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Antibody and cellular immune responses following dual COVID-19 vaccination within infection-naive residents of long-term care facilities: an observational cohort study

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Summary

Background Older age and frailty are risk factors for poor clinical outcomes following SARS-CoV-2 infection. As such, COVID-19 vaccination has been prioritised for individuals with these factors, but there is concern that immune responses might be impaired due to age-related immune dysregulation and comorbidity. We aimed to study humoral and cellular responses to COVID-19 vaccines in residents of long-term care facilities (LTCFs).

Methods In this observational cohort study, we assessed antibody and cellular immune responses following COVID-19 vaccination in members of staff and residents at 74 LTCFs across the UK. Staff and residents were eligible for inclusion if it was possible to link them to a pseudo-identifier in the COVID-19 datastore, if they had received two vaccine doses, and if they had given a blood sample 6 days after vaccination at the earliest. There were no comorbidity exclusion criteria. Participants were stratified by age (<65 years or ≥65 years) and infection status (previous SARS-CoV-2 infection [infection-primed group] or SARS-CoV-2 naive [infection-naive group]). Anticoagulated edetic acid (EDTA) blood samples were assessed and humoral and cellular responses were quantified.

Findings Between Dec 11, 2020, and June 27, 2021, blood samples were taken from 220 people younger than 65 years (median age 51 years [IQR 39–61]; 103 [47%] had previously had a SARS-CoV-2 infection) and 268 people aged 65 years or older of LTCFs (median age 87 years [80–92]; 144 [43%] had a previous SARS-CoV-2 infection). Samples were taken a median of 82 days (IQR 72–100) after the second vaccination. Antibody responses following dual vaccination were strong and equivalent between participants younger then 65 years and those aged 65 years and older in the infection-primed group (median 125 285 Au/mL [1128 BAU/mL] for <65 year olds *vs* 157 979 Au/mL [1423 BAU/mL] for ≥65 year olds; p=0·47). The antibody response was reduced by 2·4-times (467 BAU/mL; p≤0·0001) in infection-naive people younger than 65 years and 8·1-times (174 BAU/mL; p≤0·0001) in infection-naive residents compared with their infection-primed counterparts. Antibody response was 2·6-times lower in infection-naive residents than in infection-naive people younger than 65 years (p=0·0006). Impaired neutralisation of delta (1.617.2) variant spike binding was also apparent in infection-naive people younger than 65 years and in those aged 65 years and older. Spike-specific T-cell responses were also significantly enhanced in the infection-primed group. Infection-naive people aged 65 years and older (203 SFU per million [IQR 89–374]) had a 52% lower T-cell response compared with infection-naive people younger than 65 years (85 SFU per million [30–206]; p≤0·0001). Post-vaccine spike-specific CD4 T-cell responses displayed single or dual production of IFN-γ and IL-2 were similar across infection status groups, whereas the infection-primed group had an extended functional profile with TNFα and CXCL10 production.

Interpretation These data reveal suboptimal post-vaccine immune responses within infection-naive residents of LTCFs, and they suggest the need for optimisation of immune protection through the use of booster vaccination.

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See **Comment** page e449

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Introduction

A striking feature of the epidemiology of primary SARS-CoV-2 infection has been the importance of age and underlying comorbidity as risk factors for severe disease. As such, residents who live in care homes (long-term care facilities; LTCFs) have proven particularly susceptible to high rates of morbidity and mortality. This was compounded by the high prevalence of infection in some LTCFs during the early phase (January–June, 2020) of the pandemic.[1]

Over the past 12 months, several highly effective vaccines have been developed that reduce infection risk and provide substantial protection against severe clinical outcomes.[2,3] Frail older adults and people who were immune-suppressed were under-represented or excluded in the initial clinical trials of the COVID-19 vaccines;

Research in context

Evidence before this study

Residents in long-term care facilities are one of the most vulnerable populations to SARS-CoV-2 infection, but there is very little information on the relative immunogenicity of COVID-19 vaccines in this group. We searched PubMed from Dec 11, 2020, to Jan 30, 2021, with the terms "COVID-19 vaccine care home" and "COVID-19 vaccine residential". We identified no citations relating to assessment of immune response following dual vaccination. A major challenge with this research is the practical issue of obtaining blood samples from this setting.

Added value of this study

Our study collected blood samples from 488 staff and residents in the UK national study of long-term residential care homes (VIVALDI). This was, to our knowledge, the largest such global analysis. We show that around half of donors had serological evidence of previous natural infection, which greatly boosted their vaccine responses. By contrast, the older residents who were infection-naive displayed relatively impaired antibody and cellular immune responses, most probably due to immune ageing and associated comorbidity. Alternative vaccine regimens displayed differential immunogenicity in relation to antibody or cellular response.

Implications of all the available evidence

The magnitude and quality of the SARS-CoV-2-specific immune response after COVID-19 vaccination in older care home residents is greatly enhanced by previous natural SARS-CoV-2 infection. However, immune responses are suboptimal for those who remain infection-naive and should be supported by booster vaccination. Long-term assessment of vaccine-induced immune responses should be undertaken in this very high risk cohort.

therefore a need to understand the relative immunogenicity within this group remains.[4–7] Of note, real-world studies have shown relatively robust clinical protection for this vulnerable group, at least within the first few months following vaccination.[8–10]

The biological basis for the susceptibility of residents in LTCFs to severe COVID-19 remains unclear, but age alone is a very strong determinant of clinical outcome and might influence the relative efficacy of innate and adaptive immune responses across the life course.[11–14] Immune ageing refers to the deterioration in immune function associated with ageing and is probably an important factor contributing to severe disease.[15] Both the magnitude and quality of antibody and cellular responses against pathogens deteriorate with age and are likely to contribute to increased rates of COVID-19 reinfection in this group.[16] These factors also underpin the suboptimal immune responses that are seen in response to vaccines, such as the influenza[17] and herpes zoster vaccines.[18] Furthermore, many residents have multimorbidity, which increases their susceptibility to severe outcomes following infection.

Additional questions are important in relation to the assessment of vaccine efficacy in this group. Firstly, both staff and residents within LTCFs show a high prevalence of previous natural infection and several studies have shown that this acts to boost the efficacy of vaccine responses following dual vaccination.[10] Given the strong immunological priming from natural infection for subsequent vaccine responses, it now becomes important to understand how this effect is mediated to gain insight into potential approaches to boost vaccine efficacy in individuals who are infection naive.

Furthermore, two major vaccine subtypes have been deployed within the UK, the BNT162b2 (BioNTech–Pfizer) mRNA vaccine, and the ChAdOx1 nCoV-19 (Oxford–AstraZeneca) adenovirus platform.[2,3] To date, there is poor understanding of the relative immunogenicity of these two regimens in LTCF residents. As such, we aimed to assess antibody and cellular spike-specific immune responses in both staff and residents of LTCFs across England.

For the **study protocol** see https://wellcomeopenresearch.org/articles/5-232/v2

Methods

Study design and participants

The VIVALDI study (ISRCTN14447421) is a prospective cohort study which was set up to investigate SARS-CoV-2 transmission, infection outcomes, and immunity in residents and staff in LTCFs in England that provide residential and nursing care for adults aged 65 years and older. The study protocol is available online.

Eligible LTCFs were identified by the Care Provider's Senior Management Team, or by the National Institute for Health and Care Research Clinical Research Network. Pseudonymised clinical (vaccination status and PCR test results) and demographic (age, sex, and staff member *vs* resident status) data were retrieved for participants at LTCFs through national surveillance systems.

Both staff and residents were eligible for inclusion if it was possible to link them to a pseudo-identifier in the COVID-19 datastore because this enabled linkage to the vaccination records. Only participants that had both vaccine doses and had given a blood sample 6 days after the second vaccine dose were eligible for inclusion. Participants were not excluded on the basis of any underlying or ongoing comorbidities. Due to restricted PCR testing in the first wave of the pandemic, it was not possible to determine when individuals had been infected with SARS-CoV-2. Previous infection with SARS-CoV-2 was defined based on the results of MSD (Kenilworth, NJ, USA) antibody tests and Abbotts (Chicago, IL, USA) tests. Participants were stratified by age (≥65 years old *vs* <65 years old). Additionally, participants were also stratified by infection status (previous infection with SARS-CoV-2 [infection-primed group] or no previous infection [infection-naive group]).

All participants provided written informed consent for blood sample collection; if residents did not have the capacity to consent, a personal or nominated consultee was identified to act on their behalf. Ethical approval for this study was obtained from the South Central, Hampshire B Research Ethics Committee (REC Ref 20/SC/0238).

Procedures

Anti-coagulated edetic acid (EDTA) blood samples were sent to the University of Birmingham (Birmingham, UK) and a serum tube was also obtained for The Doctors Laboratory (London, UK) where anti-nucleocapsid IgG (N) testing was done. Abbott antibody test results were submitted to the COVID-19 datastore and linked to routinely held data (eg, age, sex, LTCF, and role [staff or resident]), obtained through the national SARS-CoV-2 testing programme, and to vaccination status (date and vaccine type), derived from the National Immunisations Management System. These records were linked using a common identifier based on the individuals' NHS number. Individual-level records were linked to each LTCF with the unique Care Quality Commission location identifier, allocated by the Care Quality Commission, which regulates all providers of health and social care in the UK.

Samples were processed within 24 h of receipt at the University of Birmingham. Blood was spun at 300 G for 5 min. Plasma was removed and spun at 500 G for 10 min before storage at –80°C. The remaining blood was separated using a SepMate (Stemcell Technologies, Cambridge, UK) density centrifugation tube. The resulting peripheral blood mononuclear cell (PBMC) layer was washed twice with Roswell Park Memorial Institute (RPMI) 1640 medium (Sigma, London, UK) and rested overnight in R10 (RPMI, 10% fetal bovine serum, and penicillin and streptomycin) media at 37°C in 5% CO_2.

Quantitative IgG antibody titres were measured against trimeric spike protein and nucleocapsid protein. Multiplex MSD assays were done as per manufacturer instructions (lot number K0081795). Briefly, 96-well plates were blocked and—after washing—plasma samples were diluted at 1:5000 in diluent and added to the wells with the reference standard and internal controls. After incubation, plates were washed and anti-IgG detection antibodies were added. Plates were washed and immediately read using a MESO QuickPlex SQ 120 system (MSD). Data were generated by Methodological Mind software and analysed with MSD Discovery Workbench (version 4.0) software. Presented data were adjusted for any sample dilutions.

For assessment of serological response against the SARS-CoV-2 nucleocapsid, blood samples were tested for the presence of IgG antibodies specific for nucleocapsid protein using the Abbott ARCHITECT system (Abbott, Maidenhead, UK), a semiquantitative chemiluminescent microparticle immunoassay. The assay was done at The Doctors Laboratory. An index value cutoff of 0·8 was used to classify samples as antibody-positive (≥0·8).[19,20]

Quantitative inhibition of ACE-2 binding to trimeric SARS-CoV-2 spike protein from variants of concern were measured using the V-PLEX COVID-19 ACE2 Neutralization Kit (SARS-CoV-2 Plate 13; MSD) following the manufacturer's instructions (lot number K0081795). Briefly, 96-well plates were blocked and—following washing—samples diluted 1:10 in the diluent and—alongside reference standards—added to the plate. After incubation, SULFO-TAG Human ACE-2 Protein detection protein was added to the plate and incubated for 1 h. Plates were washed before being read immediately with a MESO QuickPlex SQ 120 system. Data were generated by Methodological Mind software and analysed with MSD Discovery Workbench software. Presented data were adjusted for any sample dilutions.

For more on the **COVID-19 datastore** see https://data.england.nhs.uk/covid-19/

Peptide pools containing 15-mer peptides overlapping by 10 amino acids from either SARS-CoV-2 spike protein, nucleocapsid, envelope, and membrane protein domains were purchased from Alta Biosciences (University of Birmingham, Birmingham, UK). T-cell responses in post-vaccination samples to spike 1 (S1 region of spike protein), spike 2 (S2 region of spike protein), nucleocapsid, envelope, and membrane proteins were determined with a Human IFN-γ ELISpot PRO kit (Mabtech, Stockholm, Sweden). Isolated PBMC were rested overnight in R10 (RPMI, 10% fetal bovine serum, and penicillin and streptomycin). $2–3\times10^5$ PBMC were stimulated in duplicate with peptide mixes at 2 ng/mL per peptide, anti-CD3 and CEFX cell stimulation mix (JPT Cat:PM-CEFX-2) as a positive control, or dimethyl sulfoxide as a negative control for 16–18 h. Supernatants were harvested and stored at –80°C. Following the development of plates per the manufacturer's instructions, the plates were read using the BioSys Bioreader 5000 (BIO-SYS, Frankfurt, Germany). Mean spot counts in dimethyl sulfoxide-treated negative control wells were deducted from the means to generate normalised spot counts for all other treated wells. Cut off values were previously determined.[21]

Cytokine concentrations within ELISpot supernatants were assayed with a LEGENDplex COVID-19 Cytokine Storm Panel 1 (BioLegend, San Diego, CA, USA; lot number B332349) according to the manufacturer's instructions. Data were analysed using the LEGENDplex data analysis software suite (BioLegend).

For intracellular cytokine staining, $1·5\times10^6$ PBMC were stimulated with either SARS-CoV-2 spike 1 or spike 2, nucleocapsid, envelope, or membrane protein peptide pools at a final concentration of 2 ng/mL per peptide for 6 h. Protein transport inhibitor and CD107a-specific antibody were added after 1 h, and PBMC were washed (phosphate-buffered saline 5%, bovine serum albumin 1%, EDTA) before the addition of Brilliant Stain Buffer and surface staining at 4°C for 30 min. Cells were washed and

	Staff			Residents		
	Infection-naive group (n=117)	Infection-primed group (n=103)	All staff (n=220)	Infection-naive group (n=124)	Infection-primed group (n=144)	All residents (n=268)
Age, years						
≤64	94 (80%)	94 (91%)	188 (85%)	0	4 (3%)	4 (1%)
65–79	22 (19%)	8 (8%)	30 (14%)	25 (20%)	32 (22%)	57 (21%)
≥80	1 (1%)	1 (1%)	2 (1%)	99 (80%)	108 (75%)	207 (77%)
Median age, years	49 (37–65)	52 (42–61)	51 (39–61)	87 (81–92)	87 (79–92)	87 (80–92)
Sex						
Female	109 (93%)	88 (85%)	197 (90%)	84 (68%)	100 (69%)	184 (69%)
Male	0	23 (22%)	23 (10%)	40 (32%)	44 (31%)	84 (31%)
Vaccine schedule						
BNT162b2 recipients	95 (81%)	83 (81%)	178 (81%)	48 (39%)	61 (42%)	109 (41%)
ChAdOx1 nCoV-19 recipients	22 (19%)	20 (19%)	42 (19%)	76 (61%)	83 (58%)	159 (59%)

Data are n (%).

Table: **Cohort demographics**

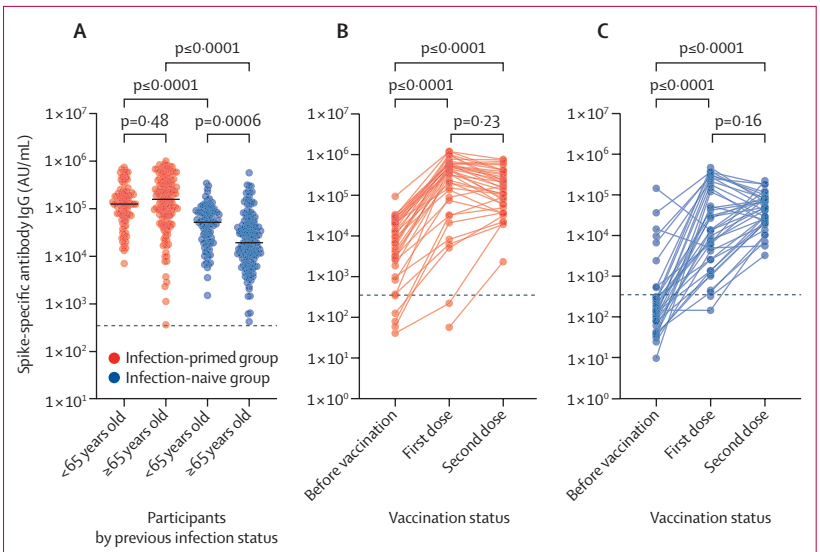

Figure 1: **Spike-specific antibody titre before vaccination, after one dose, and after two doses of COVID-19 vaccination**

(A) Spike-specific antibody titre after two doses of COVID-19 vaccine. Black solid line indicates median. Dotted line indicates assay cutoff. (B) Spike-specific antibody response of the infection-primed group before vaccination, after the first dose of vaccine, and after the second dose of vaccine (n=39). (C) Spike-specific antibody response of the infection-naive group before vaccination, after the first dose of vaccine and after the second dose of vaccine (n=37). One individual in the infection-promed group had no available baseline data.

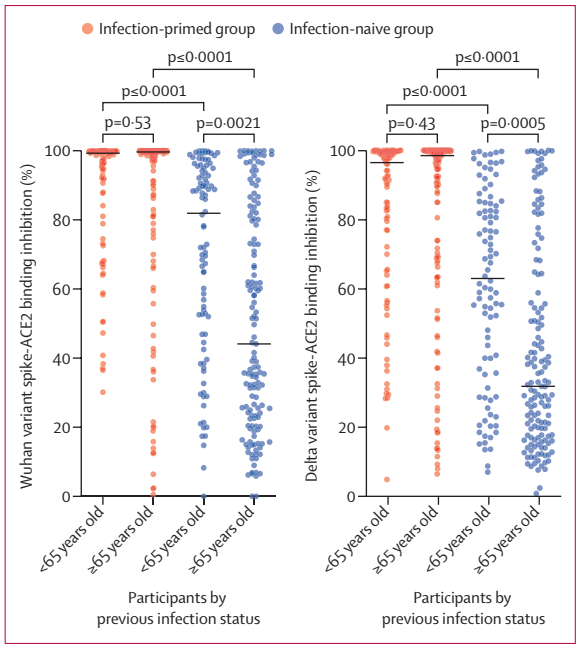

Figure 2: **Neutralisation of the original Wuhan (B.1.1.7) and delta (1.617.2) variant spike-ACE2 binding after completed vaccine schedule for residents and staff by previous infection status**

Relative inhibition of spike-ACE2 binding sera after completion of the vaccine schedule. Data are from 488 individuals. Black solid line indicates median.

See Online for appendix

resuspended in Cell Fixation Buffer (eBioscience, ThermoFisher Scientific, Waltham, MA, USA) at 4°C overnight. Cells were rewashed and human serum and saponin added to samples 5 min before the addition of cytokine-specific antibodies with Brilliant Stain Buffer (eBiosciences; appendix pp 6–7) and incubated in the dark at room temperature for 30 min. Cells were washed twice with MACS (1× PBS, 0·5% BSA, 2 mM EDTA pH 8·0) and run on a Symphony A3 flow cytometer. Analysis were done with FlowJo (version 10.7.1; appendix p 3). Cells that appeared in both the IFN-γ and IL-2 positive gates were taken as the dual IFN-γ and IL-2 positive cells.

Statistical analysis

All data were checked for normality and variance with the Kolmogorov-Smirnov test. All data were non-parametric and non-parametric statistical analysis was used. For comparative analysis with three or more groups a Kruskal-Wallis test was used, and for multiple comparisons an uncorrected Dunn's test was used. Spearman's rank correlation coefficients were calculated and tested for correlations. Data analysis was done with Graph Pad Prism (V.9.1.0 [216]). p values less than 0·05 were considered statistically significant.

Role of the funding source

The funder of the study, UK Department of Health and Social Care, had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between Dec 11, 2020, and June 27, 2021, blood samples were collected from 488 participants (220 [45%] <65 years old; 268 [55%] ≥65 years old; four [1%] of 268 residents were younger than 65 years old and 32 [15%] of 220 staff members older than 65 years old; table) from 74 LTCFs (6·6 [SD 6·4] participants per

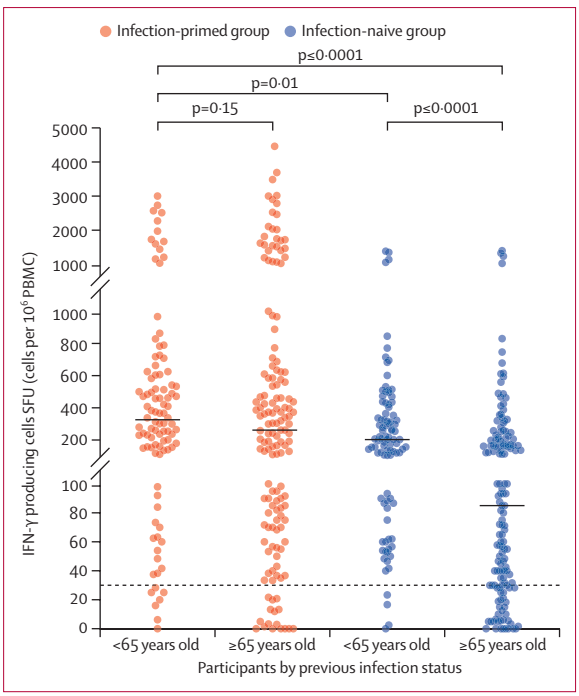

Figure 3: **Spike-specific T-cell responses following vaccination**
IFN-γ ELISpot following spike-specific PBMC. Data are from 488 individuals. Black solid line indicates median. Dotted line indicates assay cut-off. PBMC=peripheral blood mononuclear cell. SFU=spot forming units.

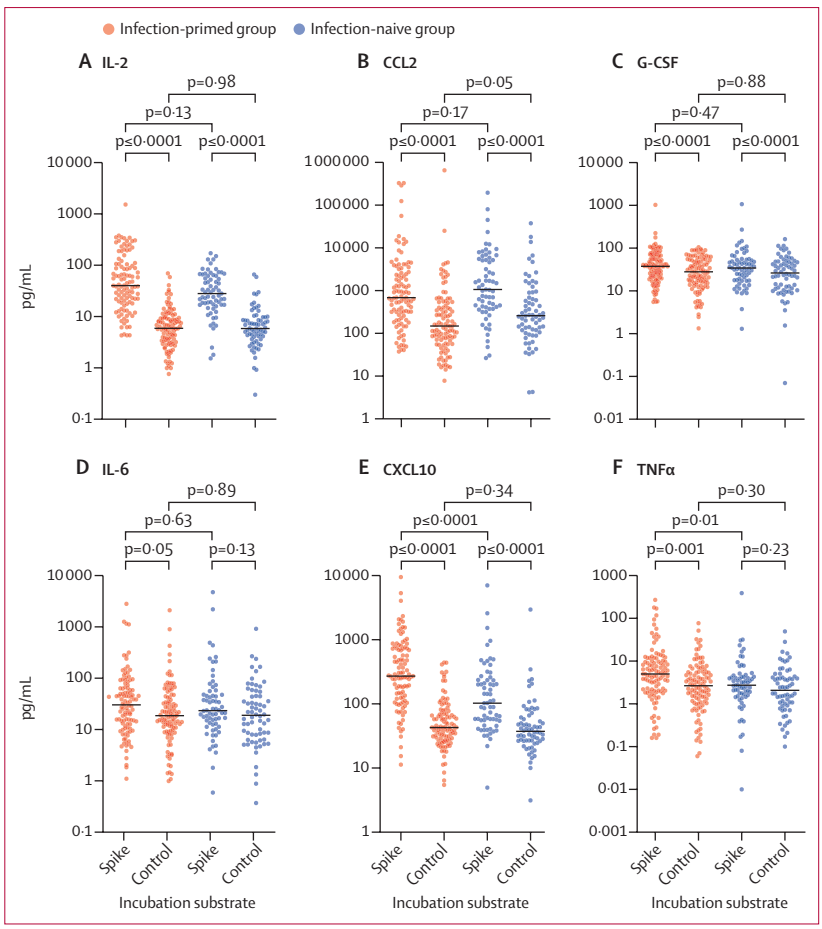

Figure 4: **Spike-specific cytokine response profile following vaccination**
Data are from 175 individuals. Black solid line indicates median. Control indicates cells were incubated with dimethyl sulfoxide only.

LTCF) who had completed their primary COVID-19 vaccination schedule (appendix p 5). Blood samples were taken with a median of 82 days (IQR 72–100) after the second vaccination. No waning of spike IgG antibody titre was observed (appendix p 1).

Both infection-primed groups had similar antibody titres (median 125 285 Au/mL [1128 BAU/mL] for <65-year-olds *vs* 157 979 Au/mL [1423 BAU/mL] for ≥65-year-olds; p=0·47; figure 1A). By contrast, antibody titres were significantly reduced in the infection-naive group. Participants younger than 65 years old in the infection-naive group had a 2·4-times lower antibody titre (51 859 Au/mL [467 BAU/mL]; p≤0·0001) compared with the infection-primed group. Participants aged 65 years or older in the infection-naive group had an 8·1-times lower antibody response (19 384 Au/mL [174 BAU/mL]; p≤0·0001) than the infection-primed group. Within the infection-naive group, antibody titres were 2·6-times lower in people aged 65 years or older compared with those younger than 65 years (p=0·0006). Across three age cohorts (<64 years old, 65–80 years old, and >80 years old) there was significant suppression of antibody responses in older donors (p=0·0006; appendix p 4).

Participants who had previously had an infection had a median spike-specific antibody titre of 7663 Au/mL (69 BAU/ml) at baseline, which increased 55·0-times after a single vaccination to 419 064 Au/mL (3775 BAU/mL) (p≤0·0001). Of note, antibody titre decreased 2·6-times to 159 753 Au/mL (1439 BAU/ml) after the second vaccine which was not statistically significant (p=0·225) (figure 1B). As expected, spike-specific antibodies were not detected before vaccine in most of the infection-naive group. In the infection-naive group, spike-specific antibody titre increased to 13 626 Au/mL (122 BAU/mL) after one vaccine (p≤0·0001) and then increased by 3·4-times to 47 535 Au/mL (428 BAU/mL) after the second vaccine (p=0·16) (figure 1B). Of note, spike-specific antibodies were seen in six people in the infection-naive group; it is possible that some of these represent people who had a previous infection but in whom waning of the nucleocapsid-specific antibody response has led to the loss of detectable response.[22]

Sera from people in the infection-primed group showed strong inhibition of spike-ACE2 binding against both the original variant (B.1.1.7; <65-year-olds 99% [IQR 88–100]; ≥65-year-olds 100% [87–100]; figure 2) and delta variant (1.617.2; <65-year-olds 96% [69–100]; ≥65-year-olds 98% [70–100]; figure 2B). Compared with their counterparts in the infection-primed group, inhibition of spike-ACE2 binding was lower in response

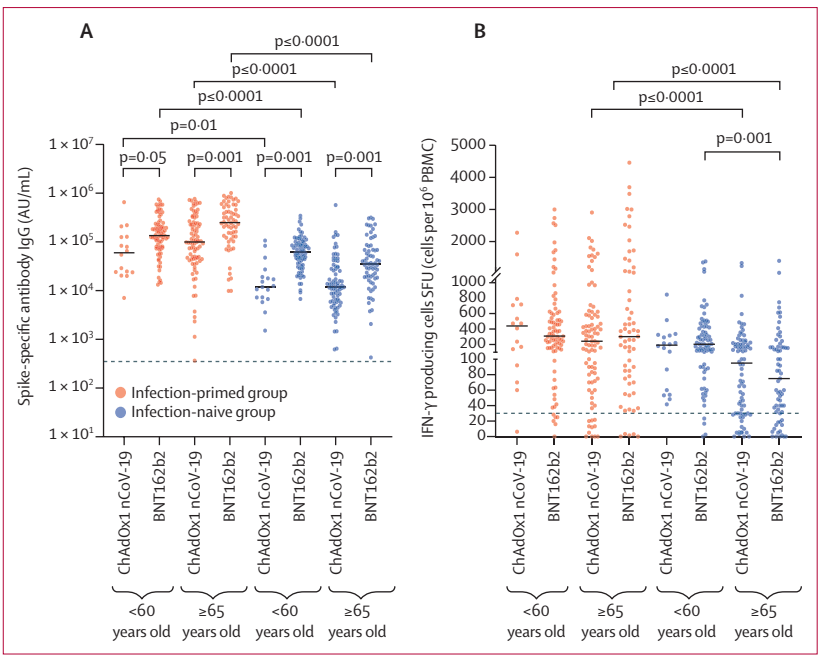

Figure 5: **Spike-specific antibody and cellular responses after completion of BNT162b2 (BioNTech–Pfizer) and ChAdOx1 nCoV-19 (Oxford–AstraZeneca) vaccine schedules**

Spike-specific antibody (A) and cellular (B) responses in long-term care facility staff and residents after dual vaccination with either ChAdOx1 nCoV-19 or BNT162b2. Data are from 488 individuals. Black solid line indicates median. Dotted line indicates assay cut-off. PBMC=peripheral blood mononuclear cell. SFU=spot forming units.

to both variants in the infection-naive group (<65-year-olds original Wuhan variant 82% [46–95; p≤0·0001]; delta variant 63% [35–85; p≤0·0001]; ≥65-year-olds original Wuhan variant 44% [23–82; p=0·0021]; delta variant 32% [17–64; p=0·0008]). These data suggest that vaccine-induced spike-specific antibodies have inferior functional activity in those who have not had a previous natural infection and this effect is greater in older people.

Median spike-specific T cell counts were broadly similar across the infection-primed group, with no evidence of cellular immune ageing (325 SFU per million [IQR 147–611] in <65-year-olds and 261 SFU per million [75–647] in ≥65-year-olds; p=0·15; figure 3). By contrast, lower cellular responses were seen in the infection-naive group (203 SFU per million [89–374] in <65-year-olds and 85 SFU per million [30–206] in ≥65-year-olds). Spike-specific cellular responses are 38% (p=0·019) lower in people younger than 65 years and 67% (p≤0·0001) lower in people aged 65 years or older in the infection-naive group compared with their counterparts in the infection-primed group. Additionally, within the infection-naive group, median spike-specific T cell count in people aged 65 years or older was 52% (p≤0·0001) lower than in people younger than 65 years, which suggests that many older individuals might develop a potentially suboptimal number of spike protein-specific T cells.

Stimulation with spike peptides led to the release of high concentrations of IL-2, in line with previous reports,[22] and this was not influenced by participant infection status (infection-primed group 40 pg/mL *vs* infection-naive group 29 pg/mL; p=0·13; figure 4). CCL2 concentration was also not influenced by infection status (infection-primed group 684 pg/mL *vs* infection-naive group 1062 pg/mL; p=0·17). Low concentrations of granulocyte colony-stimulating factor and IL-6 production were also observed, and CXCL10 concentrations were significantly increased in donors in the infection-primed group (271 pg/mL) compared with individuals in the infection-naive group (103 pg/mL; p≤0·0001). TNF-α release was also significantly increased in the infection-primed group (5 pg/mL) compared with the infection-naive group (2·7 pg/mL; p≤0·002). LEGENDplex analysis also incorporated measurement of IL-7, CCL-5, IL1-RA, CXCL8, CCL3, and IL-11, but no differences were seen between analyses (data not shown). These findings show that vaccination leads to the generation of a PBMC-released cytokine profile of IFN-γ, IL-2, and CCL2 following stimulation, while priming by natural infection triggers an extended functional phenotype that includes TNF and CXCL10.

The median duration between completion of primary vaccine schedule and analysis was 72 days (IQR 64–X79) for ChAdOx1 nCoV-19 and 97 days (81–111) for BNT162b. Median antibody response was lower for donors who had received the ChAdOx1 nCoV-19 compared with the BNT162b2 in all four subgroups (figure 5A). Median antibody response was 59 812 AU/mL (IQR 23 828–126 894; 538 BAU/ml) for people younger than 65 years and 99 561 AU/mL (45 002–242 202; 897 BAU/mL) for people aged 65 years or older in the infection-primed group who received ChAdOx1. Across the whole study population, antibody response was 44% (p=0·016) lower for people younger than 65 years and 40% (p=0·0004) lower for those aged 65 years or older who received ChAdOx1 nCoV-19 compared with the BNT162b2 (134 553 AU/mL [76 561–228 646; 1212 BAU/mL] for <65-year-olds and 249 007 AU/mL [17 015–519 623; 2243 BAU/mL] for ≥65-year-olds; figure 5A).

In the infection-naive group, median antibody concentration after ChAdOx1 nCoV-19 vaccination was 12 151 AU/mL (IQR 7097–21 094; 109 BAU/ml) for people younger than 65 years and 12 072 AU/mL (5953–30 663; 108 BAU/mL) for people aged 65 years or older. In people younger than 65 years who were infection naive, antibody concentrations were 20% lower in those who received ChAdOx1 nCoV-19 compared with those who received BNT162b2 (61 543 AU/mL [34 159–99 753]; 556 BAU/mL; p=0·0003). In people aged 65 years or older who were infection naive, antibody concentrations were 34% lower in those who received ChAdOx1 nCoV-19 compared with those who received BNT162b2 (35 297 AU/mL [18 440–68 751]; 290 BAU/mL; p=0·0005; figure 5A). Functional capacity to inhibit spike-ACE2 binding was also lower within ChAdOx1 vaccinees (appendix p 3).

Cellular responses were significantly higher in people aged 65 years or older in the infection-primed group

compared with their counterparts in the infection-naive group. In the infection-naive group, people aged 65 years or older who received BNT162b2 had a median response 2·7-times lower than people younger than 65 years (75 SFU per million PBMC for ≥65-year-olds *vs* 203 SFU per million PBMC for <65-year-olds; p=0·0002; figure 5B) suggesting BNT162b2 might result in lower cellular responses in older individuals compared with those who received ChAdOx1.

In an assessment of intracellular cytokine expression, antigen-specific responses were detected within the CD4 repertoire. Responses were generally too low for reliable detection within the CD8 pool. Spike-specific CD4 T-cell responses were detected at a frequency of 0·04% in the infection-primed group compared with 0·018% in the infection-naive group (figure 6A). The phenotype of this pool was then characterised by single or dual expression of IFN-γ and IL-2. Single cytokine positive cells were dominant: dual positive cells comprised 11% of the antigen-specific pool in the infection-naive group and 18% in the infection-primed group (figure 6B). The memory phenotype of dual cytokine-positive cells revealed that central memory populations were more than twice as common in the infection-primed group (37%) compared with the infection-naive group (18%; figure 6C). The basis for this distribution of cells is uncertain, but it could reflect secondary activation of spike-specific cells after vaccination or a longer time duration since initial activation.

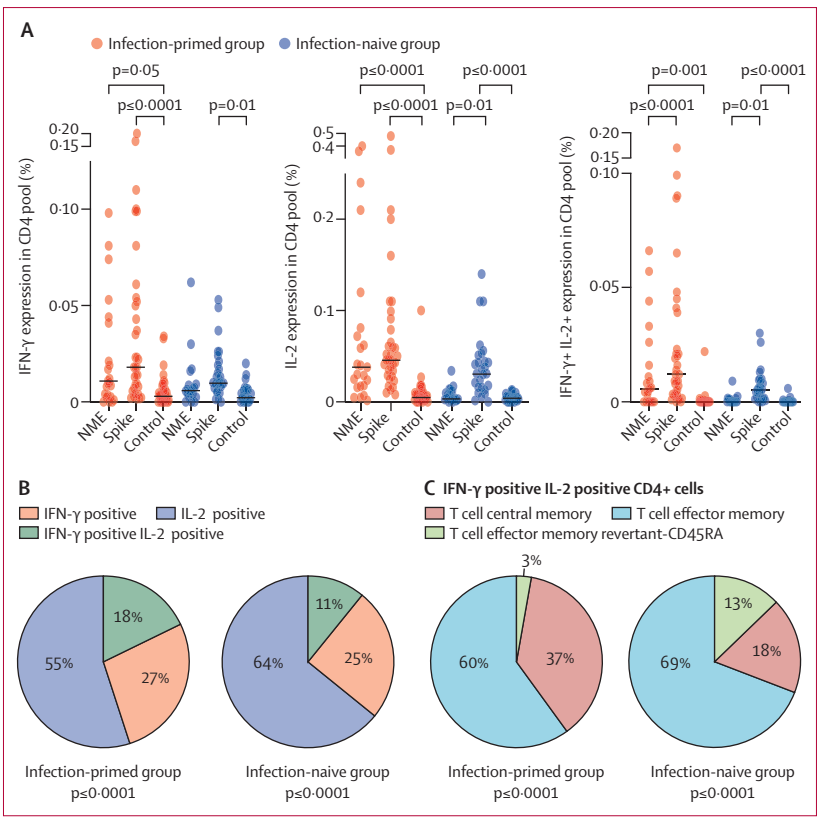

Figure 6: **Relative expression of IFN-γ and IL-2 within spike-specific CD4 T cells following dual vaccination**
(A) Detection of virus-specific CD4+ T cells, represented as a proportion of the total CD4+ repertoire, by single or dual expression of IFN-γ or IL-2 following stimulation with spike or N, M, or E peptides. Data are from 35 individuals from the infection-primed group and 27 individuals from the infection-naive group. Dimethyl sulfoxide is negative control. Black solid line indicates median. (B) Distribution of single or dual IFN-γ and IL-2 positive virus-specific CD4 T cells. (C) Memory phenotype distribution of IFN-γ and IL-2 positive CD4 virus-specific cells. E=envelope. M=membrane. N=nucleocapsid.

Discussion

Ageing is a dominant risk factor for severe COVID-19 infection, but immune ageing limits the quality of adaptive immune responses in older people. As such, assessment of the clinical efficacy and immunogenicity of COVID-19 vaccines within vulnerable populations is a research priority. Our studies of vaccine responses following dual vaccination of staff and residents in LTCFs reveal several important observations.

Both the magnitude and functional quality of adaptive immune responses were strongly influenced by previous natural SARS-CoV-2 infection. As such, there was clear evidence of impaired immune responses in the older people in the infection-naive group compared with younger people in the same infection exposure group. Median antibody titres were reduced by 8·1-times and spike-specific T cell responses reduced by 52% and relative functional impairment was also observed as assessed by spike-ACE2 inhibition in older people in the infection-naive group compared with younger people in the same infection-status group. The mechanisms that underlie immune ageing have yet to be resolved fully, but will probably reflect a combination of reduced naive cell repertoire, accumulation of an expanded memory pool, and increased tissue inflammation with ageing.[11,12,14] Suboptimal immunogenicity of vaccines, such as those against influenza or varicella-zoster, has necessitated innovations—including novel adjuvants or delivery formulations.[17,18] Our findings suggest that the new generation of COVID-19 vaccines, although highly immunogenic, remain partly susceptible to the negative influence of ageing. However, several studies of COVID-19 vaccines in older adults have shown strong immunogenicity, and it is possible that other factors, such as frailty, contribute to the severe outcomes seen following COVID-19 infection in LTCF residents.

It is important to assess these immunological findings in the context of the clinical protection afforded by vaccination in people who are care home residents. Our own studies have shown the significant protection vaccination offers against hospitalisation in older people who live in the community and those who are LTCF residents relative to unvaccinated individuals of the same age,[8] and similar findings have been reported by other groups.[23–25] An emerging feature of all COVID-19 vaccines has been the high levels of protection against severe disease and death in comparison with protection against more mild or subclinical infection. It is probable that the immune responses we observed following

vaccination are largely effective in controlling severe disease.

Our previous studies show the significant effect of the vaccine subtype in relation to spike-specific immune responses.[26] In this study, the BNT162b2 vaccine generated significantly increased antibody responses in the infection-naive group, irrespective of age. This profile has been observed in several other settings and reveals the profound immunogenicity of the mRNA platform.[26,27] By contrast, cellular responses were similar with both the mRNA and adenovirus-based platforms and probably reflect differential mechanisms of antigen presentation between these formats.

We also determined the profile of vaccine-induced spike-specific T-cell responses with multiparametric flow cytometry. These were dominated by CD4 cells, as observed in previous reports,[21] although the frequency of virus-specific responses within the CD4 repertoire was low (0·018%) in the infection-naive group, but it increased to 0·04% in the infection-primed group. IL-2 and IFN-γ were the dominant cytokines produced by these populations and indicate a T helper 1 cell (Th1) response with potential for proliferation. Of note, individuals in the infection-primed group had additional production of TNFα and CXCL10 in response to viral stimulation, potentially reflecting polarisation of Th1 phenotype. Increased serum concentrations of TNFα and CXCL10 have been seen after one vaccine following previous infection,[28] which is of note because serum CXCL10 concentrations correlate positively with post-vaccine antibody responses.[29,30] Spike-specific immune responses might support clinical protection against severe disease following SARS-CoV-2 infection and might underpin vaccine protection against viral variants, such as omicron (B.1.1.529). A future ambition will be to gain more insights into cellular response as a correlate of immune protection, but this was not defined in this study.

A striking feature was the importance of previous natural infection in enhancing both the magnitude and quality of the antibody and cellular immune response to spike protein following vaccination. A strength of this cohort is the high prevalence of natural infection, which means there are equivalent populations of infection-primed and infection-naive individuals, making it possible to assess the features of natural infection on priming for vaccine responses. The potency of this infection-priming strongly suggests that it is more significant than an incremental effect of third exposure to spike protein. As such, there are likely to be factors related to natural SARS-CoV-2 infection that train immune responses to respond effectively to subsequent spike vaccination. One of these might be the increased breadth of adaptive immune responses against viral proteins which can act to support antibody maturation and enhance T cell differentiation. Potential retention of viral protein within the gastrointestinal tract might also serve to support selection for high-affinity immune responses.[31] Of note, a confounding factor in this assessment is that people with previous natural infection are necessarily survivors of primary exposure and therefore a potential selection for people with pre-existing immune responsiveness might be in operation.

Our study has some limitations. We did not have access to the exact time or severity of primary infection for participants in the infection-primed group. Information on patient comorbidities and ethnicity were also not available. There is also the potential for some waning of spike-specific IgG titre during sampling, despite the early timepoint analysis after vaccination.[22,32] Furthermore, this was a retrospective analysis of prospectively collected data and some of the subsets are of modest size.

It is important to assess how these findings might inform the future direction of vaccine policy within the LTCF setting. The older resident population has a suboptimal immune response to the standard dual vaccine regimen and is likely to benefit from additional protection. Nevertheless, one of the encouraging findings of our studies was that antibody responses became detectable in almost all people after dual vaccination, a feature not seen in many patients with more severe forms of immune suppression.[33,34] Repeated vaccination might be less necessary in people with a previous natural infection; however, serological assessment of a previous infection is not generally ascertained in vaccine delivery programmes. Ongoing work from the VIVALDI study will address reinfection rates after dual and booster vaccinations. The emergence of the omicron variant and its subtypes (BA.1, BA.1.1, and BA.2) has led to increased reinfection in this vulnerable population and the VIVALDI team are looking into the effect of this variant of concern in LTCF settings.

In conclusion, residents in LTCFs develop suboptimal immune responses to dual COVID-19 vaccination unless they have acquired and survived natural infection. Therefore, the implementation of a third booster vaccine in this setting is appropriate at this time and should be strongly encouraged.

Contributors

LS, AC, AH, MK, GT, and PM conceptualised the study. GT, TL, and PM designed the methods. MK, BA, CF, RB, GT, CB, UA, SH, AJ, DB, MSB, MA, ES, SH, MA, ET, MS, and AI-S were responsible for project administration. GT, TL, MSB, ES, PS, NK, and CB curated and validated the data. LS, AH, AC, and PM sought study funding. GT, PM, TL, MSB, and DB wrote the original draft. All authors reviewed and edited the manuscript. GT, TL, and PM verified the underlying data. GT, TL, NK, PS, MSB, DB, and PM had full access to the data in the study. PM and GT have shared responsibility for the decision to submit for publication.

Declaration of interests

LS reports grants from the Department of Health and Social Care during the study and is a member of the Social Care Working Group, which reports to the Scientific Advisory Group for Emergencies. AH is a member of the New and Emerging Respiratory Virus Threats Advisory Group at the Department of Health. All other authors declare no competing interests.

Data sharing

Deidentified test results and limited meta-data will be made available for use by researchers in future studies, subject to appropriate research ethical approvals, once the VIVALDI study cohort has been finalised. These datasets will be accessible via the Health Data Research UK Gateway.

For the **Health Data Research Gateway** see https://www.healthdatagateway.org/

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