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Safety and humoral immunogenicity of the ChAdOx1 nCoV-19 vaccine administered as a fourth dose booster following two doses of ChAdOx1 nCoV-19 and a third dose of BNT162b2 (COV009): A prospective cohort study

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SUMMARY

Objectives: Evaluation of the safety and humoral immunogenicity of ChAdOx1 nCoV-19 as a fourth dose booster in individuals who have had two initial doses of the vaccine and a third dose of BNT162b2.
Methods: COV009 is a safety follow-up study of volunteers enroled in the pivotal pre-licensure ChAdOx1 nCoV-19. In this sub-study, 149 eligible participants were given a fourth dose of ChAdOx1 nCoV-19. Primary outcomes were reactogenicity, safety, and humoral immunogenicity. Anti-spike IgG and pseudo-neutralising antibody against multiple variants were measured from pre-first dose to 28 days post-second and post-fourth dose (third dose samples were unavailable).
Results: A fourth dose of ChAdOx1 nCoV-19 had an acceptable safety profile with no vaccine-related serious adverse events. Humoral responses against various SARS CoV-2 variants post-fourth dose were significantly increased compared with the responses after the second dose (7- to 9-fold increase for anti-spike IgG responses across variants, all $p < 0.05$). Those with lower antibody levels prior to the 4th dose had stronger responses to a 4th dose booster. Seropositivity by anti-nucleocapsid, or higher antibody responses pre-fourth dose correlated with lower infection risks six months thereafter (OR: 0.16, 95% CI: 0.05, 0.50).
Conclusions: The ChAdOx1 nCoV-19 fourth dose is well tolerated and boosts humoral immunity; this was evident as an increased humoral response across multiple variants of concern. These data support its use as a booster dose against SARS-CoV-2 infection.

Introduction

The accelerated development of vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) led to the emergency licensure of a number of vaccines including the adenoviral vector vaccine AZD1222/ChAdOx1 nCoV-19 in 2020 as a two-dose primary schedule. Subsequently, data have emerged showing that

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vaccine-induced immunity wanes following vaccination. Susceptibility to infection is further compounded by the emergence of new variants of concern (VoC) rendering vaccines designed against the original wild type Wuhan strain less protective against infection, but maintenance of protection against severe disease has been largely conserved.[1,2] A meta-analysis showed that vaccine efficacy or effectiveness (VE) against SARS-CoV-2 infection and symptomatic disease decreased by six months post-primary vaccination regardless of the vaccine platform used.[3] Furthermore, when investigating the impact of age and VE, this review found a considerable decline in VE against SARS-CoV-2 infection in older adults (≥65 years of age) after two doses of primary series vaccines (mRNA and/or adenovirus vectored).[3] Vaccination with additional doses (mRNA vaccine) augmented the immune response.[1,4] The complicated interplay between waning immunity, an aging population, and decreased protection against infection because of VoC was evident by late 2020.[5,6] More recently, emerging VoC have a higher mutational burden and significantly divergent spike proteins, unlike the first identified VoC, which had a single point nucleotide mutation of D614G.[7] The World Health Organisation declared five SARS-CoV-2 variants of concern, in November 2021 with the addition of the B.1.1.529 variant of Omicron, which exhibited greater transmissibility and immune evasion from vaccination.[8] By June 2022, there were 1.2 million cases of infection of Omicron in the UK and US. [7,8]

There has been, and still is, an ongoing policy in many countries to protect vulnerable individuals in the face of waning immunity and emerging variants.[6] Many countries have introduced booster regimens following a primary vaccination series.[9–11] The United Kingdom (UK) introduced a fourth dose vaccination against SARS-CoV-2 in April 2022 on the advice of the Joint Committee on Vaccination and Immunisation (JCVI), principally targeting older adults and clinically vulnerable groups.[12] Similarly, the European Medical Agency's COVID-19 task force (ETF) and the European Centre for Disease Prevention and Control (ECDC) also recommended a fourth dose of mRNA COVID-19 vaccination for the immune compromised individuals and people over the age of 80 based on available epidemiological data.[13] Recommendations were largely based on data from studies in Israel which indicated a reduction in the risk of severe diseases and/or death due to COVID-19 with the administration of a fourth dose mRNA vaccine as compared with those receiving only a third dose.[11] This additional vaccine was deemed safe and immunogenic, with increases observed in both binding antibodies and neutralising antibody titres.[11]

The COV-BOOST trial further assessed safety, immunogenicity and reactogenicity of multiple third doses with a sub-study evaluating mRNA based fourth doses.[9] Participants who received a primary course of two doses of ChAdOx1 nCoV-19 and a third dose of BNT162b2, were randomised to receive either full-dose BNT162b2 or half-dose mRNA-1273 as fourth dose.[9] Results demonstrate that this vaccination regimen was safe with acceptable reactogenicity, and humoral and cellular responses were enhanced following the third dose.[9] A phase 4 randomised trial conducted in China evaluated the safety and immunogenicity of a human adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) among healthy individuals who have received three doses of inactivated vaccines. This study found Ad5-nCoV, administered aerosolised or intramuscular, was safe and highly immunogenic.[14]

ChAdOx1 nCoV-19 was one of the most widely distributed vaccines used for primary vaccine series in many countries, particularly in low- and middle-income countries.[15] Whilst clinical trials conducted in the UK, Brazil, South Africa, USA, Chile and Peru have shown ChAdOx1 nCoV-19 to have an acceptable safety profile and to be efficacious against COVID-19 in two dose regimens.[16–18] In addition, a study assessing immunogenicity and reactogenicity of a third dose of ChAdOx1 nCoV-19 found that antibody titres 28 days after a third dose were significantly higher compared with the second dose and were less reactogenic compared with the first dose.[19] There are no published studies evaluating safety and immunogenicity of ChAdOx1 nCoV-19 as a fourth dose. Due to the very rare occurrence of thrombosis and thrombocytopenia (TTS or VITT) and a calculated risk-benefit analysis partially based on the availability of alternate vaccines and levels of circulating virus, alternate vaccines to ChAdOx1 nCoV-19 were recommended for people younger than 40 years of age in the UK after May 2021. Within the UK, individuals who were clinically contradicted from receiving BNT162b2 or mRNA-1273 vaccines were recommended the ChAdOx1 nCoV-19 as a booster,[20] and this vaccine was available in many other areas as a booster. Real-world evidence indicated that boosting with ChAdOx1 nCoV-19 provided enhanced protection against symptomatic infections, diseases and hospitalisations during Delta and Omicron waves of infection.[21,22]

In this study, we aimed to evaluate the safety and immunogenicity of ChAdOx1 nCoV-19 as a fourth-dose booster in individuals who received two doses of ChAdOx1 nCoV-19 as a primary schedule in the COV002 trial (ISRCTN, 15281137), followed by an external third dose of BNT162b2.

Methods

Study design and participants

COV009 was a prospective safety study designed to extend the follow-up period for participants of the COV001 and COV002 trials for an additional 12 months, recording occurrence of serious adverse events (SAEs), adverse events of special interest (AESIs), COVID-19 diagnoses and exposure to other vaccines. As part of this trial, a subset of participants who received two doses of ChAdOx1 nCoV-19 in the COV002 parent trial and a subsequent external third dose of BNT162b2 vaccine were administered ChAdOx1 nCoV-19 as a fourth dose booster. COV001, starting April 23, 2020, was a phase I/II clinical trial that enroled 1077 healthy volunteers across five sites in the UK. COV002 was a phase II/III clinical trial enroling 10,812 participants in 19 study sites in England, Wales and Scotland from May 28, 2020.

Participants were eligible for the COV009 fourth dose sub-study if they were previously enroled, randomised and received two doses of ChAdOx1 nCoV-19 in the COV002 study, had previously provided blood sample for serology at 28 days post the second dose, had received an external non-study vaccination with BNT162b2 as the third dose at least four months before planned COV009 enrolment, and could be enroled within 26 weeks of the last parent-study visit. Exclusion criteria included enrolment in another COVID-19 vaccine clinical trial, pregnancy, any allergy or other contraindication to vaccination with ChAdOx1 nCoV-19, intention to move outside the study area, or receipt of additional COVID-19 vaccines other than those listed in the inclusion criteria.

The study was reviewed and approved by the University of Oxford, South-Central Berkshire Research Ethics Committee (20/SC/026), and the Medicines and Healthcare products Regulatory Agency, and was prospectively registered with a trials database under the EudraCT number: 2021–003382-36.

Outcomes

The primary outcomes were the safety and immunogenicity of fourth dose booster vaccination with ChAdOx1 nCoV-19 after two doses of ChAdOx1 nCoV-19 as a primary schedule and a third dose of BNT162b2. Solicited and unsolicited adverse events (AEs) were collected for seven and 28 days post-vaccination, respectively, and were recorded by participants in an electronic diary. Safety reporting was completed for 6 months post enrolment with SAEs and AESIs reported to and recorded by the study team.

Humoral immunogenicity was determined as anti-spike protein, anti-RBD and anti-N IgG antibody titres, pseudovirus neutralisation antibody titres (PNA) were measured in a subset of participants (n=90, except for n=30 for PNA against Omicron BA4/5). These measurements were conducted at multiple time points: baseline before the first dose (D0), 28 days and 364 days post-second dose (PB28 and PB364), before the fourth dose (4D) and 28 days after the fourth dose (4D28). Anti-ChAdOx1 IgG antibodies were measured for in the same subset of participants (n=90).

SARS-CoV-2 infections were recorded at follow-up visits and ad hoc contact, from enrolment to 180 days post the fourth dose. Infections were self-reported by participants who had taken either a polymerase chain reaction (PCR) or lateral flow test (LFT). Systematic testing was not carried out among COV009 participants.

Procedures

ChAdOx1 nCoV-19 is a recombinant chimpanzee adenovirus codifying the full-length spike SARS-CoV-2 glycoprotein and is administered intramuscularly at standard dose of 5×10^{10} viral particles in 0.5 ml.[18]

Anti-spike, receptor binding domain (RBD), and nucleocapsid (N) responses were measured by a validated multiplexed immunoassay (8-plex ECL-based assay on the MSD platform, PPD Vaccines, Richmond, VA, USA). The lower limit of quantification (LOQ) was 69, 52, 111, 150, 143, 102 AU/ml for wild type, Alpha (B.1.1.7), Beta (B.1.351), Delta (AY.4), Gamma (P.1) and Omicron (B.1.1.529), respectively. An N value above 9787 AU/ml was considered as seropositive.[23]

Antibody neutralisation titres were measured against Wuhan and Omicron (BA4/5) with a lentivirus-based pseudovirus particle expressing the D614 SARS-CoV-2 spike protein (Monogram Biosciences, South San Francisco, CA, USA). Results are presented as inhibitory concentration of serum achieving 50% neutralisation of virus (IC50).

Sample Size

Planned sample size was 150 participants. Assuming the standard deviation for the difference between dose two and dose four is similar to the standard deviation for the difference between dose two and dose three,[19] 150 participants would provide 98% power to show non-inferiority of the fourth dose compared with the second dose, assuming a non-inferiority margin of 0.67 for the lower bound of the geometric mean fold rise, and alpha of 0.025.

Statistical analysis

Safety and reactogenicity assessments were conducted for all participants who received a fourth-dose booster and completed the electronic diary. The proportions of participants with at least one severe (grades 3–4) or one severe or moderate (grades 2–4) adverse event are presented using radial plots.[9] Reactogenicity analyses were done for all participants and stratified by sex and age group (<60 and ≥60 years).

Humoral immunogenicity analyses included all participants with available antibody data. All immune maker data were log-transformed prior to analysis. Geometric means concentrations (GMC) and 95% confidence intervals (CIs) of humoral responses were firstly summarised at each time point with measurements available. Anti-spike protein IgG and PNA 28 days post the fourth dose versus 28 days post the second dose was then compared. For each antibody and variant assayed, the fold change was calculated for each participant by dividing data at 28 days post the fourth by those post-second dose, and then the geometric mean ratio (GMR) with 95% CIs was presented. A similar approach was applied to the fold change between before the fourth dose versus 28 days post-fourth dose vaccination. Post-hoc subgroup analyses were done using GMRs from pre- to post-fourth dose by sex, age group and seropositivity at the timing of fourth dose.

As an exploratory analysis, the relationship was investigated between humoral responses before and after the fourth dose, pre-fourth dose SARS-CoV-2 infection (self-reported) and risk of infection post-fourth dose. We compared humoral immune responses prior to the fourth dose, as well as at 28 days post-fourth dose, by self-reported infection status from 14 to 180 days after fourth dose vaccination, with significance set at $p < 0.05$. Logistic regression models were further applied, with SARS-CoV-2 infection 14 to 180 days post-fourth dose evaluated as an outcome, adjusting for age group (<60, ≥60 years) and job status (healthcare workers/retired/other). Each model individually included either antibody levels before or after the fourth dose, or serostatus prior to the fourth dose, or self-reported infection six months before the fourth dose as predictors. Multiple comparisons were not adjusted due to the exploratory nature of these analyses. All analyses were conducted using R version 4.2.2.

Results

Among 4470 participants in COV009 study, 149 participants were enroled in the fourth dose sub-study and received a dose of ChAdOx1 nCoV-19 vaccine as fourth dose booster between 6 and 24 September 2022 (Fig. 1, Table 1). Participants were predominantly female (n=90, 60.4%), aged from 22 to 90 years (mean 56 years). The overall mean interval between the first and second doses of primary courses was 54 days, with females intervals slightly longer than males. The intervals between the second to third and third to fourth doses were approximate a year, averaging 385 days and 332 days, respectively.

More than 90% of participants recorded solicited AEs in the first seven days following the fourth vaccination with ChAdOx1 nCoV-19 (Table S1). The reactogenicity following the ChAdOx1 nCoV-19 vaccine's fourth dose was generally mild (Fig. 2, Table S2, Fig. S1 and Fig. S2). Injection site pain was the most common local AE, while headache and fatigue were the most common systemic AEs. Six among 148 (4.0%) participants reported severe (grade 3 or above) local and systemic solicited adverse event within 7 days of the fourth dose (Fig. S2). Reactogenicity varied by sex and age, with slightly higher proportion of female and those under 60 years of age reporting solicited AEs (Fig. S1, Fig. S2). There were seven SAEs recorded during the study period; none were considered related to study vaccination.

The trends of anti-spike IgG, anti-RBD, anti-N and PNA against variants from baseline before the first dose, 28 and 364 after the second dose, before the fourth dose and 28 days post the fourth dose are shown in Fig. 3 and Fig. S3. Substantial increases in GMC were observed when comparing antibody levels at 28 days post the fourth with the level after the second dose (Table S3 and Fig. 3). For example, the GMC for anti-spike IgG against the wild-type spike protein increased from 19,634 (95% CI: 16,515–23,342) at 28 days post the second dose to 139,531 (95% CI: 116,628–166,933) at 28 days post the fourth dose, with a fold change of 7.11 (95% CI: 5.74–8.80). When restricting to seronegative individuals across all time points, this fold change slightly increased to 7.16 (95% CI: 5.25–9.77). This pattern was consistent for anti-spike IgG against other variants tested, i.e., B.1.1.7, B.1.351, AY.4, P.1 and B.1.1.529, and anti-RBD IgG and PNA responses (Table S3).

The increase in humoral immune responses from before the fourth dose to 28 days after the fourth dose was statistically significant, with approximate 1.20–1.30-fold increase across all variants assayed for anti-spike IgG and PNA (Fig. 3, Table S3 and Fig. S3). This modest enhancement was consistent in subgroup analyses by sex

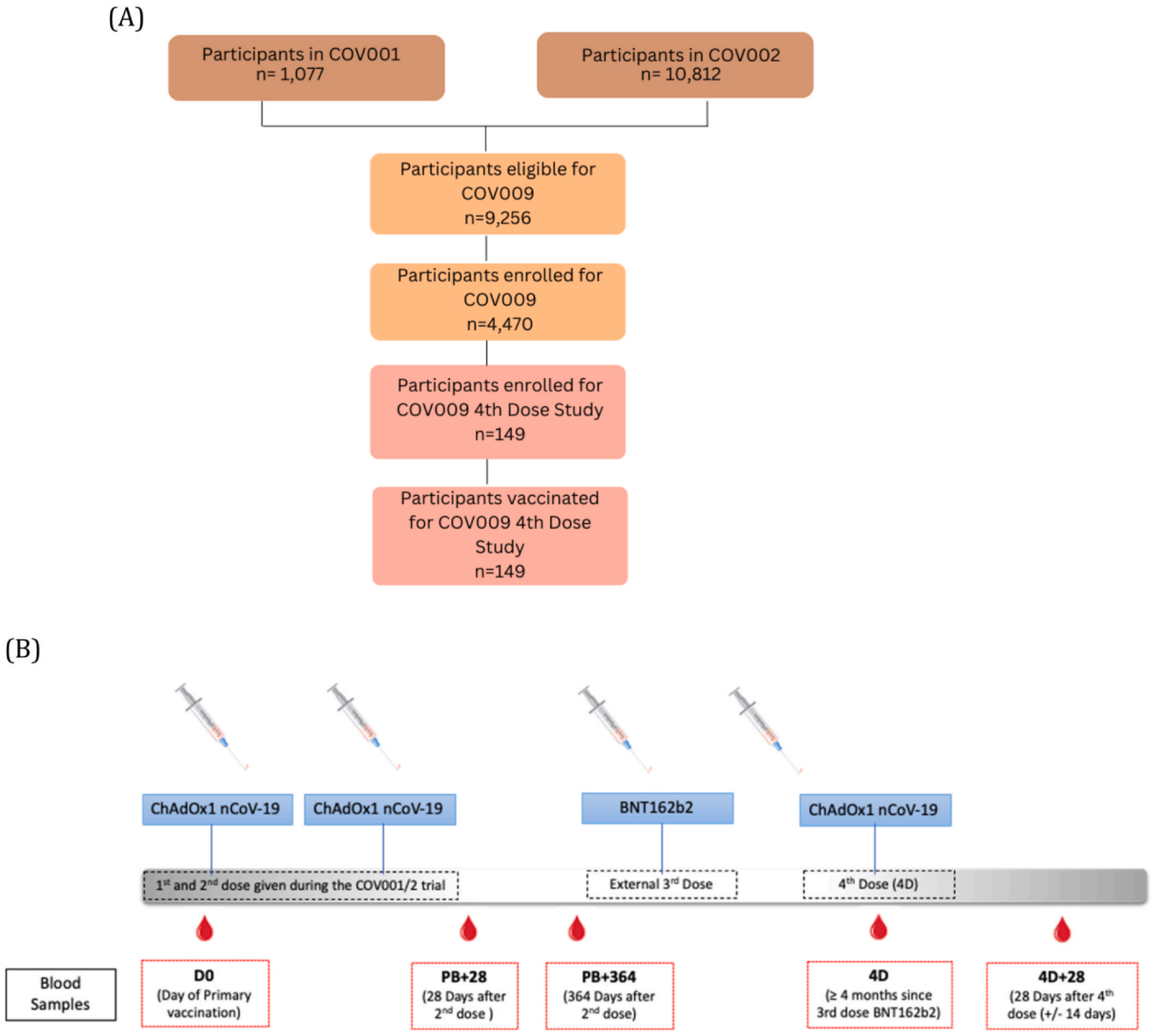

Fig. 1. (A) Flowchart and (B) Complete vaccine schedule and blood sampling for participants in COV009 fourth dose sub-study.

and age (Table S3, Fig. S4). Notably, 59.1% (88 out of 149) and 39.6% (59 out of 149) of participants reported SARS-CoV-2 infection 12 and 6 months prior to fourth dose vaccination, respectively; 37.9% (33 out of 87; 3 out of 90 anti-N assay results unavailable) participants tested seropositive by anti-N before fourth dose, indicating the existence of hybrid immunity at the timing of receiving the fourth dose. Seronegative participants had a more pronounced increase (approximate 1.4-fold or above) in antibody level after the fourth dose compared with seropositive individuals (Fig. S4). Among seropositive participants, we did not observe an increase in anti-spike and PNA GMC, suggesting an immune response plateau at the time of fourth dose vaccination.

From 149 participants, a total of 23 SARS-CoV-2 cases were reported by the participants during the follow-up visits from 14 days up to 180 days after fourth dose vaccination (Fig. S5). All cases were symptomatic and identified by LFT, with no significant differences by sex or age. Interestingly, higher levels of anti-spike IgG, anti-N IgG and PNA measured prior to the fourth dose were associated with less risk of infection from 14 to 180 days post-fourth dose (all p values < 0.05, Fig. 4, Fig. S6). Adjusting for age and job status using logistic regression analysis confirmed these results (Table S4). Being seropositive by anti-N IgG before the fourth dose, correlated with 84% infection risk reduction after the fourth dose (OR: 0.16; 95% CI: 0.05, 0.50). At 28 days post-vaccination, however, anti-N IgG level correlated with lower risk of infection ($p < 0.05$, Fig. 4, Fig. S6). We did not find any correlation between anti-vector antibody responses at pre-fourth dose and anti-spike IgG at 28 days after the fourth dose (Pearson correlation coefficient: −0.187, $p = 0.078$, Fig. S7).

Discussion

Few data exist on the safety and immunogenicity of reinforcing fourth COVID-19 vaccine doses, particularly heterologous regimes where the fourth dose is the adenoviral vectored vaccine ChAdOx1 nCoV-19. In this study, we found that fourth doses of ChAdOx1 nCoV-19 were well-tolerated, with minor variations in reactogenicity by sex and age. There were no safety concerns related to the vaccine over 180 days of follow-up. There was a modest yet significant increase in humoral responses from the day of the fourth dose booster to 28 days post-vaccination across all variants for anti-

Table 1
Baseline demographics for all fourth dose study participants, overall and by gender.

	Male	Female	Overall
	(N=59)	(N=90)	(N=149)
Age (mean, SD)	59.95 (14.1) [n=59]	54.65 (13.9) [n=90]	56.75 (14.2) [n=149]
Age (min-max)	28–90	22–75	22–90
Age group			
< 60	24 (40.7%)	56 (62.2%)	80 (53.7%)
60+	35 (59.3%)	34 (37.8%)	69 (46.3%)
Ethnicity			
White	59 (100.0%)	90 (100.0%)	149 (100.0%)
Job			
Healthcare Worker	11 (18.6%)	37 (41.1%)	48 (32.2%)
Other	27 (45.8%)	24 (26.7%)	51 (34.2%)
Retired	21 (35.6%)	29 (32.2%)	50 (33.6%)
Intervals between vaccination			
Days between 1st and 2nd dose	45.19 (21.3) [n=59]	60.11 (26.9) [n=90]	54.20 (25.8) [n=149]
Days between 2nd and 3rd dose	387.39 (50.9) [n=59]	383.30 (46.5) [n=90]	384.92 (48.1) [n=149]
Days between 3rd and 4th dose	328.71 (27.6) [n=59]	333.83 (25.0) [n=90]	331.81 (26.1) [n=149]

spike IgG and neutralising antibody. Notably, blunting of the immune response was observed among participants defined as seropositive in the anti-nucleocapsid assay at the time of the fourth dose, whilst more pronounced increases were observed in seronegative individuals. A previous mRNA fourth dose vaccine study, where participants were given the fourth dose at least four months after their third dose, has shown that antibody levels did not increase any higher than those observed at third dose and suggested that vaccines had hit an 'upper limit'.[11] Furthermore, our findings indicate a correlation between immune responses at the fourth dose vaccination and reduced risk of subsequent SARS-CoV-2 infection, underscoring the role of hybrid immunity obtained from both previous vaccination and infection.[24]

The heterologous schedule examined here had acceptable reactogenicity, with headache and fatigue the most common systemic AEs reported after vaccination. Previous studies with heterologous adenoviral-vectored and mRNA COVID-19 two dose prime-boost schedules showed increased levels of reactogenicity compared with homologous regimens; particularly fever which was reported at 41% for those that had BNT162b2 for prime and ChAdOx1 nCoV-19 for boost.[25] In this study, there was a single occurrence of a moderate fever, suggesting that the ChAdOx1 nCoV-19 vaccine as a fourth dose booster has very moderate levels of reactogenicity in the schedule studied here, although participants in our study had a wider age range than the previously published study by Liu et al. where all participants were over 50.[25] We have previously shown that reactogenicity is lower among older adults.[26]

During the study period from September 2022 to April 2023, the main circulating strain of SARS-CoV-2 in the UK was Omicron.[5] There was a relatively rapid increase in the divergence within the major lineages; the main circulating lineage was BA.5 at the start of the study, by the time the last serum samples were taken the XBB.1.5 sub-lineage had become predominant, although not to the same magnitude as BA.5.[27] At this time there was concern regarding how protective the vaccines would be against the rapidly evolving strains. Our immunogenicity data shows that binding and neutralising antibodies induced from a fourth dose booster increase beyond that measured at 28 days post second dose across all variants tested (7- to 9-fold in anti-spike IgG across variants), considering the interim additional third dose BNT162b2 vaccine and natural exposure to circulating SARS-CoV-2 as confirmed by the levels of anti-N antibodies. Comparatively, a study assessing the immunogenicity of a third homologous ChAdOx1 nCoV-19 dose in participants who had previously received two doses from the COV001 trial reported a significant 2-to 3-fold increase in anti-spike antibody levels against wild-type, Alpha, Beta, and Delta variants at 28 days post-third dose compared with 28 days post-second dose.[19] Our finding is consistent with one study involving mRNA vaccines as the fourth dose, and another study involving recombinant protein vaccine as the third dose, which reported that these booster vaccines may enhance the breadth of humoral responses across variants.[28,29]

It is likely that the modest increase in humoral immune responses across the variants tested from pre- to post-fourth dose, were due to the existing elevated level of antibody concentrations at the timing of fourth dose which blunts additional increases in response. The main circulating strain at the time of the study was Omicron and GMRs were approximately and significantly 1.24 overall for IgG against Omicron spike; delineation of this response into changes from pre- to post-fourth dose for seronegative individuals shows a more pronounced increase of GMR to 1.43, compared with a lower GMR for seropositive individuals at 0.99. This concurs with the results of studies showing that maximum levels of antibodies were observed following three doses of mRNA vaccine, although other studies have shown with a fourth dose increases in antibodies are observed, albeit with different intervals between doses and absolute antibody levels.[11,25]

An advantage of this study is that the immune responses were tracked from before first dose (vaccine naïve) until 28 days after the fourth dose vaccine. The spread of the humoral response at 364 days post second and/or third dose, reveals the wide distribution in the antibody levels across the participants, which can be attributed to antibody waning after the second dose and increases from third dose vaccination and/or natural infection. Our data show that there is an increase in the circulating anti-N antibodies between 364 days after the second dose and before the fourth dose; this suggests that natural infection boosted antibody levels significantly. Therefore, the antibody levels recorded before the fourth dose are likely due to hybrid responses from vaccine and natural infection.

Anti-spike IgG and neutralising antibody following two doses of ChAdOx1 nCoV-19 or BNT162b2 have been proven to correlate with protection against symptomatic SARS-CoV-2 infection.[30–32] In this study, we observed that anti-spike IgG responses, PNA, and anti-nucleocapsid IgG at the fourth dose were correlated with a reduced risk of SARS-CoV-2 infection during the Omicron wave over the subsequent six months as self-reported by participants. This indicates that the hybrid immunity, likely stemming from previous natural infections and a heterologous third dose of BNT162b2 administered approximately 11 months prior, offers some protection against the circulating strains of Omicron such as BA5 following the fourth dose. Self-reported COVID-19 infection prior 4th dose (6 months) did not correlate with protection, whilst seropositivity just prior to the 4th dose did; suggesting low responders following infection are more susceptible to re-infection.[33] Nevertheless, our analysis may miss those symptomatic or asymptomatic individuals who did not undergo testing, possibly biasing an association to the null, if such an association exists. We found no association between anti-vector immune responses and anti-spike IgG, suggesting that a fourth homologous boost with adenovirus-vectored vaccines does not compromise pathogen-specific immunogenicity. These data also reassuring that using ChAdOx platform for other vaccines will not be affected by anti-vector humoral responses.

Our study is limited by the absence of T cell data, which might provide insight into cellular immune responses. Furthermore, only a subset of participants was measured for humoral responses, which decreases the statistical power particularly when looking at risk of infection. Additionally, we focused on peak immune responses without assessing the waning of immunity over time in the humoral assays. Moreover, the choice of cutoff of anti-N to define seropositive may be subject to misclassification bias, as evidenced by a few

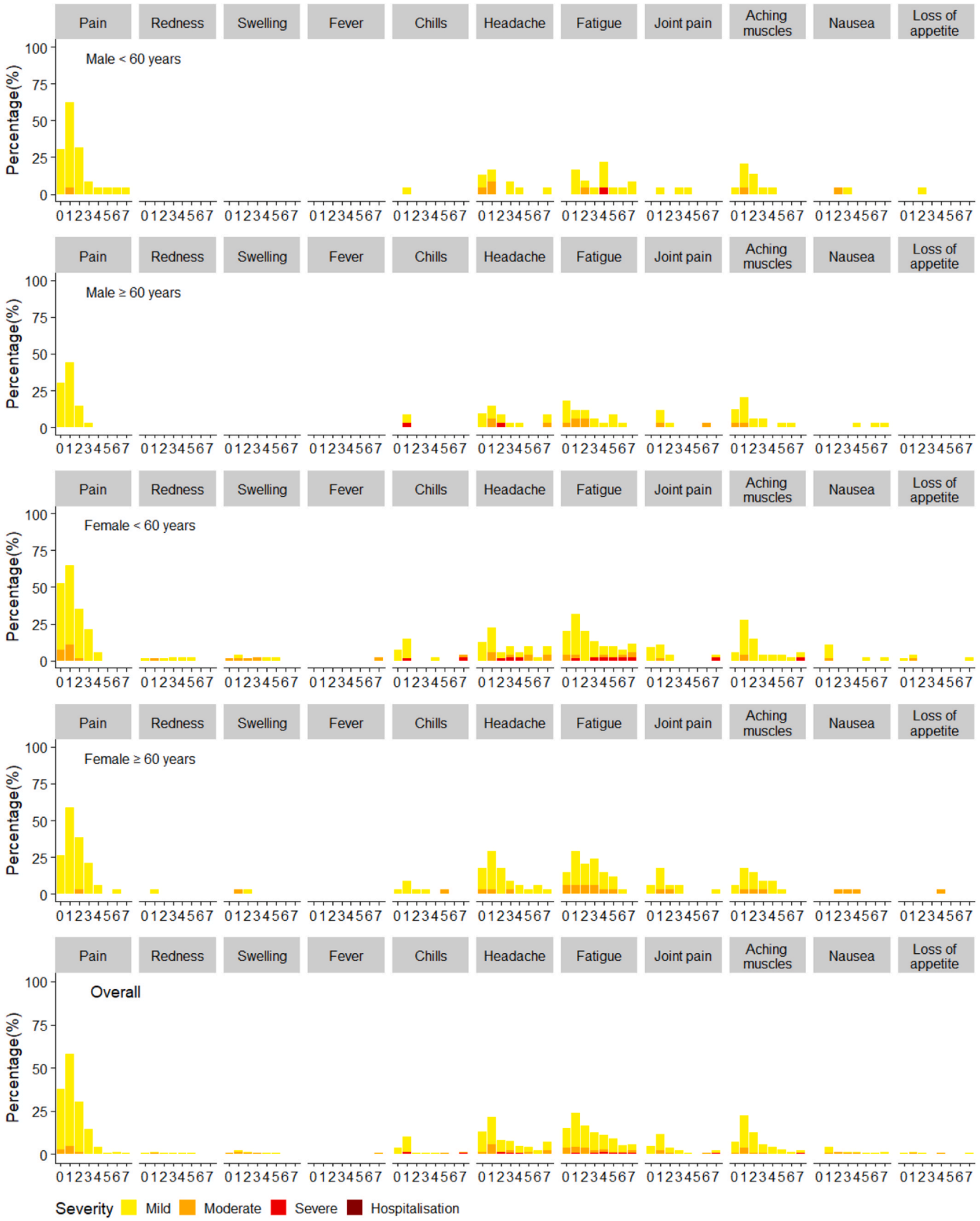

Fig. 2. Solicited local and systemic adverse reactions in first seven days after fourth dose vaccination, by gender, age and overall.

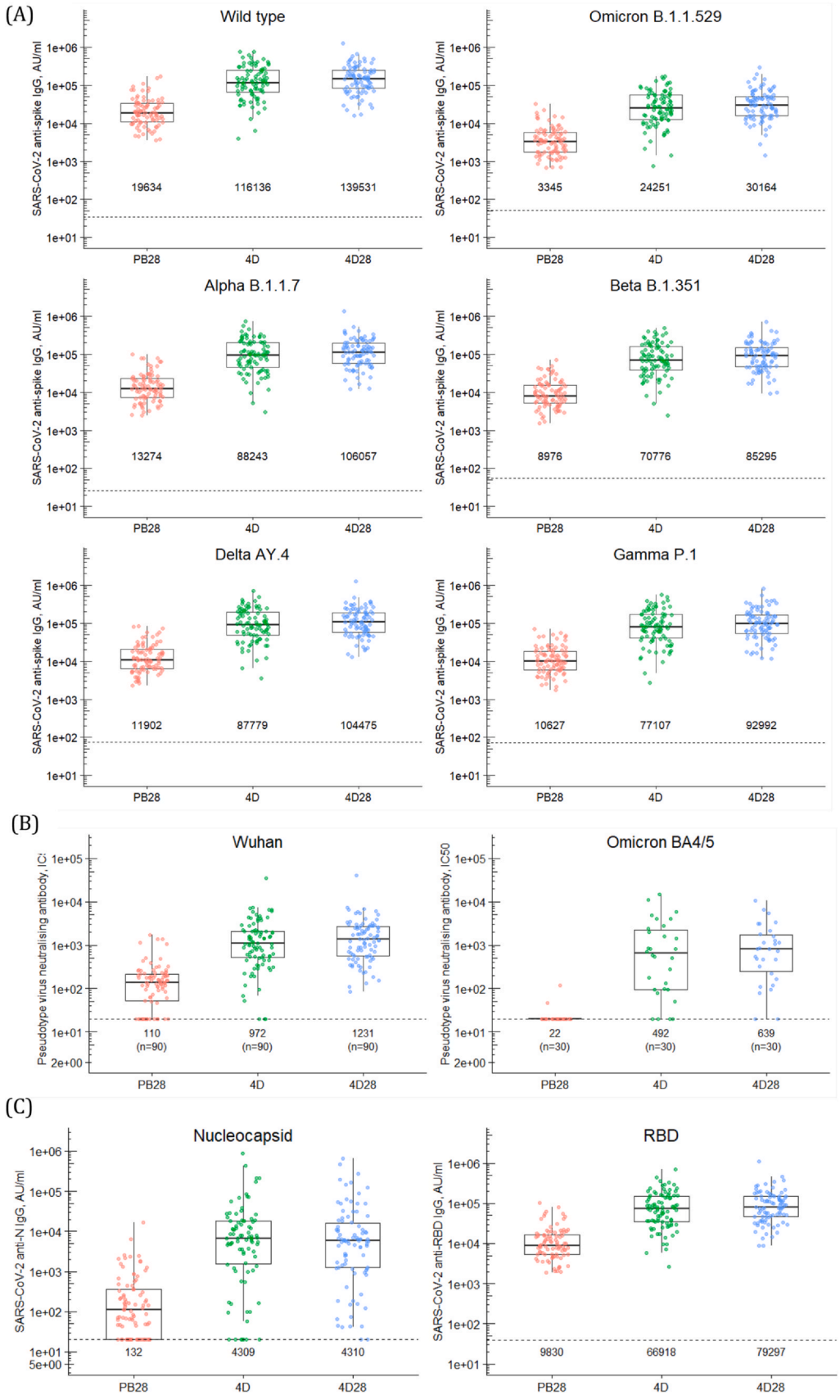

Fig. 3. (A) Anti-spike IgG (B) pseudotype virus neutralising antibody and (C) anti-nucleocapsid and anti-RBD IgG against variants measured at 28 days post the second dose, day of administering the fourth dose and 28 days post the fourth dose. PB28: 28 days post the 2nd dose; 4D: day of administering the 4th dose; 4D28: 28 days post the 4th dose.

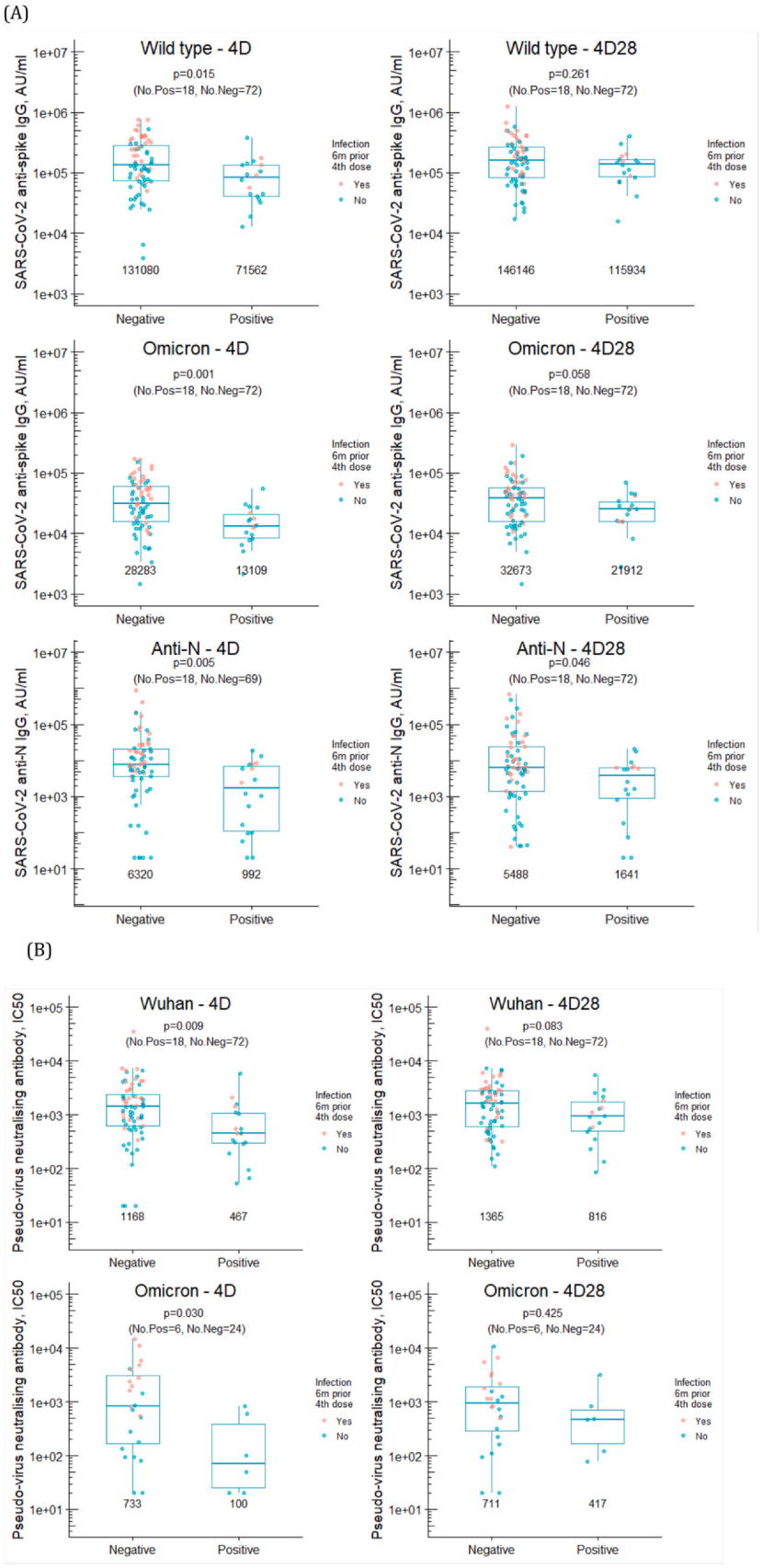

Fig. 4. (A) Anti-spike IgG against wild type and Omicron B.1.529 variants and anti-N IgG. (B) Pseudovirus neutralising antibody measured at day of administering the fourth dose, and 28 days post the fourth dose, comparing positive cases and negative non-cases occurred 14 to 180 days after the fourth dose. Different colours indicate self-reported infections from 6 months prior to the fourth dose vaccine (red) or non-infections (teal). 4D: day of administering the 4th dose; 4D28: 28 days post the 4th dose.

individuals showing significant increases in anti-N levels, suggesting possible infection, yet were categorised as seronegative according to the choice of threshold. The findings from this study derive from a white population and may not be generalisable to other ethnic or racial groups, when previous studies identified potential ethnic differences in immunological responses and vaccine efficacy.[34,35]

To our knowledge, this is the first study to report on the use of ChAdOx1 nCoV-19 as a fourth-dose booster following two ChAdOx1 nCoV-19 and one BNT162b2 doses, and demonstrates good tolerability with enhanced immunogenicity, including against other SARS-CoV-2 variants. This underscores the potential usefulness of ChAdOx1 nCoV-19 vaccine as a safe and effective reinforcing immunisation, contributing insight into vaccination strategies against COVID-19.

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Author contributions

AJP is the chief investigator. AJP, MV and TL contributed to the protocol and design of the study. SNF is study site principal investigators. SB, PA, FC, EAC, KC, AE, SF, HG, YM, NO, EP and HR contributed to the implementation of the study or data collection or laboratory experimentation. SF did the statistical analysis. SF and SB contributed to the preparation of the report. All authors critically reviewed and approved the final version.

Data availability

Anonymised participant data will be made available when the trials are complete, upon requests directed to the corresponding author. Proposals will be reviewed and approved by the sponsor, investigator, and collaborators on the basis of scientific merit. After approval of a proposal, data can be shared through a secure online platform after signing a data access agreement. All data will be made available for a minimum of 5 years from the end of the trial.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: AJP is Chair of the UK DHSC Joint on Vaccination & Immunisation (JCVI) but did not participate in the JCVI COVID19 committee during the pandemic and was a member of the Strategic Advisory Group of Experts on Immunization to the WHO (World Health Organization) until 2022. TL reports consulting fees from Vaccitech on an unrelated project, an honorarium from Seqirus, grant support from the Vaccine Taskforce for this trial, work-related investments, and is named as an inventor on a patent application for a vaccine against SARS-CoV-2. JG is an employee of, and holds stocks in, AstraZeneca. AJP, TL, SB, PA, FC, EC, SF, YM, NO, EP, HR, AS, and MV are inventors and/or contributors to the intellectual property licensed by Oxford University Innovation to AstraZeneca. All other authors declare no competing interests. The views expressed in this article do not necessarily represent the views of the funders.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2025.106423.

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