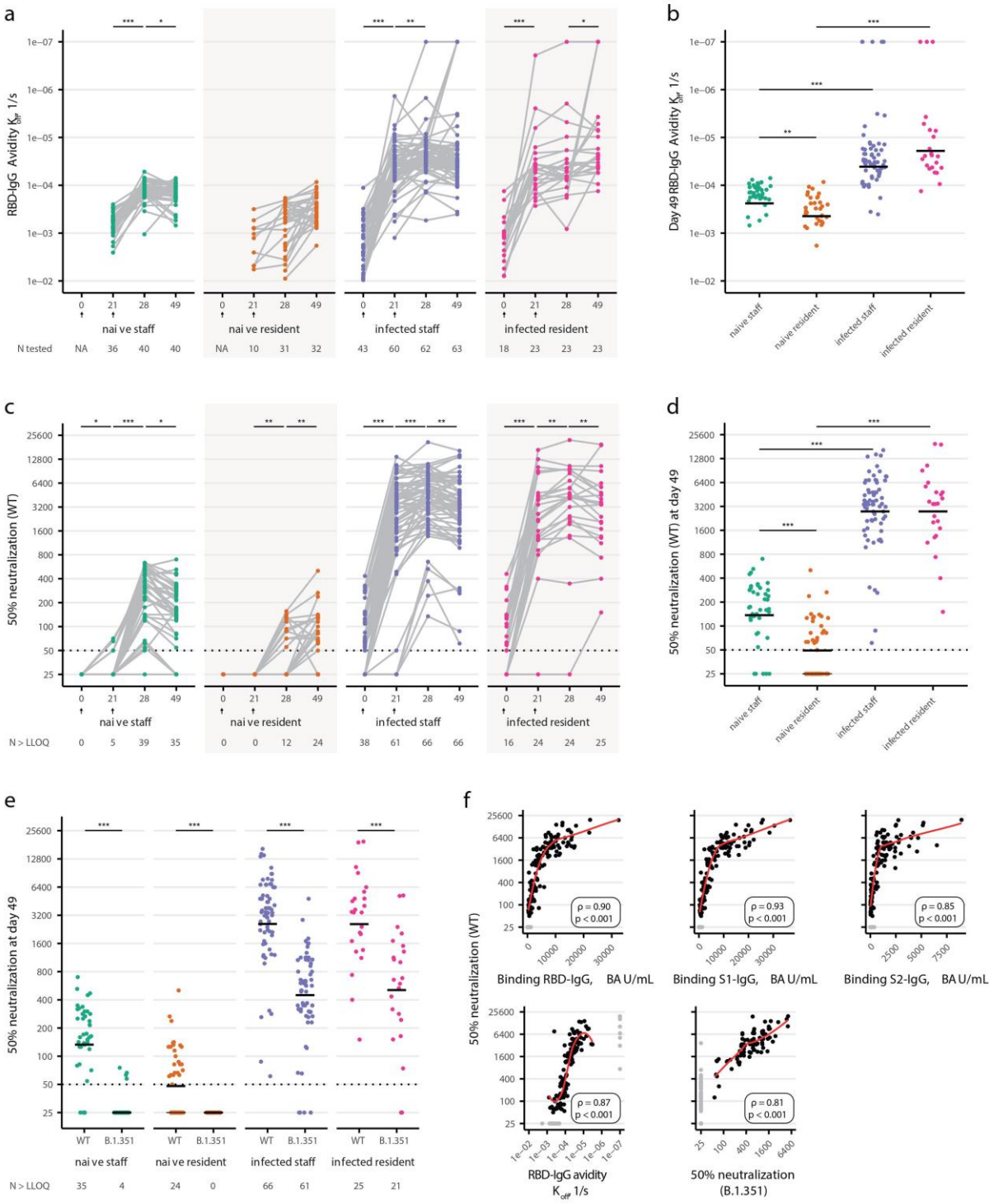
a
RBD-IgG Avidity K_{off} 1/s
naive staff
naive resident
infected staff
infected resident
N tested NA 36 40 40 NA 10 31 32 43 60 62 63 18 23 23 23
b
Day 49 RBD-IgG Avidity K_{off} 1/s
naive staff
naive resident
infected staff
infected resident
c
50% neutralization (WT)
N > LLOQ 0 5 39 35 0 0 12 24 38 61 66 66 16 24 24 25
d
50% neutralization (WT) at day 49
e
50% neutralization at day 49
WT
B.1.351
N > LLOQ 35 4 24 0 66 61 25 21
f
50% neutralization (WT)
ρ = 0.90
p < 0.001
Binding RBD-IgG, BA U/mL
ρ = 0.93
p < 0.001
Binding S1-IgG, BA U/mL
ρ = 0.85
p < 0.001
Binding S2-IgG, BA U/mL
ρ = 0.87
p < 0.001
RBD-IgG avidity
K_{off} 1/s
ρ = 0.81
p < 0.001
50% neutralization
(B.1.351)

Figure 3