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High SARS-CoV-2 antibody levels after three consecutive BNT162b2 booster vaccine doses in nursing home residents

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Abstract

Background As older age and having certain comorbidities can influence humoral responses to vaccination, we studied antibody responses after the COVID-19 booster campaigns in nursing home (NH) residents.

Methods In a two year longitudinal study with Dutch NH residents ($n=107$), aged 50 years and over, we monitored antibody responses in serum prior to and after vaccination with a third, fourth BNT162b2 (wild-type; WT), and a BNT162b2 bivalent (WT/OMI BA.1) fifth vaccine. Data on vaccinations, infections, comorbidities, and, for some participants, clinical symptoms after infection were obtained with questionnaires. Data were compared to antibody responses of BNT162b2-vaccinated, healthier community-dwelling older adults ($n=32$) from the general population.

Results The booster vaccinations substantially increased anti-WT and anti-Omicron SARS-CoV-2 Spike S1 (S1) and Spike protein receptor binding domain (RBD)-antibody concentrations of NH residents. This resulted in comparable antibody levels between NH residents and healthier community-dwelling older adults and between infection-naïve and infected NH residents, and in a decline in treatment duration and clinical symptom severity in SARS-CoV-2-infected NH residents. Between one and twelve months after the bivalent fifth dose, anti-Omicron BA.1 antibody levels of the NH residents waned faster than those against the WT strain.

Conclusions The booster vaccinations upheld humoral responses of NH residents to WT and Omicron SARS-CoV-2. This, in addition to the less virulent circulating strains, decreased symptom severity and treatment durations for SARS-CoV-2-infected NH residents. Boosting this vulnerable group should, therefore, be continued to prevent waning of humoral immunity and achieve sufficient protection especially against newly emerging variants of concern.

Keywords COVID-19, BNT162b2 booster doses, Bivalent, Geriatrics, Antibody responses, Omicron

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Background

Throughout the COVID-19 pandemic the oldest and frailest members of our communities have been hit the hardest, with high COVID-19-related deaths for residents of long-term care facilities (LTCFs) and nursing homes (NH) [1]. With age, there is a higher risk of immune system dysregulation, known as immunosenescence [2], which can increase the risk for severe outcomes of SARS-CoV-2 infection and also translate to delayed and reduced antibody responses to novel antigens in older persons (>60 years) compared to younger adults (20–40 years) [3]. Further, co-morbidities prevalent in older populations, such as renal disease and diabetes, have also been linked to a reduced antibody response [4]. In the Netherlands, the NH residents had higher rates of hospitalization and COVID-19-related deaths than the rest of the population [5].

The first two COVID-19 vaccine doses appeared to give less severe post-vaccination breakthrough infections in the older adult populations [6]. However, reported antibody responses after the primary vaccinations were still lower in NH residents and older adults compared to healthcare workers and younger controls [7]. Therefore, a COVID-19 wild-type (WT) monovalent booster vaccination (third dose) was offered to NH residents approximately ten months after completion of the primary series in the Netherlands. Afterwards, this vulnerable population was offered a second monovalent booster (fourth dose) and, subsequently, an updated bivalent booster containing both WT and Omicron BA.1 (fifth dose). Recent publications report on the antibody responses to the WT SARS-CoV-2 strain of LTCF or NH residents vaccinated with a third or fourth dose [8–11]. To the best of our knowledge, only limited longitudinal information is available on humoral responses of NH residents who received a fifth vaccination dose [12–16], while this information is crucial for assessing the long-term effectiveness of sequential booster vaccines for this vulnerable population.

We aimed to evaluate the impact of three subsequent booster vaccinations (two times BNT162b2 and one time BNT162b2 bivalent (WT/OMI BA.1)) on Dutch NH residents by reporting on the dynamics of antibody responses to SARS-CoV-2 WT and Omicron until one year after the last vaccine dose. We also report on clinical symptoms of breakthrough infections and on a comparison of humoral responses between the NH residents and healthier community-dwelling older adults of the general population who were vaccinated according to the same vaccination scheme.

Methods

Study population

Nursing home cohort

A total of 107 NH residents, aged 50 years or older and vaccinated during the primary COVID-19 vaccination series, were enrolled into this observational study; 18 from a nursing home in the northwest of the Netherlands (single location) and 89 from ten locations in the south of the Netherlands. Nursing home doctors determined if residents were mentally competent and able to provide informed consent themselves. If not, medical ethical approval for study participation was not allowed.

All residents received BNT162b2 (Comirnaty, Pfizer/BioNTech) homologous vaccination series of two doses which were given about four weeks apart (January-March 2021). NH residents also received a third (November-December 2021 or February 2022 for five (5%) SARS-CoV-2 individuals) and a fourth BNT162b2 vaccination (March 2022 or May/June 2022 for 24 (22%) SARS-CoV-2 individuals), which was followed by a fifth BNT162b2 bivalent (WT/OMI BA.1) vaccination in October 2022.

The nursing home clinicians decided which residents of their homes were eligible for possible participation on a somatic basis. Nursing home residents filled in a participation card. The duration of the study was 2 years and one month, starting in September 2021 with the last sampling timepoint in October 2023. The first sampling timepoint was six months after completion of the primary vaccination series and three months before the first booster dose. Afterwards, sampling timepoints were one month before and after the administration of a vaccine dose. Additionally, samples were collected six and twelve months after the bivalent vaccine as the fifth vaccine dose (Fig. 1A).

Blood and data sampling nursing home residents

At each timepoint, a fingerprick whole blood sample was drawn in MiniCollect® serum/gel tubes of 0,5 ml/0,8 ml with a clotting activator (Greiner Bio-one Netherlands) at the nursing home locations by a dedicated study nurse. In addition questionnaires covering demographic factors, comorbidities, COVID-19 vaccination brand and dates and, if applicable, information on SARS-CoV-2 confirmatory testing (PCR testing of a nose/throat swab) were filled in by the nursing home clinicians. By fingerpricks a volume of 200–400 ul whole blood was sampled. Samples were kept at RT and transported to the laboratory. After centrifugation the tubes for 10 min at 3000 rpm, a serum volume of about 100–200 ul per sample have been stored at -20 °C. The volume of just a few blood samples resulted in serum volumes lower than 100 ul or even too low for further analysis.

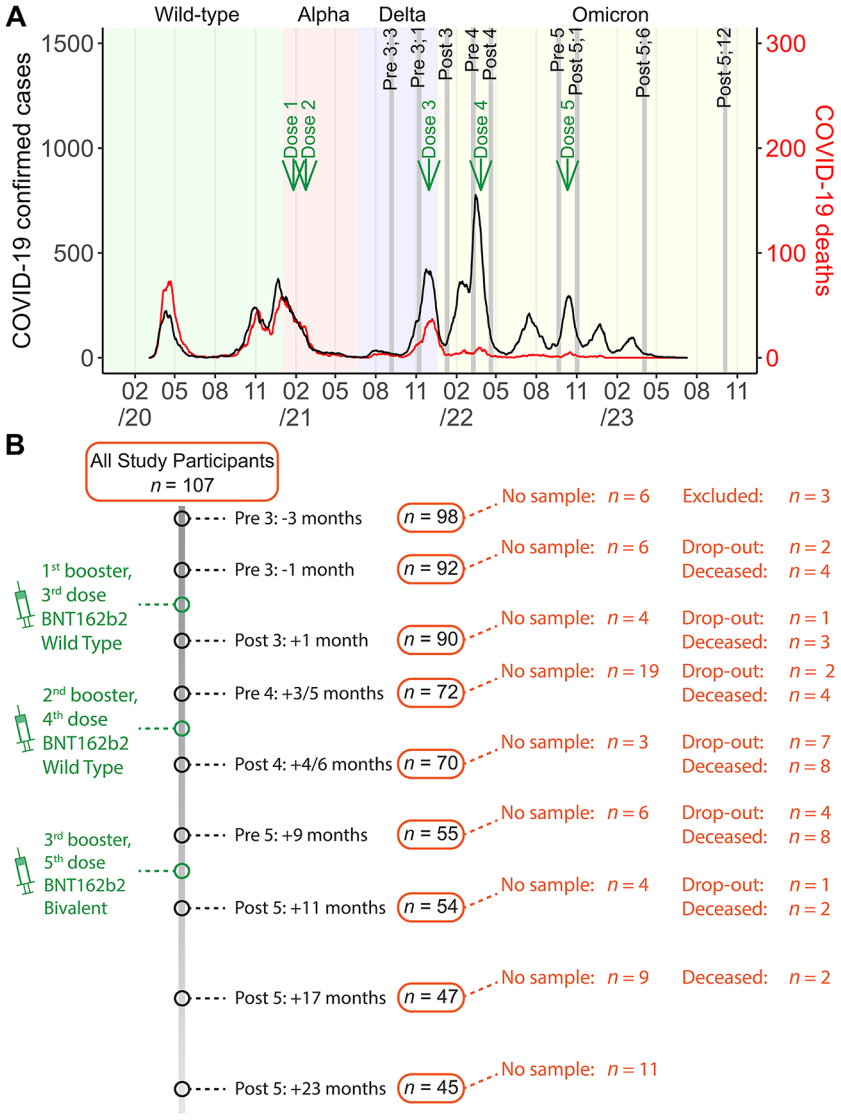

Fig. 1 Study design with number of study participants, vaccine and sampling timepoints, and the different dominant SARS-CoV-2 variant periods. (**a**) Information on the number of COVID-19 confirmed cases and deaths of older adults aged 70 years or over who live in a nursing home in the Netherlands. Data was collected until the 11th of July 2023 and obtained from data.rivm.nl/covid-19/. Additionally, information on vaccine dose administration, sampling timepoints, and circulating SARS-CoV-2 variants of concern have been indicated in the graph. (**b**) A flowchart depicting vaccine dose administration and sampling timepoints together with information on the number of study participants per timepoint

SARS-CoV-2 infection-related clinical data nursing home residents

Clinical data of NH residents from eight locations in the south of the Netherlands with a test-confirmed SARS-CoV-2 infection (n=35) were collected by the nursing homes clinicians until the end of the study and independently reviewed by two healthcare workers. This included use and duration of dexamethasone, fraxiparine, oxygen, and IV fluids. Data regarding the presence and duration of fever (>37.5 °C), maximum temperature recorded during the fever episode (measured every morning and evening) were also recorded, together with the date(s) of positive COVID-19 test(s) and the duration of isolation periods. These data were grouped based on circulating SARS-CoV-2 variants (Fig. 1A); WT (before the primary series), Delta (timepoints before the third dose), or Omicron (timepoints after the third dose until one year after the fifth dose). None of the participants had a SARS-CoV-2 infection when the SARS-CoV-2 Alpha variant was dominant.

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Cohort of community-dwelling older adults from the general population

Fingerprick blood samples of participants of the general population and part of the Doetinchem Cohort Study

(DCS) [17] who were 50+years of age and also received either three, four, or five BNT162b2 vaccination doses, similar to the NH residents, were included in the community-dwelling older adult cohort[18]($n=32$). Participants of the general population indicated the specific vaccine received at every study timepoint in the questionnaires. In addition, vaccination was controlled by the national vaccination registry (CIMS). Timepoints of blood and data sampling completely matched that of the NH cohort, only the sampling timepoint before the fourth vaccine dose was not available due to logistical constraints. Questionnaires covering demographic factors, comorbidities, COVID-19 vaccination brand and dates and, if applicable, information on SARS-CoV-2 confirmatory testing (PCR testing of a nose/throat swab) were filled at every study timepoint.

The age of all study participants was the age in years at first BNT162b2 vaccination and sex is defined as the sex assigned at birth.

Antibody detection

Anti-SARS-CoV-2 Spike S1 (S1)-, Nucleoprotein (N)-, and Spike protein receptor binding domain (RBD)-specific immunoglobulin G (IgG) antibody concentrations against the WT strain were measured using a previously described multiplex bead-based assay [19]. In short, serum samples (25 µl) diluted in SM01 buffer (Surmodics, Eden Prairie, MN, USA) containing 2% fetal calf serum were mixed with beads coated with either SARS-CoV-2 WT monomeric spike S1 (40591-V08H), RBD (40592-V08B), or N (40588-V08B) proteins or recombinant SARS-CoV-2 B1.1.529.1, also referred to as BA.1, (Omicron) Spike S1 (40591-V08H41) or RBD (40592-V08H121) proteins (all Sino Biological, Beijing, China) and incubated for 45 min at room temperature in the dark while rotating (750 rpm). Thereafter, samples were washed three times with phosphate-buffered saline, incubated with phycoerythrin-conjugated goat anti-human IgG (Jackson ImmunoResearch, West Grove, PA, USA) for 30 min, and, thereafter, washed and analyzed with an LX200 (Luminex, Genk, Belgium). Concentrations were interpolated using a 5-parameter logistic fit using pooled sera calibrated against the WHO international standard (NIBSC 20/136) for WT antigens and expressed as binding antibody units per ml (BAU/ml). The threshold for seropositivity was set at 10.1 BAU/ml for S1[20], 14.3 BAU/ml for N [18], and 30.0 BAU/ml for RBD [20]. Anti-Omicron antibody concentrations were expressed as arbitrary units per ml (AU/ml).

SARS-CoV-2 infection status definition

Individuals were classified as either infection-naïve or SARS-CoV-2-infected. Participants were considered to have had a SARS-CoV-2 infection when a positive test was reported in the questionnaire, when their concentration of anti-N antibodies exceeded the cut-off value of 14.3 BAU/ml or were re-infected when this anti-N antibody concentration was four-fold higher than the previously reported concentration. Infections scored at a given timepoint took place before the given timepoint. A naïve participant who became infected got switched to the SARS-CoV-2-infected group during the further study follow up. Eleven participants were infected twice with SARS-CoV-2 during the study. Individuals that had anti-N antibody seroconversion without reported SARS-CoV-2 infection at the first timepoint were excluded from the study given that the exact date of the infection was unknown. As the period in which the infection occurred was known, the dominant SARS-CoV-2 variant (WT, Delta, or Omicron) of that period was also known from the information on the number of COVID-19 confirmed cases and deaths of older adults aged 70 years or over who live in a nursing home in the Netherlands. Data was collected until the 11th of July 2023 and obtained from data.rivm.nl/covid-19/ (Fig. 1).

Statistical analyses

Statistical analyses and visualization were performed in R 4.4.0 and R studio 2024.04.1. The distribution of the data was assessed with a Shapiro-Wilk test and QQ-plots. When the data did not pass these checks or had a small group size, a non-parametric statistical test was used. Anti-WT and anti-Omicron antibody concentrations were both expressed in AU/ml for the Omicron versus WT S1-IgG ratio calculations. A non-parametric Kruskal-Wallis ANOVA with a Dunn's multiple comparison corrected post-hoc test (Holm's method; Rstatix v 0.7.0) was used to assess differences in antibody concentrations or ratio's. Significant differences of discrete clinical data were evaluated with a pairwise chi-square test (Rstatix).

We constructed two linear mixed effects models (LMEMs) to investigate covariate influence on the log10-transformed anti-S1 antibody concentrations in infection-naïve and infected individuals separately (lme4 v1.1-30). Using these models potential associations between clinical data of the NH residents collected by the nursing homes clinicians (Table S2) and antibody concentrations at all study time points were further assessed. For both models, the fixed effects were specified as age, sex, using prednisone, and whether someone had diabetes, cancer, rheumatoid arthritis, or any lung, bowel, immune, kidney, cardiovascular, neurological, skin, or mental disease. Participant numbers and sampling timepoints were specified as random effects both models model. For the LMEM of infected individuals, an additional random effect was specified as the timepoint that an individual became infected. A forest plot was generated displaying the coefficient estimates and multiple

comparison testing adjusted *p*-values (Holm's method) for each model (sjPlot v2.8.11).

LMEMs were also constructed to assess changes in log10-anti-S1 antibody concentrations following the third, fourth, and fifth vaccine dose in infection-naïve and infected individuals separately. Only individuals that received a third, fourth, and fifth vaccine dose were included in the models. For these antibody decay and increase LMEMs, we excluded the study participants who had an SARS-CoV-2 infection during the analyzed time period. In the constructed LMEMs, the time since the vaccine dose was specified as a fixed effect and the number of participants was specified as a random effect. Differences between infection-naïve and previously infected individuals in antibody decay rates and rates in which antibody levels increased were assessed with a one-way ANOVA (lmerTest v3.1-3). Antibody half-life, in time in days since the last vaccine dose, was calculated with the predicted anti-S1-antibody concentrations of the fitted LMEM. Differences between slopes was tested with a Welch modified two-sample t-test (BSDA v1.2.2).

Other R packages that were used for data analysis and visualization were; dplyr v1.0.10, tidyr v1.2.1, tidyverse v1.3.2, reshape2 v1.4.4, zoo v1.8-10, and janitor v2.2.0 for data manipulation/organization, gglot2 v3.4.0, RColorBrewer v1.1-3 and ggh4x v0.2.3 for data visualization, and qwraps2 v0.5.2 for generating tables.

Analyses with *p*-values of <0.05 were considered statistically significant.

Ethical approval Ethical approval and oversight was carried out by the Medical Research Ethics Committee (METC) NedMed, Utrecht (METC number: 21/056, study number: NL76551.041.21, EudraCT: 2021-001976-40). All participants provided written informed consent.

Role of the funding source The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Demographics of the cohorts

One hundred seven BNT162b2 vaccinated NH residents were enrolled (Fig. 1B) of which 98 (92%) individuals were part of the first timepoint. Three (3%) NH residents were excluded from the study due to anti-N antibody seroconversion without reported SARS-CoV-2 infection (Figure S1) and a further six (5%) individuals had a missing serum sample or insufficient sample volume at the first timepoint. The median age of the NH residents was 85 years (interquartile range (IQR), 77–89), 70 (67%) were over 80 years of age, 76 (73%) were female, and 93 (62% with two or three conditions and 27% with more than four conditions) had multiple comorbid conditions (Table S1 and S2). During the study period, 31 (29%) of the NH residents passed away. Information on average time between vaccine doses is enlisted in Table S1. For NH residents the time interval between the first two vaccines was 28 days. This was 35 days for the community dwelling older adults. In the Netherlands, a four to five weeks interval between the first and second vaccine had been adopted.

Eight (8%) NH residents used prednisone (5 mg/day for long-term usage (>10 days) or 20 or 30 mg/day for short-term usage (<10 days)). Only sex associated with anti-S1 antibody concentrations of infection-naïve NH residents (coefficient estimate, 0.45 BAU/ml [95% confidence interval (CI), 0.17–0.72], $p=0.021$; Figure S2A); infection-naïve NH males had significantly lower anti-WT S1 antibody concentrations than females over all timepoints, despite the low numbers of males (Figure S2B). The number of comorbidities between male and female infection-naïve NH residents did not influence this effect and the two groups were similar in age.

To compare the humoral responses of NH residents to those of healthier older adults, we included a small cohort of community-dwelling older adults who had a median age of 71 years (IQR, 67, 78) and had zero or one comorbid condition(s) (Table S3).

SARS-CoV-2 antibody concentrations of nursing home residents were substantially boosted and comparable to those of healthier community-dwelling older adults

Before the first booster dose and just six to seven months after the primary vaccination series, anti-S1 antibody concentrations were generally low for the NH residents (median 42.4 BAU/ml [IQR, 16.8-120.4]; Fig. 2A). After the third vaccine (first booster) dose, anti-WT S1 antibody levels significantly increased a 14-fold and even a 98-fold for infected and infection-naïve NH residents respectively, compared to values before the third dose ($p=0.014$ and $p<0.001$).

Anti-WT S1 antibody levels of both infection-naïve and infected NH residents also significantly increased after dose four ($p=0.043$ and $p=0.001$, respectively), whereas these subsequently decreased in both groups ($p=0.081$ and $p=0.007$, respectively) in the three to five months between the fourth and the fifth vaccination.

The fifth vaccine dose (first bivalent vaccine) did not significantly increase anti-WT S1 antibodies (infection-naïve; $p=0.400$ and infected; $p=0.277$), but did significantly increased anti-S1 antibody levels against Omicron with a 5.3-fold for infection-naïve and with a 2.3-fold for infected NH residents ($p<0.001$ and $p=0.002$, respectively; Fig. 2B) compared to pre-dose five values. Consequently, infection-naïve and WT- or Delta-infected NH residents had a higher Omicron versus WT S1-IgG ratio after the fifth dose compared to before this dose

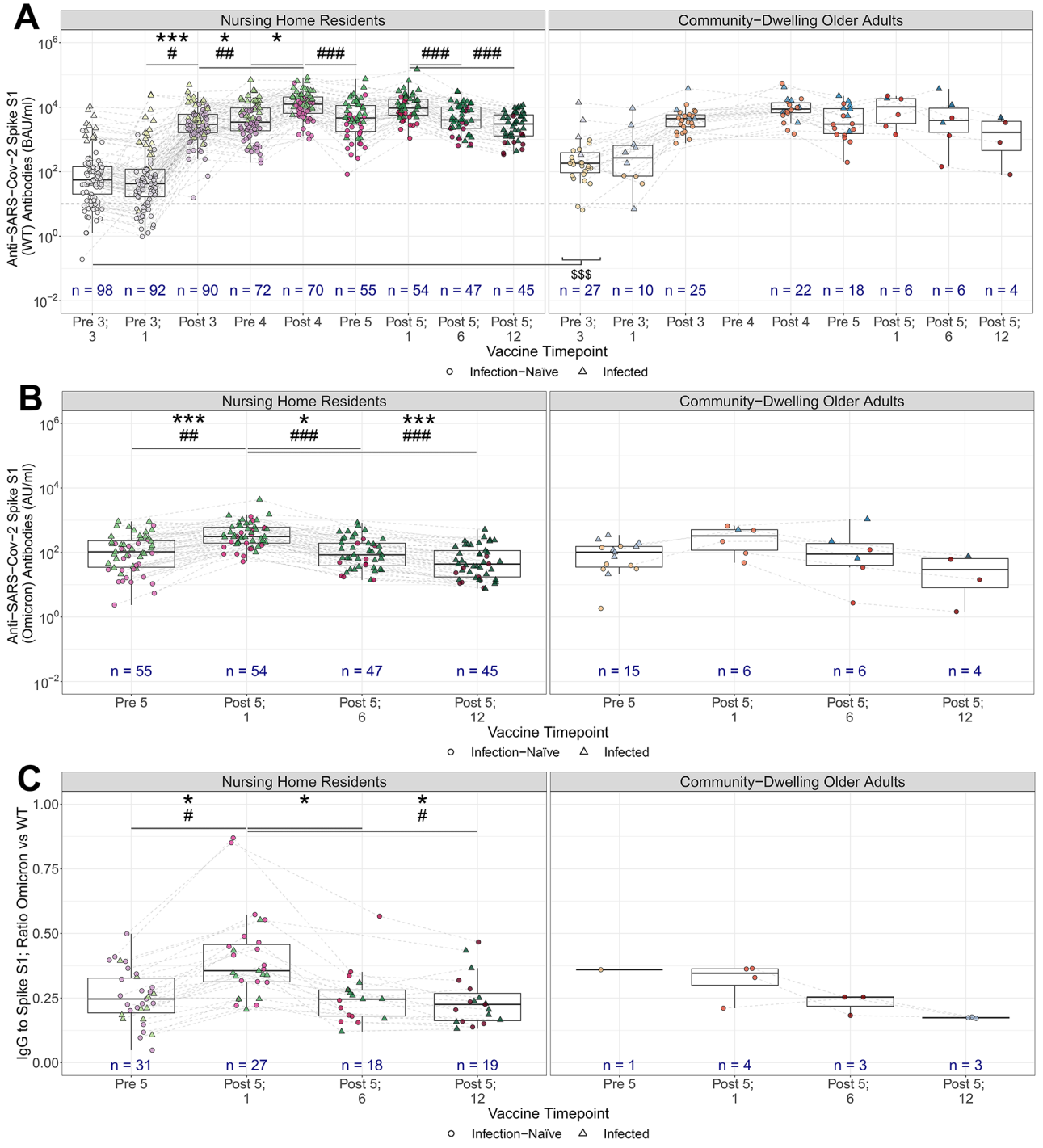


Fig. 2 Anti- SARS-CoV-2 Spike S1 antibody levels of nursing home (NH) residents substantially boosted. (**a**) Antibody responses to SARS-CoV-2 Spike S1 wild-type (WT) protein of NHNH residents (left) and community-dwelling older adults (right) before and after the third, fourth, or fifth BNT162b2 vaccine doses were administered. Antibody concentrations are expressed in international binding antibody units (BAU) using the 20/136 NIBSC standard and the horizontal dashed line represents the threshold for seropositivity to Spike S1. (**b**) Anti-SARS-CoV-2 Spike S1 (Omicron BA1.1.529) antibody levels before and after the bivalent fifth vaccine dose of NH residents (left) or community-dwelling older adults (right). Antibody concentrations are expressed in arbitrary units (AU). (**c**) Ratios of anti-S1 antibodies against Omicron versus anti-WT S1 antibodies before and after the bivalent fifth vaccine dose of NH residents (left) or community-dwelling older adults (right) (COA). Infection-naïve individuals are indicated with an circle (NH in red, COA in orange) and SARS-CoV-2 infected persons are shown with an triangle (NH in green, COA in blue). Data are medians with 75% quartiles and the number of study participants per timepoint are summarized within the graph. Significance for statistical comparisons with data of infection-naïve individuals are marked by an asterisk, whereas for infected persons a hashtag was used. Statistical comparisons between NH residents and community-dwelling older adults are marked by a dollar sign. ***, ###, or $$$ $p < 0.001$, ## $p < 0.01$, and * or # $p < 0.05$

($p=0.018$ and $p=0.037$, respectively; Fig. 2C). This was solely due to the BNT162b2 bivalent booster dose as no significant higher Omicron versus WT S1-IgG ratio was observed before or after monovalent vaccine dose four (Figure S3A). Between one and six months after the fifth dose, anti-S1 antibody levels against WT and Omicron decreased for infection-naïve (Omicron; $p=0.042$; Fig. 2B) and for infected NH residents (WT; $p=0.007$ and Omicron; $p=0.008$; Fig. 2A-B). Also, the Omicron versus WT S1-IgG ratio of infection-naïve and WT- or Delta-infected NH residents became smaller six months after the fifth vaccine dose compared to one month after this dose ($p=0.026$ and $p=0.075$, respectively; Fig. 2C). One year after the fifth dose, antibody levels against both WT and Omicron decreased even further for infection-naïve (Omicron; $p<0.001$; Fig. 2B) and infected NH residents (both $p<0.001$; Fig. 2A-B) compared to one month after the fifth dose.

The antibody levels for infection-naïve NH residents differed from those of community-dwelling infection-naïve older adults three months before the first booster dose (median 45.6 BAU/ml [IQR, 14.3-100.5] vs. median 151.5 BAU/ml [IQR, 79.7-216.5], respectively; $p<0.001$; Fig. 2A). Afterwards, the anti-WT S1 antibody levels did not significantly differ between both infection-naïve and infected NH residents and community-dwelling older adults. Also, anti-Omicron S1 antibody concentrations and the Omicron versus WT S1-IgG ratio before and after the fifth vaccination were comparable between NH residents and the community-dwelling older adults (Fig. 2B-C).

Similar trends were observed for anti-RBD antibody concentrations before and after the three booster doses (Figure S4A-B), only no significant difference in Omicron vs. WT ratio of anti-RBD antibody levels between pre- and post- vaccine dose four and five was observed (Figure S32B and S4C). Overall, the booster vaccine doses resulted in an increase of antibody levels in NH residents, making them comparable to those of healthier community-dwelling older adults.

SARS-CoV-2-related clinical symptoms and antibody responses of NH residents with a SARS-CoV-2 (breakthrough) infection

Although vaccinated against SARS-CoV-2, 58 (56%; $n=104$) NH residents experienced a breakthrough SARS-CoV-2 infection during the study of which eleven (11%; $n=104$) individuals got even infected twice. In total, sixteen (15%; $n=104$) individuals were infected prior to being vaccinated. Only 9 (20%; $n=45$) of NH residents were infection-naïve at the end of the study (Table S4).

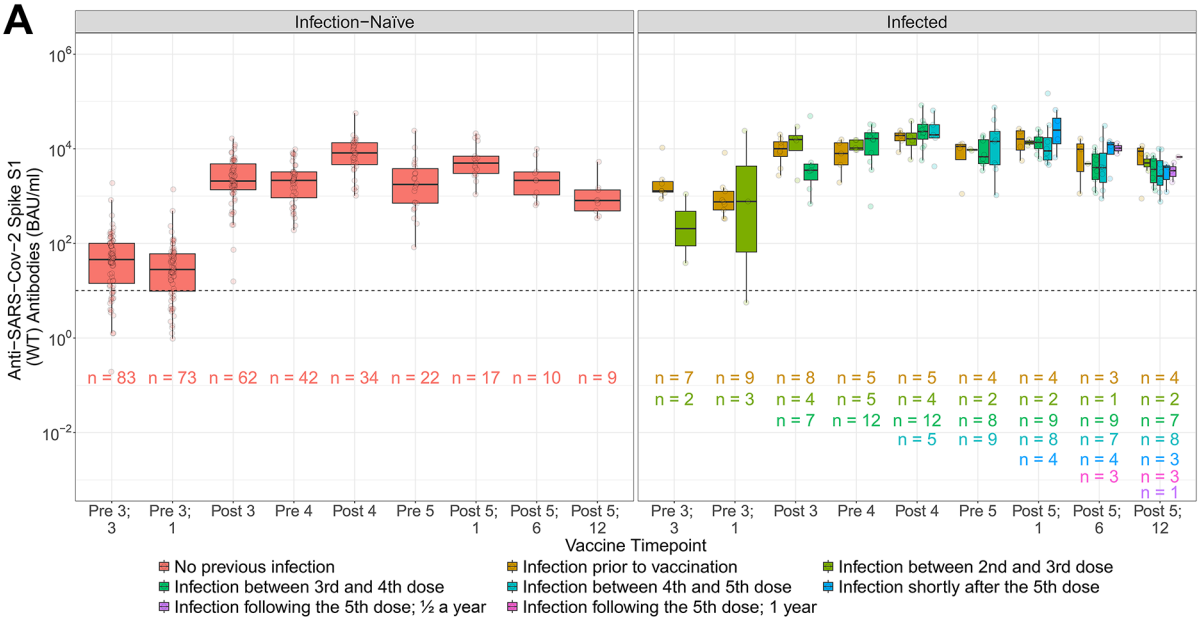
Most NH residents (93%) with a SARS-CoV-2 infection before the primary series experienced fever (>37.5 °C; WT period; Table 1) and these individuals had higher anti-WT S1 antibody concentrations than infection-naïve NH residents before the third dose (both $p<0.001$; Fig. 3A-B). Also, after the primary series and before the third dose, all SARS-CoV-2 breakthrough-infected NH residents had fever (Delta period; Table 1), but they did not have different anti-WT S1 antibody concentrations post vaccination as those of infection-naïve NH residents (Fig. 3A-B). After the third vaccine dose, fewer infected NH residents (64%) experienced fever and the isolation time decreased significantly compared to before the first two doses (vs. WT $p=0.001$; Omicron period; Table 1). At this timepoint, anti-WT S1 antibody concentrations of individuals infected before vaccination still differed from those of infection-naïve NH residents ($p=0.018$;

Table 1 Syndromic profile and medication usage of nursing home residents with a SARS-CoV-2 Wuhan infection or Delta or omicron breakthrough infection

	Prior to vaccination Wuhan ($n=16$)	Before the third dose Delta ($n=6$)	After the third dose Omicron ($n=11$)	After the fourth dose Omicron ($n=9$)	After the fifth dose Omicron ($n=6$)
Fever > 37.5°C	14 (93%)	6 (100%)	7 (64%)	6 (67%)	5 (83%)
Fever > 37.5°C duration	4.5 (2.5, 8.3)	4.3 (2.5, 6.4)	3.5 (2.3, 4.3)	**1.0 (1.0, 1.8)[a]****	3.0 (1.0, 3.0)
Placed in isolation	16 (100%)	6 (100%)	11 (100%)	9 (100%)	6 (100%)
Isolation duration	24.5 (20.3, 29.0)	16.8 (13.6, 18.8)	**10.0 (9.5, 11.0)[a]****	**6.0 (5.0, 9.0)[a]***, [b]***	**5.5 (5.0, 8.3)[a]***, [b]***
Oxygen used	9 (60%)	2 (33%)	3 (27%)	1 (11%)	2 (33%)
Oxygen duration	15.5 (9.0, 19.0)	18.5 (12.3, 24.8)	3.5 (3.3, 6.5)	1.0 (1.0, 1.0)	6.5 (6.3, 6.8)
IV fluids used	2 (13%)	0	0	0	0
IV fluids duration	7.3 (4.6, 9.9)	NA	NA	NA	NA
Anticoagulant used	2 (13%)	0	0	0	0
Anticoagulant medication duration	11.0 (11.0, 11.0)	NA	NA	NA	NA
Dexamethasone used	4 (27%)	1 (17%)	0	0	0

Data of 35 infected NH residents was grouped based on circulating SARS-CoV-2 variant being either Wuhan, Delta, or Omicron. Data are n (%) or median (interquartile range). Data in bold is significant. * $p<0.05$, ** $p<0.01$, and *** $p<0.001$ compared to [a]Wuhan- or [b]Delta-infected NH residents

A



B

	Infection-Naïve	Infection prior to vaccination	Infection between 2nd and 3rd dose	Infection between 3rd and 4th dose	Infection between 4th and 5th dose	Infection shortly after the 5th dose	Infection after the 5th dose; ½ a year	Infection after the 5th dose; 1 year
Pre 3; 3	45·6 (14·3, 100·5)	**1288·6 (1220·8, 2030·8)*****	570·8 (304·6, 837)					
Pre 3; 1	28·1 (9·8, 60·3)	**756·1 (510·2, 1264·1)*****	770·6 (388·1, 12406·3)					
Post 3	2083·3 (1365·9, 4816·5)	**10129·3 (7243·9, 14100·5)***	15716·4 (11787·2, 19701)	3521·2 (2400·8, 4763·3)				
Pre 4	2149·9 (924·9, 3251·9)	7962·7 (4586·5, 13378·7)	**10349·9 (9414·1, 15347·5)***	**16412 (7499·9, 21818)*****				
Post 4	8175·8 (4658·9, 13445·5)	19153·6 (14808·7, 21515·7)	16358·2 (12729·6, 22971·4)	**23227·9 (16244·3, 32798)****	19757·4 (16976·4, 32278·1)			
Pre 5	1764·8 (708·8, 3836)	11280·6 (8025·3, 12390·4)	9512·9 (9318·8, 9707·1)	6815·5 (4863·7, 15579·1)	**14322·8 (4609·2, 23121·3)***			
Post 5; 1	4988·1 (3026·9, 6992·7)	17180·9 (9775·5, 24005·5)	13747·7 (12733·4, 14762)	13648·4 (10513·9, 21354·6)	9082 (5835·9, 18380·5)	2733·4 (13588·6, 45639·2)		
Post 5; 6	2148·2 (1064·1, 3241·4)	9770·4 (5449·5, 13110·6)	4836·1 (4836·1, 4836·1)	4019·7 (2248·4, 7767·9)	3994·7 (2118·4, 9978)	1236·5 (9154·6, 13581·8)	10346 (9129·8, 11697·8)	
Post 5; 12	807·1 (487·4, 1349)	8959·2 (6157·8, 10379·2)	5399·1 (4415·5, 6382·7)	3674·25 (1979·2, 6162·5)	2709·8 (1733·7, 5941·3)	4091·3 (2655·4, 4392·6)	3443·1 (2705·2, 4254·5)	6723·7 (6723·7, 6723·7)

Fig. 3 (See legend on next page.)

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Fig. 3 Antibody responses to wild-type SARS-CoV-2 Spike S1 protein of infection-naïve or SARS-CoV-2 (breakthrough)-infected nursing home residents. (**a**) Boxplots of infection-naïve individuals are shown in red (left) and of infected persons (right) are yellow for an infection before vaccination, light green for an infection between the second and third dose, dark green for an infection between the third and fourth dose, turquoise for an infection between the fourth and fifth dose, blue for an infection shortly after the fifth dose, purple for an infection half a year after the fifth dose, and pink for an infection one year after the fifth dose. Antibody concentrations are expressed in international binding antibody units (BAU) using the 20/136 NIBSC standard and the horizontal dashed line represents the threshold for seropositivity to Spike S1. Data are medians with 75% quartiles and the number of study participants per timepoint and per infection status group are summarized within the graph. (**b**) A table with median anti-S1 antibody concentrations with interquartile ranges of the NH residents per timepoint and per infection status group. Data in bold are significant. *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$

Fig. 3A-B). SARS-CoV-2-infected individuals received IV fluids, anticoagulants, and dexamethasone, as a treatment of a SARS-CoV-2 infection only before the third vaccine dose (WT and Delta period; Table 1). Before vaccine dose four, anti-WT S1 antibody concentrations of individuals with a breakthrough SARS-CoV-2 infection between the second and third or third and fourth vaccine dose were significantly higher than those of infection-naïve NH residents ($p=0.036$ and $p<0.001$, respectively; Fig. 3A-B). The latter difference was still observed after the fourth vaccine dose ($p=0.003$; Fig. 3A-B). Furthermore, NH residents with a breakthrough SARS-CoV-2 infection between the fourth and fifth vaccine dose had significantly higher anti-WT S1 antibody concentrations than infection-naïve individuals before the fifth vaccine dose ($p=0.039$; Fig. 3A-B), but these individuals did have a shorter duration of fever and isolation than nursing home residents who experienced an infection before the third dose (vs. WT $p=0.010$, vs. WT $p<0.001$, and vs. Delta $p=0.011$, respectively; Omicron period; Table 1). However, after the fifth vaccine dose (bivalent), infection-naïve NH residents had similar anti-WT S1 antibody concentrations as previously infected individuals and the isolation duration shortened even more compared to before the third dose (vs. WT $p<0.001$ and vs. Delta $p=0.011$; Omicron period; Table 1).

SARS-CoV-2 spike S1 antibody increase and decay before and after subsequent booster doses

Lastly, we examined antibody kinetics pre- and post-vaccine dose three, four, and five for infection-naïve and previously SARS-CoV-2-infected NH residents (Fig. 4). The antibody concentrations of infection-naïve NH residents increased after the third dose significantly steeper than previously infected study participants, who did start out with higher antibody levels (βi 0.0991 vs. 0.0418, respectively, $p<0.001$; Fig. 4A). Thereafter, the antibody levels of both infection-naïve and previously infected NH residents decayed slowly (βi -0.0088 vs. -0.0032, respectively, $p=0.083$; Fig. 4B). Antibody concentrations did increase slightly more rapidly for the infection-naïve individuals than previously infected persons after the fourth vaccine dose (βi 0.0375 vs. 0.0201, respectively, $p=0.056$; Fig. 4C). The half-life of anti-WT S1 antibodies from approximately one to five months after the fourth vaccination dose was 61.8 days for infection-naïve NH residents compared to 85.2 days for previously infected individuals (βi -0.0162 vs. -0.0117, respectively, $p=0.049$; Fig. 4D). Interestingly, the increase in anti-WT and anti-Omicron S1 antibody concentrations from before to after the fifth (bivalent) vaccine dose for infection-naïve individuals was also significantly steeper than for previously infected individuals (βi 0.0474 vs. 0.0195, $p<0.001$ and 0.0630 vs. 0.0368, $p=0.002$, respectively; Fig. 4E-F). The half-life's of anti-S1 antibodies after the fifth (bivalent) vaccine dose were 116.2 days (WT) and 85.8 days (Omicron) for infection-naïve while those of previously infected NH residents were 150.7 days (WT) and 111.6 days (Omicron) (βi -0.0086 vs. -0.0066, $p=0.110$ and −0.0166 vs. -0.0090, $p=0.097$, respectively), which also highlights that humoral responses against Omicron of infection-naïve and infected NH residents waned quicker than those against WT after the fifth (bivalent) vaccine dose (βi -0.0086 vs. -0.0166, $p=0.091$ and −0.0066 vs. -0.0090, $p=0.046$, respectively; Fig. 4G-H).

Discussion

In this study, we evaluated humoral responses to SARS-CoV-2 of NH residents after two SARS-CoV-2 booster doses with the BNT162b2 vaccine and a subsequent booster dose with the BNT162b2 WT/Omicron BA.1 adapted bivalent vaccine. These additional boosters substantially increased antibody concentrations to SARS-CoV-2 of NH residents; especially the first booster (third vaccine dose) diminished differences in antibody levels between NH residents and healthier community-dwelling older adults and between infection-naïve and previously infected NH residents, and reduced clinical symptom severity and anti-SARS-CoV-2 treatment usage of infected NH residents. Peak antibody levels were reached after the fourth vaccine dose for the NH residents. Furthermore, the bivalent fifth vaccine dose was of added value by increasing Omicron-specific antibody concentrations, but these did wane faster than WT-specific antibody concentrations of the NH residents in the one year post vaccination.

Before receiving the third dose, anti-WT SARS-CoV-2 antibody levels were lower for the NH residents (age 85±9.7) compared to healthier community-dwelling older adults (age 71±8.2). This suggests that aside from age, also the health status of a person may affect antibody responses. Indeed, frailty has been negatively associated

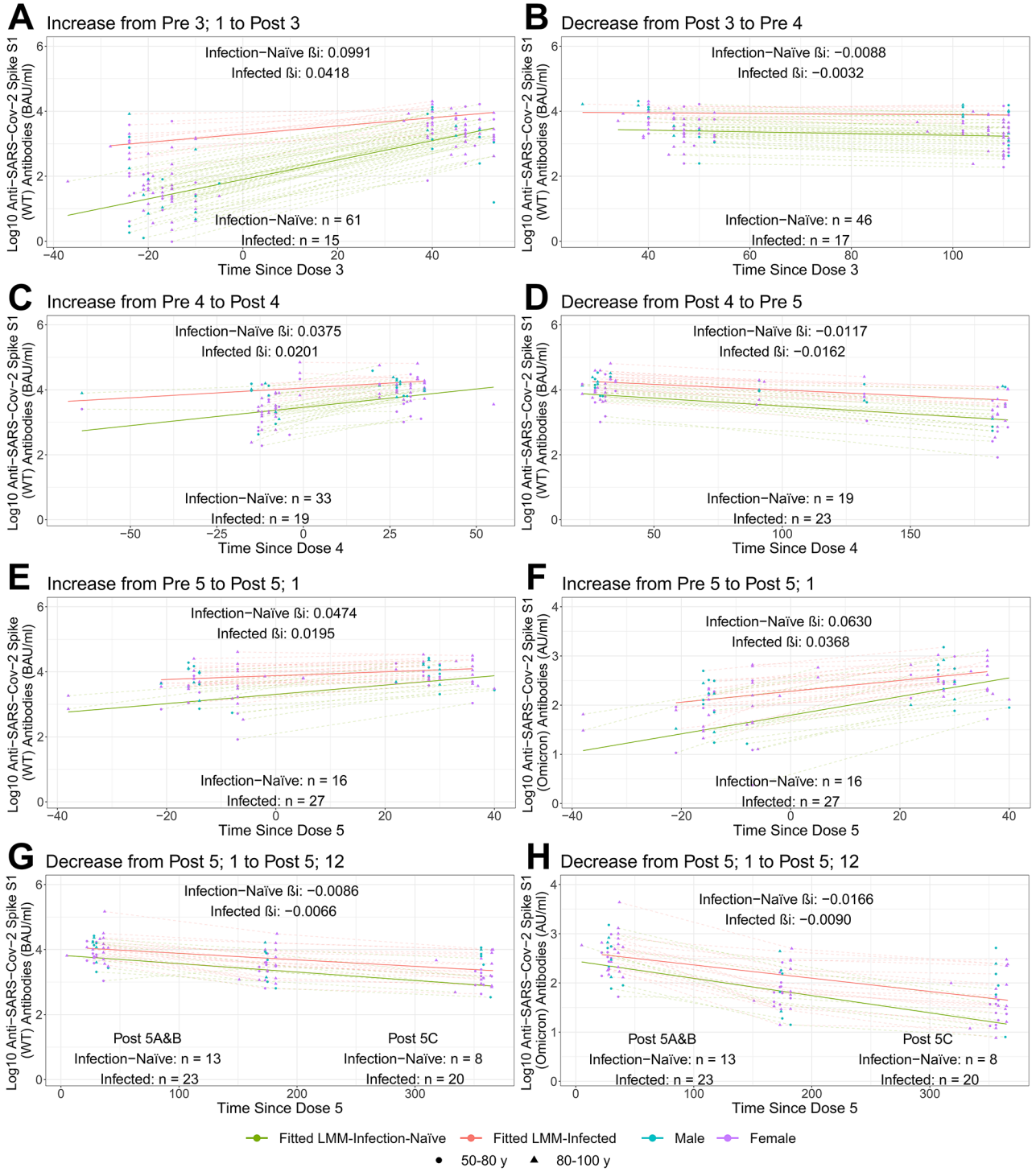

Fig. 4 Differences in anti-SARS-CoV-2 Spike S1 antibody kinetics between infection-naïve and previously SARS-CoV-2-infected nursing home residents. (**a**) Increase due to the third vaccine dose, (**b**) decay after the third vaccine dose, (**c**) increase due to the fourth vaccine dose, (**d**) decay after the fourth vaccine dose, and (**e**) increase due to the fifth vaccine in anti-wild-type (WT) S1 antibodies. (**f**) Increase in anti-S1 antibodies to Omicron due to the fifth vaccine dose. Decays to one year post vaccine dose five of anti-S1 antibodies against (**g**) the WT or (**h**) Omicron SARS-CoV-2. The anti-WT S1 antibody concentrations are expressed in international binding antibody units (BAU) using the 20/136 NIBSC standard and anti-S1 antibody concentrations against Omicron are expressed in arbitrary units (AU). The green line shows the fitted linear mixed effects model (LMEM) of the data from infection-naïve nursing home residents and the red line that from previously infected individuals.Individual data points from males are shown in blue and the female ones in purple. Circles represent individuals of 50–80 years of age, whereas triangles depict persons of 80–100 years of age. Slopes (βi) and number (n) of study participants per timepoint are given within the graphs

with anti-SARS-CoV-2 antibody levels of older adults before the booster doses [21]. After the third dose, antibody responses against WT S1 and RBD were tremendously boosted for especially infection-naïve, but also for previously infected NH residents, being in line with previous reports [8–10]. Thereafter, humoral responses of the NH residents and the healthier community-dwelling older adults were comparable and also the duration of fever, oxygen use, and isolation was shorter for boosted and SARS-CoV-2 breakthrough-infected NH residents compared to those with an infection at earlier timepoints. Others also reported lower rates of hospitalization, severe illness, and death for at least 11 weeks [22] and 12 weeks [23] for residents of LTCFs or NHs that received the third vaccine dose compared to those who only received two doses. Nevertheless, clinical features probably have also been influenced by the less virulent virus variants, since also in the Netherlands the Omicron variant was dominant in the period after the third vaccine dose and that the regulations regarding isolation duration were less strict from February 2022 onwards. Interestingly, dexamethasone and anticoagulants were only used as treatment of a SARS-CoV-2 infection before the third vaccine dose and not afterwards, which may indicate a lower disease burden for boosted NH residents. Overall, these data show the benefit of these additional booster doses in increasing immunity against SARS-CoV-2 of this vulnerable population.

Interestingly, the fifth vaccine dose containing the first bivalent vaccine boosted anti-Omicron S1 antibody levels more substantial for infection-naïve NH residents than for SARS-CoV-2-exposed persons, making these two groups comparable after this dose. The antibody levels of the infected NH residents might have plateaued, known to occur for healthy, infected adults after two vaccinations [18], potentially due to a negative feedback loop where pre-existing antibodies can reduce a new wave of humoral responses against SARS-CoV-2 by inhibiting the recruitment of naïve B-cells [24] or because overactivation of the B-cells is prevented [25]. Another interesting difference was the faster waning of Omicron-specific antibodies compared to WT-specific antibodies. Although described for neutralizing antibodies [26], Omicron-specific binding antibodies might have waned faster than those against the WT strain due to immune imprinting to this ancestral strain. Others also reported longer half-life's of anti-S1 antibodies after the first booster compared to after the primary series, which was attributed to potential recruitment after the booster of more long-lasting plasma cells with a long half-life and less newly generated plasma cells with a shorter half-life [27, 28]. Additional booster doses to newly emerging SARS-CoV-2 variants would be of value for the NH residents to stay protected against these newer variants.

Strengths of this study are the two year longitudinal follow-up of the same NH residents boosted three times with the same vaccine type, our comparison to healthier community-dwelling older adults, and that we report on epidemiological and clinical data in combination with SARS-CoV2 antibody responses (WT and after the fifth dose also Omicron). Also, measuring anti-N antibodies to SARS-CoV-2 allowed us to validate the infection status of the individuals additive to the questionnaires or adjust the infection status in case no positive test was reported.

This study has some limitations. We only enrolled somatic self consenting NH residents. However, since living in a nursing home is only possible by being frail and most NH residents showed multiple comorbidities, this population was the most frail one to be enrolled. Therefore, we also lost a part of our study participants during the two year time span of the study that resulted in low .

Furthermore, after correction for the low numbers of males at the end of the study by the linear mixed model, infection-naïve NH males showed lower anti-WT S1 antibody concentrations than NH females over all timepoints. This sex difference in SARS-CoV-2 antibody responses even after the booster vaccinations might be caused by possible sex-based specific comorbidities in the older more frail population as proposed by others [29].

Additionally, we did not measure neutralization titers because of low blood volumes as we chose to use minimal invasive fingerprick sampling for the fragile NH residents. However, the clinical data does suggest a build-up of immunity against and protection from SARS-CoV-2 as boosted and Omicron-infected NH residents needed less treatment and had less clinical symptoms than WT- and Delta-infected NH residents. Furthermore, a recent study in NH residents did demonstrate that S-specific IgG against the WT SARS-CoV-2 strain can serve as a correlate of protection even against infections with other SARS-CoV-2 variants [11]. We also observed a high correlation between binding IgG antibodies and neutralization of RBD of both SARS-CoV-2 WT and Omicron BA.1 after the fifth booster vaccination (bivalent) in individuals aged 64–85 years (unpublished data). Still, the newer Omicron variants e.g. BA.4/BA.5, XBB.1, and JN.1 have a greater ability to escape from neutralizing antibodies present in serum due to the acquired mutations in RBD such as F486P and F456L [30, 31] that hinder antibody binding [32]. This might also explain why there was no clear increase in Omicron versus WT RBD-IgG ratio from before and after the fifth vaccine dose, while this difference was detected for anti-S1 antibodies. Also, data on antibody responses of the community-dwelling older adults before the fourth vaccine dose were missing and the sample size became small towards the end of the study.

In conclusion, we showed that NH residents benefited from the three additional booster doses, which prompted high antibody responses to SARS-CoV-2. This, in addition to the less virulent circulating strains, decreased symptom severity and treatment durations for SARS-CoV-2-infected NH residents. Nevertheless, we also observed considerable waning of WT-specific and even faster waning of Omicron-specific antibodies one year after the last vaccine dose, suggesting the need for further boosting of this vulnerable population.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12979-024-00495-4.

Supplementary Material 1

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Author contributions

M.H.: conceptualization, methodology, formal analysis, visualization, writing of original draft, writing review and editing. J.K. and A.P.: conceptualization, formal analysis, writing of original draft, writing review and editing. E.Z., M.W., and G.J.: conceptualization, investigation, writing review and editing. L.R. and G.S.: data curation, investigation and writing review and editing. L.L.H: conceptualization, methodology, formal analysis and writing of original draft.G.H.: conceptualization and writing review and editing. A.B.: conceptualization, funding acquisition, project supervision, project administration, writing review and editing.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study was conducted according to the principles of the World Medical Association Declaration of Helsinki and its amendments since 1964, and in accordance with the Medical Research Involving Human Subject Act (WMO). The study protocols were approved by the Medical Ethics Committee of the University Medical Center (METC) Utrecht, NedMed, Utrecht (METC number: 21/056, study number: NL76551.041.21, EudraCT 2021-001976-40. All participants signed the informed consent to participate in the study for finger prick blood sampling at all study timepoints (NL76551.041.21, EudraCT 2021-001976-40).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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