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Third dose of COVID-19 mRNA vaccine closes the gap in immune response between naïve nursing home residents and healthy adults

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ABSTRACT

Background: Nursing home residents, a frail and old population group, respond poorly to primary mRNA COVID-19 vaccination. A third dose has been shown to boost protection against severe disease and death in this immunosenescent population, but limited data is available on the immune responses it induces.
Methods: In this observational cohort study, peak humoral and cellular immune responses were compared 28 days after the second and third doses of the BNT162b2 mRNA COVID-19 vaccine in residents and staff members of two Belgian nursing homes. Only individuals without evidence of previous SARS-CoV-2 infection at third dose administration were included in the study. In addition, an extended cohort of residents and staff members was tested for immune responses to a third vaccine dose and was monitored for vaccine breakthrough infections in the following six months. The trial is registered on ClinicalTrials.gov (NCT04527614).
Findings: All included residents (n = 85) and staff members (n = 88) were SARS-CoV-2 infection naïve at third dose administration. Historical blood samples from 28 days post second dose were available from 42 residents and 42 staff members. Magnitude and quality of humoral and cellular immune responses were strongly boosted in residents post third compared to post second dose. Increases were less pronounced in staff members than in residents. At 28 days post third dose, differences between residents and staff had become mostly insignificant. Humoral, but not cellular, responses induced by a third dose were predictive of subsequent incidence of vaccine breakthrough infection in the six months following vaccination.
Interpretation: These data show that a third dose of mRNA COVID-19 vaccine largely closes the gap in humoral and cellular immune response observed after primary vaccination between NH residents and staff members but suggest that further boosting might be needed to achieve optimal protection against variants of concern in this vulnerable population group.

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1. Introduction

Due to older age and frailty, residents of nursing homes (NH) are at higher risk of severe COVID-19 and death, and have consequently suffered from particularly high hospitalization and mortality rates in the beginning of the pandemic [1–2]. Fortunately, primary COVID-19 vaccination has proven to be very effective at

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preventing disease not only in the general population but in NH residents as well [3–4].

Nevertheless, we and others have shown that immune responses to two doses of COVID-19 mRNA vaccination in SARS-CoV-2 naïve NH residents are relatively poor as compared to those observed in the general, younger, and healthy population [5–7]. In addition, their vaccine-induced immunity has been reported to wane faster than in younger people [8]. Together with the threat of steadily emerging variants which become ever more effective at escaping immune responses, boosting with a third vaccine dose has been widely implemented in many countries.

A third vaccine dose, often administered around six months after the first, has in turn been shown to effectively protect against severe disease, even in contexts dominated by Delta or Omicron variants [9–12]. Importantly, several studies reported that the gap in immune response between NH residents and healthy, younger people observed after two doses becomes much smaller or disappears altogether after administration of a third vaccine dose [13–18].

Here we present an extensive and detailed comparison of vaccine-induced immune responses in naïve NH residents and staff members, investigating not only quantitative but also several qualitative measures of the humoral response, in addition to the characterization of cellular responses through T and memory B cell quantification. We also report on how these immune parameters relate to the incidence of breakthrough infections (BTI) in the six months following third dose administration.

2. Material and methods

2.1. Study design and approvals

PICOV-VAC is a prospective, multi-center, observational cohort study investigating the immune response to BNT162b2 mRNA COVID-19 vaccination (BioNTech/Pfizer, hereafter called BNT) in NH residents and staff members. We previously reported on the response to the first two doses in both previously infected and naïve participants [19,5]. Here we report on the comparison of the immune response to a third versus a second dose of BNT, and explore correlates of protection by linking levels of immunity post third dose with incidence of vaccine BTI in the six months following the third dose. The study was approved by the Ethics Committee of the Hôpital Erasme, Brussels, Belgium (reference P2020/424; A2021/138), the Federal Agency for Medicines and Health Products (2021–000401–24), and is registered on ClinicalTrials.gov (NCT04527614).

2.2. Inclusion and exclusion criteria

Residents and staff members having previously received two doses of BNT and willing to receive a third dose of the same vaccine were eligible to participate. Participants were considered previously infected based on [1] a previous positive molecular SARS-CoV-2 PCR test [2], detectable SARS-CoV-2 nucleocapsid-specific antibodies at any of the available time points before the time of third dose administration, or [3] an increase in titer of SARS-CoV-2 receptor binding domain (RBD) antibodies between any available time points not encompassing a vaccination event. Main exclusion criteria for NH residents included a previous diagnosis of dementia, a mini-mental state examination (MMSE) score $\leq$ 18/30, and life expectancy < 6 months.

2.3. Vaccination, sample collection, and surveys

All subjects received a third dose of 30 μg BNT vaccine, around eight months after the administration of the first vaccine dose. Blood samples were collected on the day of vaccination, four weeks, and six months later. For previous time points, historical samples were used as described previously [5]. Survey data were collected and managed using REDCap (research electronic data capture) tools hosted at Sciensano [20–21].

2.4. Breakthrough infections

All participants were followed up during six months after third dose administration. Participants were considered to have experienced a BTI if they either [1] reported a positive molecular or antigenic SARS-CoV-2 test [2], registered an increase in titer of SARS-CoV-2 RBD antibodies between day 28 and month six post dose 3 time points, or [3] had detectable SARS-CoV-2 nucleocapsid specific antibodies at the six month time point.

2.5. Procedures

SARS-CoV-2 anti-RBD specific IgG concentrations were measured by enzyme linked immunosorbent assay (ELISA; Wantai SARS-CoV-2 IgG ELISA (Quantitative); CE-marked; WS-1396; Beijing Wantai Biological Pharmacy Enterprise Co., Ltd, China) and results are reported as Binding Antibody Units [BAU]/mL. Neutralizing antibody titers (nAbs) against SARS-CoV-2 Wuhan (2019-nCoV-Italy-INMI1, reference 169 008 V-03893) and Omicron BA.1 and BA.5 variants (hereafter called BA.1 and BA.5, respectively) were measured with a live virus neutralization assay (VNA, reported as reciprocal 50% neutralization titer, NT50) [5]. The VNA was only performed against BA.1 and BA.5 for samples with an NT50 (Wuhan) titer > 400. Antibody binding avidity specific to SARS-CoV-2 Wuhan RBD was measured through bio-layer interferometry. IgG subclasses and antibody dependent complement deposition were measured with Luminex technology. SARS-CoV-2 specific memory B cell frequencies were determined with an *in house* B cell ELISpot assay and expressed in Spot Forming Cells (SFC) per 10^6 input cells. SARS-CoV-2 spike subunit 1 (S) and 2 (S2) specific T-cell frequencies were determined by IFN-γ enzyme-linked immunosorbent spot assay. Detailed methods can be found in supplementary material.

2.6. Statistical analyses

Categorical data are presented in percentages and frequencies, continuous variables in means and standard deviation or geometric mean titer (GMT) and its 95% confidence interval (95% CI). The proportion of participants (and 95% CI) with a response lower than the limit of detection (LOD) was calculated at day 28 post dose two and post dose three. Non parametric tests for repeated measures data in factorial design (F1-LD-F1) were used to compare groups at different time points. The risk factors at day 28 post third dose associated with BTIs appearing in the six months following third dose were assessed using univariate and multivariate logistic regressions. Analyses were done using R [22]. Detailed statistical methods can be found in supplementary material.

3. Results

Blood samples were collected from 173 NH residents and staff members at 28 days post third vaccine dose administration (Table 1, complete cohort). Median age of the residents was 85 years (IQR = 54·0 – 100, n = 85) and of the staff 50 years

Table 1
Demographics.

	Complete cohort (n = 173)		Immunogenicity cohort (n = 84)	
	staff	**resident**	**staff**	**resident**
Participant, N	88	85	42	42
Sex				
female	74 (84%)	46 (54%)	33 (79%)	23 (55%)
male	14 (16%)	39 (46%)	9 (21%)	19 (45%)
Age (years)				
mean (SD)	48 (11)	83 (11)	47 (10)	82 (12)
median (range)	50 (24–78)	85 (54–100)	48 (24–65)	85 (54–99)
Ethnicity				
European	85 (97%)	83 (98%)	40 (95%)	40 (95%)
Asian	1 (1%)	0 (0%)	0 (0%)	0 (0%)
NA	2 (2%)	2 (2%)	2 (5%)	2 (95%)
BMI				
< 18.5	0 (0%)	6 (8%)	0 (0%)	6 (15%)
≥ 18·5 and < 25	46 (53%)	36 (45%)	25 (60%)	15 (37%)
≥ 25	41 (47%)	38 (47%)	17 (40%)	20 (48%)
Smoke				
never	70 (80%)	69 (81%)	32 (76%)	33 (78%)
stopped	5 (5%)	6 (7%)	3 (7%)	2 (5%)
smoke	13 (15%)	10 (12%)	7 (17%)	7 (17%)
Comorbidity[¶]				
yes	5 (6%)	55 (65%)	3 (7%)	30 (71%)
no	82 (94%)	30 (35%)	38 (93%)	12 (29%)
Time lapse between dose 1 and 3 (days)				
mean (SD)	253 (18)	247 (36)	238 (11)	249 (15)
median (range)	252 (228–319)	251 (79–280)	236 (228–268)	240 (236–273)

[¶] At least one self-reported co-morbidity.

(IQR = 24·0 – 78·0, n = 88). The interval between the first and the third dose was 251 days (IQR: 79 – 280) for residents and 252 days (IQR: 228 – 319) for staff. Paired historical blood samples collected at 28 days post second dose were available from 42 residents and 42 staff members showing no evidence of previous SARS-CoV-2 infection (Table 1, immunogenicity cohort). In the following paragraphs, we start by comparing 2nd and 3rd dose responses including data from the immunogenicity cohort only (n = 84). Next, correlation analyses of immune parameters with incidence of BTI are performed on the complete cohort (n = 173).

3.1. Normalization of humoral immune levels in nursing home residents after a third vaccine dose

We investigated whether the lower responses observed after two doses became comparable to those of healthy adult staff after a third dose by evaluating a wide panel of quantitative and qualitative immune parameters. Anti-RBD specific binding IgG were boosted significantly by a third dose in residents (GMT = 732BAU/mL post D2 versus 2252BAU/mL post D3, p < 0·001) but plateaued in staff (GMT = 2641BAU/mL post D2 versus 2749BAU/mL post D3, p = 0·9) (Fig. 1A, Table S1). As a result, IgG titers post D3 were not significantly different anymore between residents and staff (p = 1). Of note, the wider range of IgG titers post D2 in residents persisted post D3, with several values now even exceeding those of the younger healthy staff (Fig. 1A). While anti-RBD IgG titers correlated with age post D2, this correlation also disappeared post D3 (SFigure 1).

SARS-CoV-2 Wuhan neutralizing antibody titers (nAbs) were strongly boosted post D3 compared to post D2, in both residents (GMT = 1112 versus 58, respectively, p < 0·001) and staff (GMT = 1580 versus 169, respectively, p < 0·001) (Fig. 1B, Table S1). Again, the difference between residents and staff became much smaller and statistically insignificant (p = 0·1) after a third dose (2·3 to 1·4 fold difference, respectively)(Fig. 1B). Importantly, all residents had detectable nAbs post D3, where this was the case for only 57% of residents post D2. Participants with NT50 values > 400 were also tested against variant BA.1. Post D2, none of the participants had detectable titers of BA.1 specific nAbs. This increased to 86% and 60% post dose 3 in staff and residents, respectively (Table S1). In contrast to WT specific nAbs, there was still a significant, albeit small, difference between staff and residents in BA.1 specific nAbs (Fig. 1C). In terms of antibody avidity, a measure of total binding strength to the cognate antigen, we also observed that the difference post D2 between residents and staff disappeared after administration of a third dose, after a significant boost in both residents and staff (Fig. 1D).

Next, we examined the levels of IgG antibody subtypes 1 and 3, typically potent triggers of anti-viral effector mechanisms. Wuhan and BA.1 RBD specific IgG1 were boosted post D3 compared to post D2 in residents and staff. Post D3, there were no significant differences anymore between residents and staff (Fig. 2A,D). IgG3 antibody titers were not different either post D2 or post D3 in staff and residents. For both, Wuhan RBD specific IgG3 titers decreased between D2 and D3, while BA.1 RBD specific IgG3 decreased in staff but increased in residents. As for IgG1, BA.1 specific IgG3 titers were much lower than for Wuhan (Fig. 2B,E). Finally, antibody dependent complement deposition (ADCD) levels were measured. ADCD levels were higher post D3 than post D2 for both staff and residents, but the boost was much stronger in the latter. The difference between both remained significant, however. BA.1 specific ADCD responses were generally low (Fig. 2C,F).

3.2. Memory B cells are strongly boosted in nursing home residents after a third vaccine dose

Frequencies of Wuhan and BA.1 RBD specific memory B cells (MBC) were measured 28 days after second and third dose administration. Interestingly, we observed that MBC responses were significantly boosted, not only in the younger and healthy staff but also in the older and frail residents of nursing homes. The GMT of Wuhan RBD specific MBC frequencies increased from 45 SFC/10^6 input cells post D2 to 427 SFC/10^6 input cells post D3 in staff (n = 24), and from 20 SFC/10^6 input cells post D2 to 433

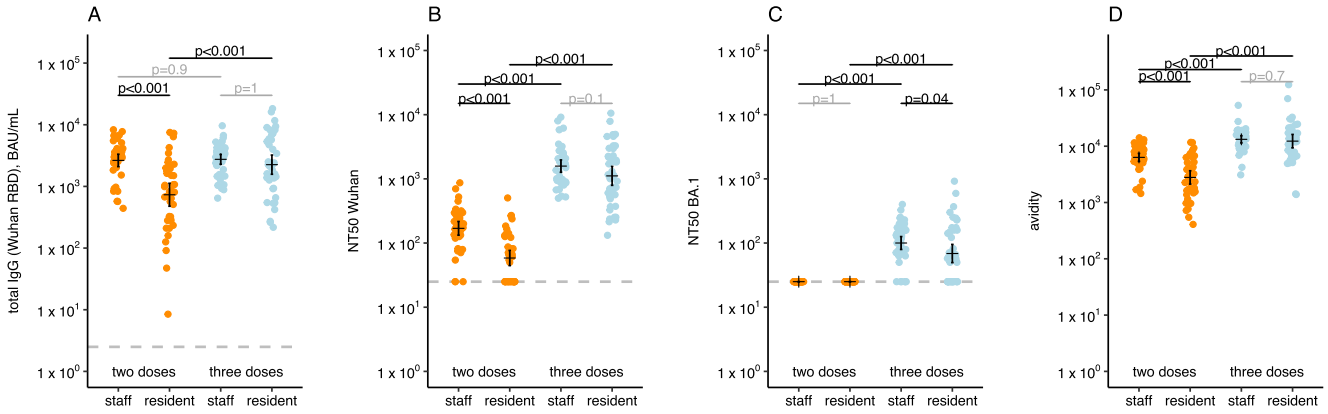

Fig. 1. Humoral immune responses after two (orange) and three (blue) vaccine doses in nursing home staff members and residents of the immunogenicity cohort. (**A**) SARS-CoV-2 Wuhan anti-RBD binding IgG titers (in BAU/mL) measured by ELISA (limit of detection, LOD = 5.4 BAU/mL); (**B**) neutralizing Ab titers of SARS-CoV-2 Wuhan (in NT50) measured by live VNA (LOD = 50 NT50, reciprocal titer); (**C**) neutralizing Ab titers of SARS-CoV-2 Omicron BA.1 (in NT50) measured by live VNA, only samples with NT50 (Wuhan) > 400 were tested; (**D**) Avidity of SARS-CoV-2 Wuhan RBD-specific IgG measured by bio-layer interferometry (1/Koff in s). Each data point represents a serum sample (N = 42, except for resident's avidity where N = 40). Black bars indicate geometric mean titers with 95% CI. Statistical significance of differences i) between doses by study groups was determined by the Wald-type statistics test [29]; ii) between study groups at a time point was determined using a pairwise Wilcoxon rank sum tests with Holm correction for multiple testing. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

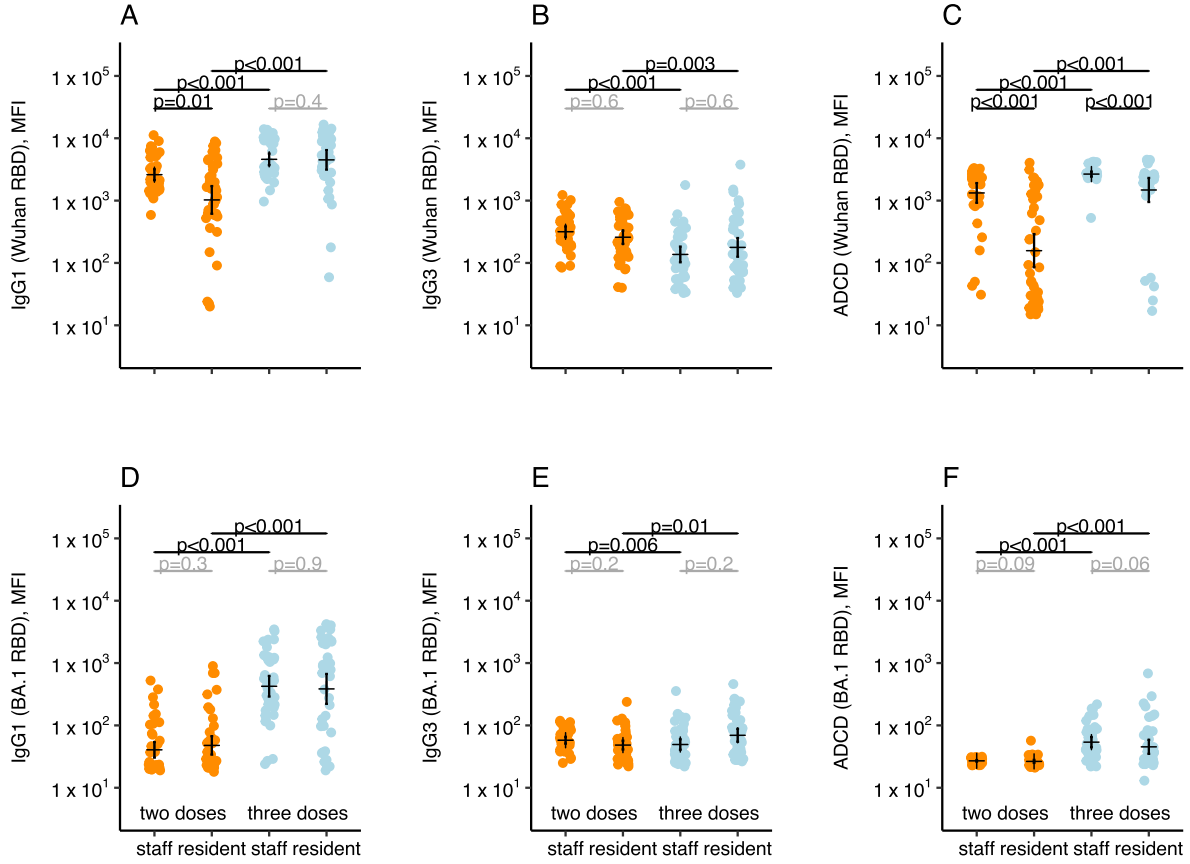

Fig. 2. IgG subclasses and antibody dependent complement deposition (ADCD). Levels of IgG1 (**A,D**), IgG3 (**B,E**) and ADCD (**C,F**) after two (orange) and three (blue) vaccine doses in staff members and residents of nursing homes, measured by multiplex technology specific for SARS-CoV-2 RBD Wuhan and Omicron BA.1, and reported as Mean Fluorescence Intensity (MFI). Each data point represents a serum sample (n = 42 in each condition). Black bars indicate geometric mean titers with 95% CI. Statistical significance of differences i) between doses by study groups was determined by the Wald-type statistics test [29]; ii) between study groups at a time point was determined using a pairwise Wilcoxon rank sum tests with Holm correction for multiple testing. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

SFC/10^6 input cells post D3 in residents (n = 26)(Fig. 3A)(Table S1). Post D3, all tested participants had a detectable MBC responses, which did not differ anymore between staff and residents. BA.1 RBD specific responses were much lower, with significant responses post D3 only detectable in staff (Fig. 3B)(Table S1). Insufficient cells were available to test Wuhan S1 and S2 specific MBC responses at both time points.

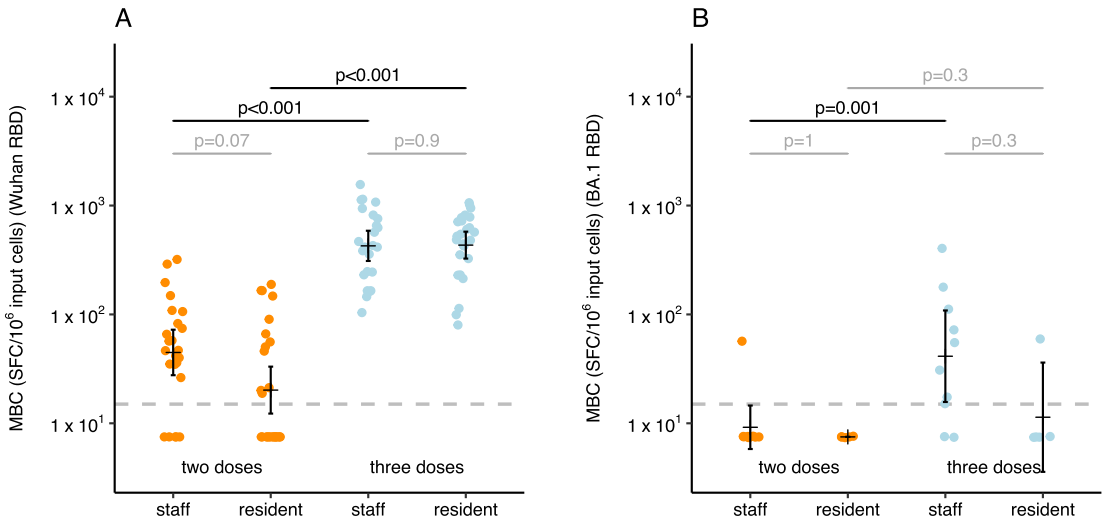

Fig. 3. Frequency of memory B cells (MBC) after two (orange) and three (blue) vaccine doses in staff members and residents of nursing homes, measured by B cell ELISpot and expressed as Spot Forming Cells (SFC) per million input cells. (**A**) Frequency of MBC to SARS-CoV-2 Wuhan RBD (n = 24 staff members and 26 residents) and (**B**) to SARS-CoV-2 Omicron BA.1 RBD (n = 10 staff members and 5 residents). Each data point represents a PBMC sample. Black bars indicate geometric mean titers with 95% CI. Statistical significance of differences i) between doses by study groups was determined by the Wald-type statistics test [29]; ii) between study groups at a time point was determined using a pairwise Wilcoxon rank sum tests with Holm correction for multiple testing. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Correlates of vaccine breakthrough infections post third dose

In the six months following third dose administration, 173 participants (85residentsand88staff) were monitored for the incidence of BTI. A total of 81 BTIs have been registered, 38 (44,7%) in residents and 43 (48,9%) in staff. Risk factor analyses of BTI incidence were performed in this cohort of 173 staff and residents. Immune parameters at 28 days post D3 were compared between those participants developing a BTI and those who did not. These included binding and neutralizing antibody titers, antibody avidity, levels of IgG subtypes and ADCD, memory B cell frequencies and SARS-CoV-2 specific T-cell frequencies as measured by IFN-γ ELISpot. GMTs between both groups were significantly different for total IgG (Wuhan RBD), nAbs (Wuhan and BA.1), avidity, IgG1 (Wuhan and BA.1 RBD) and ADCD responses (Wuhan RBD) (Table S2), indicating that these were all individually predictive of incidence of BTI post D3. Univariate logistic regression analysis showed a significant odds ratio ($p < 0{\cdot}1$) for total IgG (Wuhan RBD), nAbs (Wuhan and BA.1), avidity, IgG1 (Wuhan and BA.1 RBD) and ADCD responses (BA.1 RBD)(Fig. 4). These were then introduced in a multivariate logistic regression, adjusted by age and BMI, which had a linear relationship with the immunological variables of interest (Figure S2). Forward and backward stepwise selection led to a model with just one variable predictive of vaccine BTI incidence, namely anti-RBD Wuhan antibody avidity (in log10), with an OR = 0·33 (0·13-0·74) ($p = 0{\cdot}01$) (Table S3). This model had an accuracy of 62·9% (55·1%-70·2%), a sensitivity of 48·1% and a specificity of 75·6%.

4. Discussion

We and others have previously reported lower humoral and cellular vaccine responses to primary COVID-19 mRNA vaccination in older and frail NH residents compared to healthy younger adults [5–7]. In this study we compared immune responses to second and third doses of mRNA vaccination in naïve NH residents, and observed significant increases of binding and neutralizing antibodies (Wuhan and BA.1), RBD-specific antibody avidity, as well as non-neutralizing antibody activity as measured by antibody-dependent complement deposition levels. In addition, at the cellular level, we observed strong boosting of MBC frequencies in response to a third dose. Importantly, we show that the gap in immune response observed between naïve NH residents and staff members after primary vaccination, was abolished for most immune parameters after the third dose. This was especially true for vaccine-strain specific responses, as Omicron specific responses still remained lower in residents versus staff. Previous reports of the normalization of vaccine responses in NH residents have been limited to binding and neutralizing antibodies, and frequencies of antigen-specific T-cells [17,13–15]. Strong boosting and converging levels of avidity have also been reported after a third dose, but only in healthy people above 65 years old compared to people below 65, and not in NH residents which have a distinctly higher prevalence of frailty and are known to have poorer vaccine responses than home-dwelling older people [23–24].

Of note, peak total RBD-specific IgG titres post D3 were not higher than post D2 in staff members, suggesting that a maximum response was reached. We show here that this level, possibly a plateau, was reached after two doses in naïve younger people, while three doses were required in naïve NH residents. This is not very different from other published data showing similar or only slightly higher total IgG titres post D3 versus D2 while observing more significant gains in neutralization capacity, amongst others [23,25].

Indeed, while the quantity of total antibodies specific for SARS-CoV-2 might have reached its peak, significant gains were still observed in terms of antibody quality, both in NH residents and staff members. Peak levels of virus neutralization capacity post D3 were much higher than post D2, both against the original Wuhan strain as well as the BA.1 variant. The significantly improved (cross-)neutralizing capacity of the antibodies is likely due to their improved avidity, two immune parameters correlating strongly with each other (Figure S3) [5,26–27]. These data therefore suggest that a third dose induces strong antibody affinity maturation in NH residents. In addition, the lower levels of IgG3 and higher levels of IgG1 are illustrative of continued IgG class-switch, similar to what has been described before in healthy individuals Irrgang et al., 2022 Dec 22;8 [28]:eade2798.. Taken together, two important conclusions can be drawn. First, responses

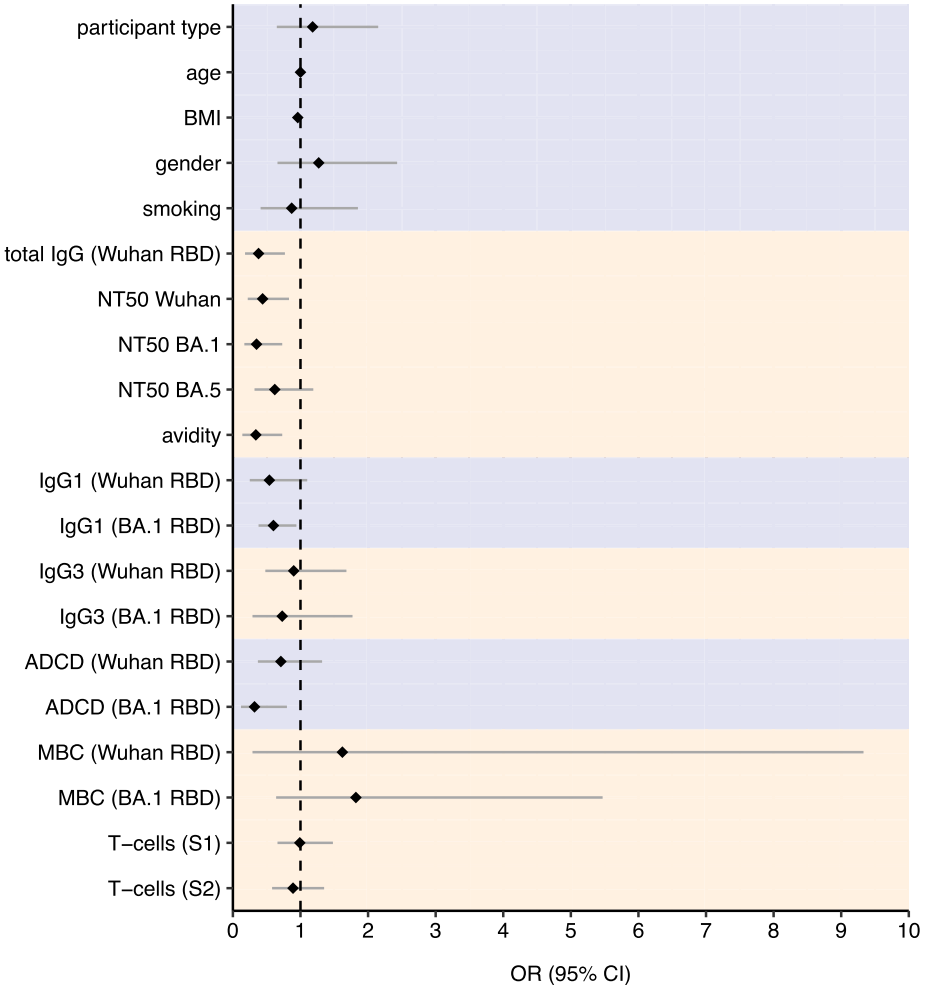

Fig. 4. Univariate logistic regression analysis. Odds ratios (95% CI) of developing a vaccine breakthrough infection between 28 days and six months after third dose are shown for all available immune parameters measured at 28 days post third dose.

to the third dose are superior to those to the second dose and eliminate differences between NH residents and staff. Second, antibody quality is significantly increased and as a result lower antibody levels may be needed to achieve similar neutralization capacity. Despite similar rates of antibody waning, immunity therefore may be sustained for a longer time after third versus second dose administration.

On the cellular side of the response, SARS-CoV-2 specific MBC frequencies were strongly boosted post D3, by an order of magnitude compared to post D2. It is interesting that old and frail people, with a senescent immune system, were still able to mount MBC responses equivalent to younger and healthy people, provided they were given enough time and were exposed repeatedly to the antigen. Unfortunately, the limited number of available cells prevented us from evaluating BA.1 RBD specific MBC responses with sufficient statistical power. From our limited data, we can only hypothesize that a third dose was not able to induce a significant BA.1 specific MBC response in residents, while such responses were detected in staff members. As is the case for humoral responses, differences between residents and staff disappeared after the third dose for vaccine strain specific responses but seemed to persist for variants such as BA.1 at the cellular level as well.

The incidence of BTI was monitored for a duration of six months after third dose administration, a period encompassing two large SARS-CoV-2 infection waves in Belgium, caused respectively by the Delta and the BA.1 variants of concern. Nearly half (46·8%) of all study participants experienced a BTI, but none were severe. The proportion of residents (44·7%) and staff (48·9%) registering a BTI was equivalent. When comparing immunity at 28 days post D3 between participants who went on to develop a BTI and those who did not, a clear picture emerged of consistently higher mean values of all studied humoral, but not cellular, SARS-CoV-2 specific immune parameters in the group without BTI. However, none of these parameters were actually able to predict the incidence of BTI with a meaningful accuracy, as can be expected from their largely overlapping distributions between the BTI and non-BTI groups. Indeed, our final multivariate model based on the avidity of anti-RBD antibodies had an accuracy of 62·9%, which is only marginally better than a model predicting the most frequent class, which has an accuracy of 53·9%. These observations speak to the complexity and difficulty of defining a simple and clear correlate of protection. Despite having identified a number of immune markers which are strongly correlated with BTI incidence, it is likely that there are several important confounding factors determining whether a person will be infected or not, such as amount of exposure, infection dose, circulating viral variant, genetic susceptibility, time since vaccination, amongst others.

Limitations of the study include the smaller sample size of the immunogenicity cohort compared to the full cohort, which includes additional participants recruited after second dose administration and for whom historical samples were therefore not available. From the historical samples, limited cellular material was

available, restricting the number of cellular assays and antigen specificities that could be tested. The control population of our cohort of NH residents consists of NH staff members, which are not representative of the general population. In terms of BTI correlation analyses, while we did have a considerable number of cases (n = 81), all were mildly symptomatic. While this speaks to the effectiveness of the vaccine induced immunity, it is presumably easier to find a correlate of protection against severe disease than mild disease, let alone asymptomatic infection. In addition, the lack of information on the infecting variant strains was an extra confounding factor which could not be taken into account in the correlation analyses.

In conclusion, our data show that a third BNT162b2 dose generates superior humoral and cellular responses compared to the second dose in NH residents and reduces the gap in vaccine induced immunity with staff members. While their primary immune response proved to be diminished and delayed, the immune response of this frail and immunosenescent population group to booster mRNA vaccination appears strong and largely comparable to that of younger healthy adults. Nevertheless, we revealed that Omicron specific responses were still lower than in healthy adults, warranting further boosting with follow-up vaccinations. Humoral immune responses measured one month post third dose were strongly correlated with but poorly predictive of vaccine BTI in the following six months.

CRediT authorship contribution statement

Pieter Pannus: Conceptualization, Data curation, Formal analysis, Investigation. **Stéphanie Depickère:** Data curation, Formal analysis, Investigation. **Kemlin Delphine:** Investigation. **Daphnée Georges:** Investigation. **Sarah Houben:** Data curation, Investigation. **Véronique Olislagers:** Investigation. **Alexandra Waegemans:** Investigation. **Stéphane De Craeye:** Investigation. **Antoine Francotte:** Investigation. **Félicie Chaumont:** Investigation. **Celien Van Oostveldt:** Investigation. **Leo Heyndrickx:** Investigation. **Johan Michiels:** Investigation. **Elisabeth Willems:** Investigation. **Emilie Dhondt:** Investigation. **Marharyta Krauchuk:** Investigation. **Marie-Noëlle Schmickler:** Investigation. **Mathieu Verbrugghe:** Investigation. **Nele Van Loon:** Investigation. **Katelijne Dierick:** Investigation. **André Matagne:** Investigation. **Isabelle Desombere:** Conceptualization, Funding acquisition, Investigation. **Kevin K. Ariën:** Conceptualization, Investigation. **Arnaud Marchant:** Conceptualization, Investigation, Formal analysis, Funding acquisition. **Maria E. Goossens:** Conceptualization, Investigation, Funding acquisition.

Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Role of the funding source

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2023.03.047.

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