Homologous & heterologous COVID-19 vaccine priming schedules: An analysis of systemic & mucosal immunity

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Abstract

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Most two dose vaccination schedules, including UK-licensed SARS-CoV2 vaccinations, are licensed as homologous schedules. At the point of UK emergency licensure of the first two SARS-CoV2 vaccines, global demand for vaccine outstripped supply. National immunisation programmes faced logistical challenges to ensure sufficient doses of the same vaccine could be distributed to each person in the manufacturer-recommended timeframe.

Two randomised control trials (Com-COV1 and Com-COV2) assessed safety, tolerability and non-inferiority of humoral immunogenicity of heterologous priming schedules using vaccines available to the UK immunisation program – AstraZeneca, Pfizer, Moderna and Novavax.

Heterologous schedules are more reactogenic than their equivalent homologous schedules, however, all schedules are tolerable and without safety concern.

Not all heterologous schedules are non-inferior to their relevant homologous schedule in terms of serum anti-SARS-CoV2 spike binding IgG, but they do always achieve a robust immunological response at least as large as the least immunogenic schedule studied (homologous AstraZeneca), which has proven efficacy against both severe disease and symptomatic infection.

Differences between vaccine platforms are also qualitative with clear differences in the capability of each schedule to produce neutralising responses. T-cell responses differ markedly with the greatest responses seen in those receiving heterologous schedules with AstraZeneca as the first dose.

Increasing priming interval from one to three months resulted in a modest increase in antibody response and a decrease in cellular response.

Mucosal responses were evaluated as an exploratory endpoint. There was no evidence that mucosal anti-SARS-CoV2 spike IgA was induced by intramuscular vaccination. There is evidence that nasal mucosal responses can occur in the absence of systemic responses, possibly due to asymptomatic mucosal SARS-CoV2 exposure. Existing mucosal IgA responses against seasonal coronaviruses may confer some degree of protection against SARS-CoV2 infection.

These results support the use of heterologous schedules as viable alternatives to homologous schedules and support flexibility in priming interval. They have informed UK and global immunisation policy. There is a need for alternative vaccination strategies, such as mucosally delivered vaccines, which induce mucosal responses that may reduce transmission.

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Publications

The following is a list of first-author or joint-first author publications, which have arisen out of the work conducted for my DPhil

- **Shaw, R. H.**, Greenland, M.et al (2023). Persistence of immune response in heterologous COVID vaccination schedules in the Com-COV2 study – A single-blind, randomised trial incorporating mRNA, viral-vector and protein-adjuvant vaccines. *Journal of Infection*,
- **Shaw, R. H**., Liu, X. et al (2022). Effect of priming interval on reactogenicity, peak immunological response, and waning after homologous and heterologous COVID-19 vaccine schedules: exploratory analyses of Com-COV, a randomised control trial. *The Lancet, Respiratory Medicine*
- Dejnirattisai, W., **Shaw, R. H**. et al (2021). Reduced neutralisation of SARS-CoV-2 omicron B.1.1.529 variant by post-immunisation serum. *The Lancet*
- Stuart, A. S., **Shaw, R. H**. et al (2021). Immunogenicity, safety, and reactogenicity of heterologous COVID-19 primary vaccination incorporating mRNA, viral-vector, and protein-adjuvant vaccines in the UK (Com-COV2): a single-blind, randomised, phase 2, non-inferiority trial. *The Lancet*
- Liu, X., **Shaw, R. H**. et al (2021). Safety and immunogenicity of heterologous versus homologous prime-boost schedules with an adenoviral vectored and mRNA COVID-19 vaccine (Com-COV): a single-blind, randomised, non-inferiority trial. *The Lancet*
- **Shaw, R. H.**, Stuart, A. et al. (2021). Heterologous prime-boost COVID-19 vaccination: initial reactogenicity data. *The Lancet*

Additional publications relevant to, but not directly related to this thesis. First/Last author publications marked with an asterix (*).

- ***Shaw, R. H.** (2023). Nasally delivered SARS-CoV-2 vaccines: future promise and challenges. Invited Commentary. *The Lancet, Respiratory Medicine*
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- Voysey, M., Costa Clemens, S. A., et al (2021). Single-dose administration and the influence of the timing of the booster dose on immunogenicity and efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine: a pooled analysis of four randomised trials. *The Lancet*

Conferences & Oral Presentations

- Oral Presentation (2021) "The effect of interval on vaccine immunogenicity & reactogenicity" *British Infection Association*
- Regular oral presentation (2020-2023) to the UK Vaccine Taskforce COVID meetings
- Oral presentation (2023) as an independent external expert to the Trial Coordination Board of the combined EU funded projects EU-RESPONSE, RECOVER, ECRAID-Prime and VACCELERATE

Academic Prizes & Awards

- Ralph A. Lewin Prize for DPhil, St Cross College, University of Oxford, 2024
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- Clarendon Scholar, University of Oxford, 2021

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Abbreviation	Definition
A&E	Accident & Emergency
AE	Adverse Event
AESI	Adverse Event of Special Interest
aGMR	Adjusted Geometric Mean Ratio
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
Anti-N IgG	Anti-nucleocapsid IgG
Anti-S IgG	Anti-SARS-CoV2 spike IgG
ARDS	Acute Respiratory Distress Syndrome
BAU	Binding Antibody Units
BCG	Bacille Calmette Guerin
BIPAP	Bi-level Positive Airway Pressure
BSA	Bovine serum albumin
C19P	COVID-19 Pathway
CEPI	Coalition for Epidemic Preparedness Innovations
CI	Confidence Interval
CL2 / CL3	Containment Level 2 / Containment Level 3
COVID / COVID-19	Coronavirus infectious disease / Coronavirus infectious disease 2019
CPAP	Continuous Positive Airway Pressure
CRO	Contract research organisations
CRP	C-reactive protein
CT	Computerised tomography
CV	Coefficient of variation
DCMO	Deputy Chief Medical Officer
DPBS	Dulbecco's Phosphate Buffered Saline
D-PBS	Dulbecco's Phosphate Buffered Solution
DSMB	Data Safety Monitoring Board
ECLIA	Electrochemiluminescence assay
eDC	Electronic data capture system
ELISA	Enzyme linked immunosorbant assay
ELISpot	Enzyme linked immunosorbant spot assay
ELU	ELISA laboratory units
EMA	European Medicines Agency
EudraCT	European Union Drug Regulating Authorities Clinical Trials
FBC	Full Blood Count
FDA	Food & Drug Administration
$FRNT_{50}$	50% neutralisation titre in the Focus Reduction Neutralisation Assay (FRNT)
GMC	Geometric Mean Concentration
GMR	Geometric Mean Ratio
GMT	Geometric Mean Titre
gp	glycoprotein

GSTT	Guys & St Thomas' Hospital, London
HCoV	Human Coronavirus
HIV	Human Immunodeficiency Virus
HPAI	Highly Pathogenic Avian Influenza
ICS	Intracellular cytokine staining
ID	Intradermal
IFA	Incomplete Freund's adjuvant
IFNγ	Interferon gamma
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IHR	International Health Regulations
IL	Interleukin
IM	Intramuscular
IMP	Investigational medicinal product
IRAS	Integrated Research Application System
ISRCTN	International Standard Randomised Controlled Trial Number
ITT	Intention to treat
JAK	Janus Kinase
JCVI	Joint Committee on Vaccination and Immunisation
LFA	Lateral Flow Antigen
LFT	Liver Function Test
LMIC	Low and middle income country
LRTI	Lower Respiratory Tract Infection
MAE	Medically Attended Adverse Event
MAIT	Mucosally associated invariant T cells
MERS-CoV	Middle Eastern Respiratory Syndrome - Coronavirus
MHRA	Medicines and Healthcare products Regulatory Agency
mITT	Modified Intention to treat
mL	Millilitre
MLF	Mucosal lining fluid
MNA	Micro neutralisation assay
MSC	Microbiological Safety Cabinet
MSD	Mesoscale Diagnostics
MVA	Modified Vaccinia Ankara
NAAT	Nucleic acid amplification test
NaCl	Sodium chloride
nCoV	Novel Coronavirus
NHS	National Health Service
NIHR	National Institute of Health and Care Research
NT_{50}	50% neutralisation titre
ORF-1	Open Reading Frame 1
OVG	Oxford Vaccine Group
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PHA	Phytohaemagglutinin
PHE / UKHSA	Public Health England (now United Kingdom Health Security Agency)

PHEIC	Public Health Emergency of International Concern
PNA	Pseuotype virus neutralisation assay
PPE	Personal protective equipment
RBD	Receptor Binding Domain
RBM	Receptor Binding Motif
RCT	Randomised Control Trial
RLU	Relative luminescence units
RNA	Ribonucleic acid
rpm	Revolutions per minute
RT	Room temperature
RT-PCR	Reverse transcription polymerase chain reaction
SAE	Serious Adverse Event
SAM	Synthetic absorbable matrix
SARS	Severe acute respiratory syndrome
SARS-CoV / SARS-CoV1	Severe acute respiratory syndrome – Coronavirus / Severe acute respiratory syndrome – Coronavirus 1
SARS-CoV2	Severe acute respiratory syndrome – Coronavirus 2
SC	Subcutaneous
SFC	Spot forming cells
SGUL	St George's University London
sIgA	Secretory IgA
SIV	Simian Immunodeficiency Virus
T1RF	Type 1 Respiratory Failure
T2RF	Type 2 Respiratory Failure
TMB	Tetramethylbenzidine
TNF	Tumour Necrosis Factor
TPA	Tissue plasminogen activator
TSG	Trial Steering Group
U&E	Urea & Electrolytes
UCLH	University College London Hospitals
UKHSA / PHE	United Kingdom Health Security Agency (formerly Public Health England)
URTI	Upper Respiratory Tract Infection
VAED	Vaccine associated enhanced disease
VITT / VATT	Vaccine-induced thrombocytopaenic thrombosis / Vaccine-associated thrombocytopaenic thrombosis
VTF	Vaccine Taskforce
WHO	World Health Organisation
WT	Wild-type

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Declaration & Contribution

All data and analyses presented in this thesis are solely my own unless otherwise stated by reference or acknowledgement and have not been submitted for any other degree or professional qualification.

Prof Xinxue Liu prepared the statistical analysis plan for the trial and undertook the analyses of immunological outcomes presented in the two primary outcome publications and interval publication. He also reconciled assay result discrepancies that arose from the results from the various contract research organisations. Analyses in this thesis are separate and my own.

Ms Melanie Greenland undertook the reactogenicity analyses from the published papers. Analyses in this thesis are separate and my own.

Samples were processed by a number of different commercial clinical research organisations for the assays that formulated the primary and secondary end points of the study. Methodologies for the assays conducted were written with advice from the scientists from each organisation:

- Nexelis laboratories, Canada: Anti-SARS-CoV2 spike IgG ELISA, Pseudo-typed virus neutralisation assay
- UK Health Security Agency: Live virus neutralisation assay, Anti-nucleocapsid ECLIA
- Oxford Immunotec: T-cell ELISpot, Intracellular cytokine staining

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Chapter 1 Introduction

1.1 Global Health Threats

Over the centuries, increased global trade and travel have led to greater prosperity and a greater mixing of communities around the world. This increased mixing has also resulted in increased opportunity for pathogen transmission and has led to various infectious epidemic outbreaks (1). The recognition that infectious disease control required trans-national cooperation led to fourteen International Sanitary Conferences, the first of which occurred in Paris in 1851 (2), where the diseases of focus were plague, yellow fever, small pox and cholera. These conferences had variable impacts and continued until the outbreak of the Second World War. The balance of protecting health and maintaining individual freedoms and business was always at the forefront of discussion.

Following on from the Second World War, the World Health Organisation (WHO) was formed in 1948 to promote health globally. In 1969 the first iteration of the International Health Regulations (IHR), was adopted by the World Health Assembly (the decision-making body of the WHO) and covered six diseases (plague, cholera, yellow fever, typhoid, small pox and 'relapsing fever'). It has since been revised in 1973, 1981 and 2005 to address the changing threats to health from the emergence and re-emergence of different diseases that have accompanied increased international trade and travel.

Following the Severe Acute Respiratory Syndrome (SARS) outbreak of 2002-2004 (3) caused by the virus Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), the IHR were updated again in 2005. With this came the concept of a Public Health Emergency of International Concern (PHEIC) defined as *"an extraordinary event which is determined to constitute a public health risk to other states through the international spread of disease and to potentially require a coordinated international response"* (4).

Since 2005 there have been a number of PHEIC declared (5) including Influenza A H1N1pdm09 (2009), Ebola (West African outbreak 2014-2016, Democratic Republic of Congo 2018-2020), poliomyelitis (2014 – 2023), Zika (2016), COVID-19 (2020 – Present) and latterly Mpox (previously Monkeypox, 2022-2023). There have been other serious outbreaks which did not pass the definition threshold for PHEIC, including Middle Eastern Respiratory Syndrome Coronavirus (MERS-CoV) in 2012, and the 2019-2020 Kivu Ebola outbreak, whilst the threat of other pathogens such as highly pathogenic avian influenza (HPAI) H5N1 remain in the background (6).

In addition to the above pathogens that have already required coordinated international responses, it is recognised that there are other pathogens including Crimean-Congo haemorrhagic fever and Lassa fever that carry a large public health risk either due to their transmissibility and pandemic potential or due to the lack of countermeasures that could mitigate spread, prevention or treatment (7).

Some of these illnesses such as MERS were characterised by high mortality, but poor transmissibility (8), whilst others such as H1N1pdm09 were more transmissible, but had a lower mortality (9), in part thanks to existing immunity in some of the older population, who had likely previously been exposed to an antigenically similar virus.

1.2 SARS-CoV2

In December 2019, an outbreak of a cluster of cases of a novel severe respiratory syndrome of unknown cause was described in Wuhan, Hubei province, People's Republic of China (10). Over the ensuing days and weeks, it rapidly became apparent that this was a highly transmissible disease which was associated with a high mortality. Initial attempts to contain the global spread of disease were largely unsuccessful (11).

Soon afterwards in January 2020, the aetiological pathogen, a novel coronavirus was isolated , sequenced and made publicly accessible (12). It was subsequently designated

Severe Acute Respiratory Syndrome – Coronavirus 2 (SARS-CoV2) due to its close sequence homology with SARS-CoV (henceforth in this thesis referred to SARS-CoV1).

The original wild-type strain (WT) was named Wuhan-Hu-1 (13), however, another Wuhan-Hu-1 related sample, taken early on in the pandemic in Australia, was named Victoria (SARS-CoV-2/human/AUS/VIC01/2020) (14). The literature surrounding wild-type SARS-CoV2 variably uses either of these extremely closely related strains to denote wild-type. Given the close similarity of these 'wild-type strains', I will, for simplicity, refer to the wild-type only in this thesis.

1.3 Coronaviruses

Coronaviruses are part of the large family of viruses called *Coronaviridae (***Figure 1***).* They are enveloped viruses containing single-strand positive sense ribonucleic acid ($ssRNA^{+}$). Their genomes are some of the largest of any RNA viruses (27-31 kilobases long). There are currently seven coronaviruses recognised to cause disease in humans – three epidemic and four endemic, which are phylogenetically classified as either alpha or beta coronaviruses. Prior to the 2003 global outbreak of SARS-CoV1, there were only two recognised human coronaviruses (HCoV) designated OC43 and 229E. Both were considered to cause only mild upper respiratory tract infections such as the 'common cold'. Following on from SARS-CoV1, global perception of coronaviruses and their importance with regards to their impact on human health changed. Two further coronaviruses were later isolated, designated NL63 & HKU1 (15–17), which again predominantly caused upper respiratory tract illness. MERS-CoV was isolated in 2012 following on from a series of high mortality pneumonias (18).

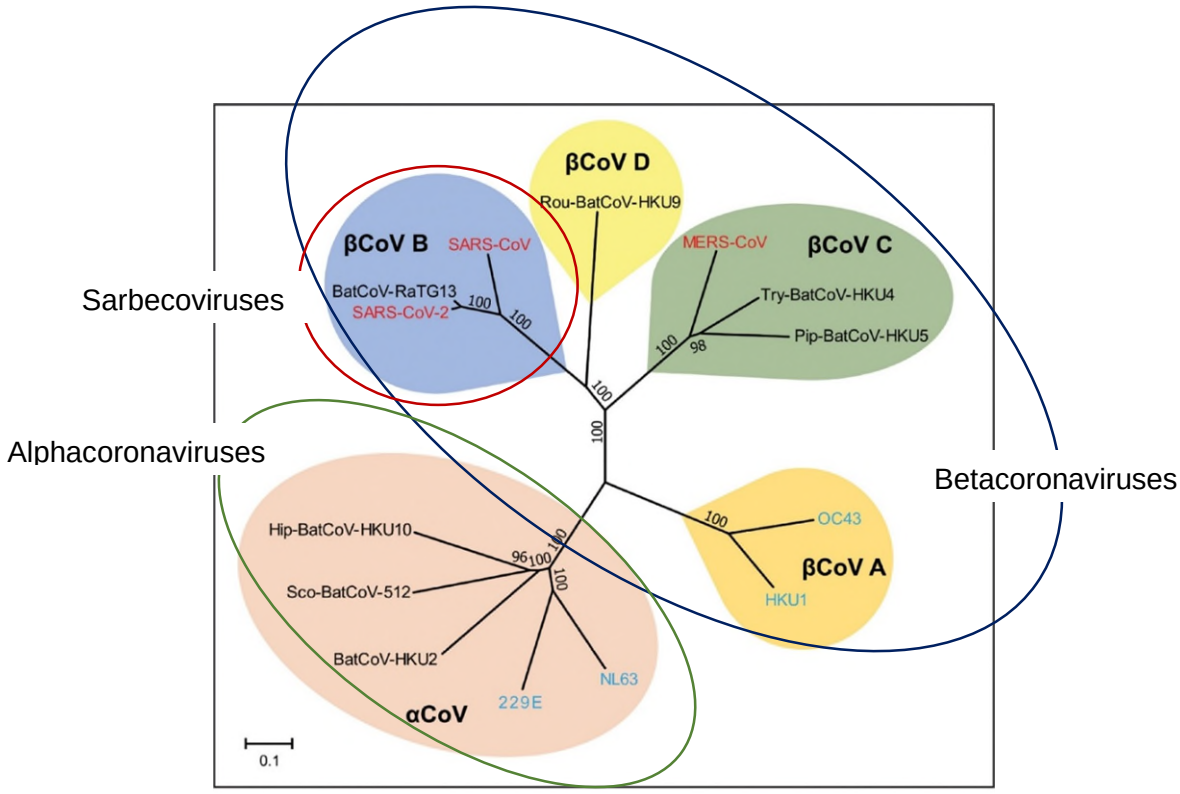

Figure 1 *– Phylogenetic tree of the Coronavirus family. Modified from the paper "Does Cross-neutralization of SARS-CoV-2 Only Relate to High Pathogenic Coronaviruses?" by Ma et al. published in Trends in Immunology, 2020* (19)

1.4 COVID-19

COVID-19 (coronavirus infectious disease 2019) was the name given to the syndrome caused by this novel pathogen, SARS-CoV2.

SARS-CoV2 infection causes a range of syndromes from asymptomatic/sub-clinical infection to a mild upper respiratory tract infection (URTI) or a lower respiratory tract infection (LRTI) causing severe inflammatory lung damage.

Symptomatic infection in an unvaccinated population with the wild-type virus typically caused a biphasic syndrome (20), which consisted of an initial mild viral illness hallmarked by fever, new persistent cough and a change or reduction in sense of smell/taste (often in the absence of nasal congestion or other coryza). Sore throat, fatigue, myalgia, arthralgia, headache, coryza, anorexia, nausea, diarrhoea and shortness of breath were all also commonly described (21). The duration of this initial illness would normally be less than a week, often with near-complete recovery, followed by a potentially more severe, immune-mediated phase of illness occurring 7-12 days

following the initial onset of symptoms, associated with respiratory deterioration and oxygen desaturation. It was this phase that typically caused hospitalisation and death. During this second phase, there was sometimes a disconnect between the level of hypoxia and the severity of symptoms, with some patients appearing relatively comfortable despite very low oxygen saturations.

Radiologically, severe SARS-CoV2 infection of the lower respiratory tract was characterised by bilateral peripheral ground glass changes and/or consolidation on chest x-ray or computerised tomography (CT) (22).

The term COVID-19 has been inconsistently used by the media, the general public and medical professionals to describe both the virus itself, as well as any infection caused by the virus, regardless if the infection was asymptomatic, a mild URTI or a severe LRTI. In this thesis, I will therefore refer to SARS-CoV2 infection and classify severity, rather than using the term COVID-19 which has some associated ambiguity.

1.5 Diagnosis

Prior to the wide availability of a reliable molecular diagnostic test, most diagnoses were made on a syndromic basis (new onset fever, persistent cough, anosmia). Epidemiological risk (recent contact with a confirmed or suspected positive case or travel to a geographic area with a high incidence of disease) was initially used as part of the diagnostic algorithm to ascertain likelihood of diagnosis. However, as SARS-CoV2 spread more widely, this rapidly ceased to be a very useful metric in diagnosis. Radiographic findings were also used to inform the likelihood of a positive case although these lacked both sensitivity for infection and specificity for the cause of lung changes.

The first widely available assay reliably to detect SARS-CoV2 was a nucleic acid amplification test (NAAT), the first of which was a reverse transcriptase polymerase chain reaction (RT-PCR). This involved a combined nasopharyngeal sample taken with

a flocked swab which was stored in viral transport medium and detected the presence of viral RNA. Although highly sensitive, there were several issues with the PCR test: Firstly inadequately administered tests (either by the patient or healthcare professionals) results in reduced sensitivity. Secondly, sensitivity is dependent on timing of testing according to the syndrome, with peak sensitivity occurring concurrently with the first phase of illness. Thirdly it was seen that viral RNA had the potential to be present for prolonged periods following recovery from infection with viral clearance – up to 3 months in some cases (23). Many PCR platforms had more than one target (e.g. Spike, Nucleocapsid and ORF1) (24) – as new variants emerged, the primers targeting the most mutable genes (spike) bound with lower affinity. Hence, diagnosis algorithms had to change (so-called 'S-gene drop-out' or 'S-gene target failure' were used as a correlate to diagnose new variants) (25). Despite these caveats, with PCR primers needing to be redesigned in keeping with emerging variants, PCR remained one of the most reliable ways for mass detection of SARS-CoV2 available to the UK population.

Routine whole genome sequencing (WGS) of samples started in 2020, soon after the pandemic began. This provided more detailed genetic information about transmission and new variants (26). It also did not suffer from the problems of S-gene target failure.

The same nasopharyngeal swab samples could also be tested via lateral flow antigen (LFA) tests which detected viral protein, although the precise make up of which proteins or proteins subunits that were detected remain unknown due to the proprietary nature of these kits. Sensitivity and specificity varied between different commercial products (27). It is likely that there is also variation of protein targets between products. These products, however, did not require specialised equipment or operators, as PCR did and so were a useful diagnostic adjunct for mass home testing.

The diagnostic method of choice varied from country to country according to government policy. These policies changed over the course of the pandemic as different modalities

of testing were up- and down-scaled and as vaccination was rolled out with efforts to reduce social restrictions. Each method of diagnosis had its own merits in terms of ease of use, rapidity of test result, inference of transmissibility, expense, sensitivity and specificity (28).

Serology (anti-spike immunoglobulin G [anti-S IgG] or anti-nucleocapsid [anti-N] IgG) is typically not regarded as a helpful diagnostic tool in the acute phase of illness, taking approximately 1-2 weeks to become positive following infection (29). Additionally, anti-S IgG remains raised for prolonged periods following vaccination (as does anti-N IgG for inactivated vaccines), whilst both remain raised for prolonged periods following natural infection, making it difficult to define re-infection (30).

1.6 Risk factors for severe illness in the unvaccinated

Some groups of unvaccinated people were reported as more at risk of severe SARS-CoV2 infection and death than others. Particular risk factors included increased age, male sex, non-white ethnicity, cardiovascular co-morbidity and immunocompromise as well as certain genetic conditions such as Down's syndrome (31,32). The association of non-white ethnicity with severe outcomes of SARS-CoV2 infection might have been feasibly explained by a protective genetic trait, rather than there being several deleterious traits across the heterogenous population termed 'non-white'. However, it is far more likely that the apparent effect of ethnicity is confounded by other factors such as occupation, household structure and co-morbidity (33).

1.7 Complications of Infection

1.7.1 Death

What set wild-type SARS-CoV2 apart from other pathogens of pandemic potential, including the closely related SARS-CoV1 and MERS-CoV, was its high transmissibility coupled with a high mortality. R_0 (the basic reproduction rate of a pathogen at the start of an outbreak) is a measure of its transmissibility and represents the number of further secondary cases each case results in with a number of greater than one representing a spreading infection. Estimates for R_0 for SARS-CoV2 (~2.5) were greater than for both SARS (~2.0) and MERS (~0.6), highlighting its high transmissibility (34). Broadly the mortality estimates of wild-type SARS-CoV2 infection in an unvaccinated population varied between 0.3-3% (35,36). Notably the mortality rate or infected fatality rate for SARS-CoV2 infection is notoriously difficult to establish. The case fatality rate is often used as a surrogate to inform estimates of mortality rates, however, this is subject to over-estimation bias as it is dependent on the total number of people tested for infection. Mortality estimates used data from many different sources and included mathematical modelling, but were further confounded by non-uniform changes in social distancing practises, changes in the circulating SARS-CoV2 variant and differential speeds of vaccine roll out.

1.7.2 Thrombosis

Many infective syndromes induce a pro-thrombotic state with increases in coronary and cerebral arterial infarcts during the acute episode. Increased risks of both arterial and venous thrombosis even up to a year after recovery were also seen. SARS-CoV2 infection, in particular, was noted to have high rates of thrombotic complication – up to 22 times greater than for those admitted with non-SARS-CoV2 infective diagnoses (37,38) particularly pulmonary emboli.

1.7.3 Myocardial injury

Raised serum troponin levels were frequently measured in those hospitalised with acute SARS-CoV2 infection. This likely represents myocardial damage both by direct viral invasion as well as immune-mediated injury (39). It is not clear what the long term sequelae and implications of these injuries are.

1.7.4 Secondary infection

Secondary bacterial infection (such as bacterial pneumonia) were relatively rare in patients admitted with SARS-CoV2, but were managed by concomitant treatment with appropriate empirical or targeted antibiotic therapy (40).

1.7.5 Dermatological manifestations

Various dermatological manifestations associated with SARS-CoV2 infection have been described including maculopapular, erythematous, urticarial vesicular and pustular eruptions as well as pseudo-chillblains (“COVID toe”). The timing of the onset of the skin manifestation of rash varies according to the specific rash, and at least some are likely to be immune-mediated, but it is unclear whether there are any long-lasting impacts from these dermatological issues (41).

1.7.6 Long COVID

SARS-CoV2 infection has also been associated with significant co-morbidity in recovery with significant numbers of patients experiencing prolonged symptoms, termed “Long COVID”. Syndromes are heterogenous, frequently multi-system and include prolonged fatigue, exertional malaise, cognitive dysfunction and memory issues (42). There are data to suggest that there may be some physiological changes in rates of pulmonary gas exchange post-SARS-CoV2 infection, although it is not yet clear if this sequala is

specific to SARS-CoV2 infection (43). There is some evidence to suggest that vaccination may have a protective effect against the development of “Long COVID” (44).

1.8 Healthcare facility saturation

Pre-symptomatic viral shedding and asymptomatic infection contributed to the increased transmissibility of SARS-CoV2 (45). The result of this was that SARS-CoV2 spread quickly across the globe and infected large numbers of people. Although the estimates for mortality of SARS-CoV2 were not as high as for SARS-CoV1 and MERS-CoV (46), the increased transmissibility (47) meant that large proportions of the population were infected leading to large absolute numbers suffering from severe disease and requiring medical care. This led to a rapid saturation of healthcare resources in the early phase of the pandemic leading to a disproportionate increase in mortality in those who were unable to receive optimal care (48).

1.9 Transmission Prevention / Infection Prevention & Control

SARS-CoV2 is predominantly a respiratory pathogen and its main mode of transmission is through short-range airborne droplets, although there is still likely to be transmission via aerosol in crowded and poorly ventilated areas as well as via contact with fomites in communal areas (49). Many countries around the world implemented various infection control measures in order to curb transmission and ‘flatten the epidemiological curve’ of the pandemic and ultimately mitigate the disproportionate mortality increase (50).

1.9.1 Personal Protective Equipment (PPE)

The wearing of face coverings or masks in public places became common place in order to reduce transmission (51), whilst in healthcare settings, increased levels of PPE including gowns, aprons, gloves and eye protection were additionally worn depending on the availability of PPE and a graded risk assessment of transmission.

1.9.2 Social distancing & public health measures

Countries implemented social distancing measures, encouraged working from home, furloughed employees, restricted occupancy of public events and mandated some business closures (52). Periods of self-isolation were introduced for those diagnosed with possible or confirmed infection (53). The introduction and partial uptake of a decentralised contact tracing app aided the implementation of these measures to some degree (54).

1.9.3 Travel restrictions

Several countries restricted population movements within their state borders. Restrictions also came into being when crossing borders between countries, with many countries employing a tiered system (55) that included denying entry to travellers from certain countries with high transmission rates, to mandating quarantine in state-run facilities, to allowing ‘free’ access if sufficient diagnostic evidence of lack of infection was provided (56).

1.10 Secondary pandemic injuries

Non-pharmaceutical measures were partially successful in reducing the density of new infections per period of time. In other words, they did “flatten the curve” to some degree in order to reduce some of the pressure on healthcare facilities. They did, however, carry a large economic cost with the furlough scheme and the closure of several businesses (57). It is likely, however, that these costs were less than no intervention at all (58). The restrictions on individual freedoms were also viewed sceptically by some, including in the UK (59). Self-isolation and the prohibition on national travel and meeting with friends and family had a large impact on schooling (60) and the psychological well-being of the nation, although evidence for the latter is inconsistent (61).

In addition to the above, there were also significant impacts on physical health. Many routine appointments and elective surgeries were cancelled or delayed (62). In acute hospitals, emergency or urgent care pathways suffered disruption as hospital remodelled their methods of care delivery to take into account infection prevention and control measures. Patients' fear of being exposed to SARS-CoV2 infection in healthcare settings led to the delayed presentation of many illnesses including coronary artery disease (63)

1.11 Treatments

At the start of the pandemic, treatments were lacking and were entirely supportive, predominantly respiratory, with the evidence for benefit to these patients often lacking. Incredible efforts from a series of platform studies including RECOVERY and REMAP-CAP (64,65) resulted in the identification of treatments which improved mortality. A number of pharmaceutical companies successfully brought to emergency licensure several directly acting antiviral drugs including remdesivir as well as a selection of different cocktails of monoclonal antibody.

Many of these treatments were expensive and it was frequently difficult to assess which patients were most likely to deteriorate and therefore have the greatest amount to gain from treatment. The availability of anti-viral and immunomodulatory treatments for different patient groups in the UK was frequently dictated on a national level by governments, rather than by local clinicians and was, in part, determined by availability and cost.

1.11.1 Supportive

1.11.1.1 Oxygen therapy

Extrapolation from wider data in hypoxia (not specific to COVID-19) has informed the convention of supplemental O2 to achieve 92-96% in hypoxic patients with T1RF. It is unclear whether supplemental Oxygen therapy is beneficial to those with SARS-CoV2 infection and low oxygen saturations, in terms of mortality or progression to intubation, however, it is a challenging decision clinically, not to give Oxygen to patients who are overtly hypoxic.

In those for whom there is symptomatic relief of breathlessness, it may be more clearly beneficial, as well as for those in whom other comorbidity (e.g. cardiac and respiratory) may be destabilized by low oxygen saturations

1.11.1.2 Ventilatory pressure support

Continuous positive airway pressure (CPAP) was used empirically as a way to maintain critical care capacity. There is some limited evidence that CPAP may reduce progression to intubation (66) and was reasonably used as first line supportive therapy in those for whom supplemental oxygen alone was insufficient, however, a clear mortality benefit has not yet been established. Patients with pre-existing conditions and type two respiratory failure should still be considered for bi-level positive airway pressure support.

1.11.1.3 Thromboprophylaxis

Many healthcare systems globally, instituted SARS-CoV2 infection specific anti-coagulation policies, without much evidence and, indeed, even now, clear convincing evidence of a benefit is lacking, although some RCTs showing possible benefit in some subgroups (67,68).

1.11.2 Anti-viral agents

It should be noted that that many of the effect sizes outlined in this section are not directly comparable as the comparator arm was the standard of care, which rapidly evolved during the early days of the pandemic.

1.11.2.1 Remdesivir (Veklury, Gilead)

Remdesivir is an adenosine nucleotide prodrug that was initially developed as an anti-viral drug against the filovirus, Ebola. It is used in the treatment of adults and children with SARS-CoV2 infection with pneumonia requiring supplemental oxygen or who are at increased risk of progressing to severe COVID-19 (69). Although the effect size varied by subgroup, Remdesivir was found to reduce 29-day all-cause mortality (hazard ratio [HR], 0.73, 95% CI 0.52 to 1.03) and reduced time to recovery (10 days (95% CI, 9 to 11) vs 15 days (95% CI, 13 to 18).

1.11.2.2 Nirmatrelvir with ritonavir (Paxlovid, Pfizer)

Nirmatrelvir, a protease inhibitor, boosted with ritonavir, is used in both adults and children with symptomatic COVID-19 not requiring supplemental oxygen and who are at increased risk of progressing to severe COVID-19 as well as in non-hospitalised patients (70). Paxlovid resulted in a reduction in a combined end-point of hospitalization or death by day 28 (HR 0.89).

1.11.2.3 Sotrovimab (Xevudy, GlaxoSmithKline)

Sotrovimab is a recombinant human IgG1 monoclonal antibody (mAb) targeting the receptor binding domain (RBD) of the SARS-CoV2 spike protein. Prescribed in patients >12 who do not require supplemental oxygen as well as in non-hospitalised patients (71). Monoclonal antibody efficacy is often dependent on the circulating strain. Sufficient mutation in the RBD can result in a loss of efficacy, which has been the case for several

previous mAbs (72). In its original setting, Sotrovimab prevented disease progression that would have lead to hospitalization or death (relative risk reduction, 85%; 97.24% CI, 44 to 96).

1.11.2.4 Molnupiravir (Lageviro, Merck)

Molnupiravir's mode of action is by induction of mutagenesis in the viral RNA genome, leading to the non-viability of virus. It was used only in the community in the UK, having originally been shown to be efficacious in an unvaccinated population (73). Molnupiravir resulted in a reduction in a combined end-point of hospitalization or death by day 28 (HR, 0.69; 95% CI, 0.48 to 1.01). However, the PANORAMIC trial then failed to show superiority above the standard of care in a vaccinated population (74).

1.11.3 Immunomodulatory

1.11.3.1 Dexamethasone

One of the first big developments in treatments for those with SARS-CoV2 infection was the discovery that dexamethasone, a cheap, generic, readily available drug, had a dramatic 18-36% reduction in mortality depending on level of respiratory support (75). Low dose steroid was just as efficacious as high dose steroid (76). Equivalent doses of Prednisolone 40 mg or hydrocortisone can be considered in special situations including where dexamethasone is contraindicated.

1.11.3.2 Tociluzimab

Tociluzimab is an anti-IL-6 monoclonal antibody which improved survival regardless of level of respiratory support (77). It increased the chances of discharge within 28 days (rate ratio 1·22; 95% CI 1·12–1·33). Amongst those not receiving invasive mechanical ventilation at baseline, tocilizumab resulted in a reduced requirement of invasive ventilation or death (hazard ratio 0·84; 95% CI 0·77–0·92)

1.11.3.3 Baricitinib

A selective and reversible inhibitor of Janus kinase 1 and 2 (JAK1/JAK2) (78). Baricitinib reduced death (age-adjusted hazard ratio 0·87; 95% CI 0·77−0·99).

1.11.3.4 Inhaled Budesonide

The PRINCIPLE trial demonstrated inhaled budesonide to be efficacious in shortening time to recovery by 2.94 days in those at risk of severe illness. It also showed a possible reduction in hospital admissions (relative ratio 0·75 [95% BCI 0·55 to 1·03]). Interventions based on these results were not routinely implemented in the UK (79).

1.12 Vaccine development

The anti-viral and immunomodulatory treatments were successful in reducing mortality in those already infected, however, they are expensive, have adverse reactions and have varying levels of interactions with other medications, which are particularly relevant for the most co-morbid and therefore the most at-risk populations.

The previously mentioned non-pharmaceutical measures described mitigate only the mortality increase due to healthcare facility saturation. Alone they do not suffice to protect those most at risk of poor outcomes after SARS-CoV2 infection, as they would eventually be exposed to the virus. The only way to protect this at-risk population was by immunisation through the roll out of an effective vaccination programme.

There was an unprecedented global effort by academic institutions and industry alike to produce a safe, efficacious vaccine resulting in 183 vaccines in various clinical phases of development by March 2023 (80).

On 2nd December 2020, the Medicines and Healthcare products Regulatory Agency (MHRA) granted emergency authorisation for Comirnaty (BNT162b2, tozinameran, manufactured by Pfizer-BioNTech) – an mRNA lipid nanoparticle vaccine (81). This was

followed by emergency authorisation by the European Medicines Agency (EMA) on 21st December 2020. Soon afterwards Vaxzevria (ChAdOx1 nCoV-19, AZD1222 manufactured in a collaboration between the University of Oxford and AstraZeneca) – an adenovirus vectored vaccine – also received emergency authorisation from the MHRA on 29th December 2020 (82).

The MHRA then similarly granted emergency authorisation for another mRNA COVID-19 vaccine, Spikevax (mRNA-1273 manufactured by Moderna) (83). Whilst these three vaccines were amongst the first to receive emergency authorisation, Nuvavoxid (NVX-CoV2373, manufactured by Novavax), an adjuvanted protein vaccine, had published early promising efficacy results, but remained under rolling review by the MHRA (84).

These vaccines all received their emergency authorisation as 2-dose homologous immunisation schedules at 3 week (BNT162b2, NVX-CoV2373) and 4-12 week (ChAdOx1 nCoV-19, mRNA-1273) intervals. These two-dose schedules formed what subsequently became known as the 'priming immunisation course' and is the focus of this thesis.

The race by different nations to acquire vaccine doses from manufacturers started well before emergency licensure became a reality and each individual nation made its own decisions as to which vaccines it would aim to purchase. The UK appointed the Vaccine Taskforce to manage this process. The vaccines that would therefore likely form part of any UK national immunisation programme were the ones already mentioned.

1.12.1 ChAdOx1 nCoV-19 / AZD1222 (Vaxzevria/Covishield, University of Oxford/AstraZeneca) – shorthand ChAd

ChAd is an adenovirus vectored vaccine using the recombinant chimpanzee ChAdOx1 virus that has had the E1 gene deleted and modification of the E3 and E4 genes to enhance immunogenicity and optimise production of vaccine (85). It is therefore able to infect cells and use native host cellular machinery to produce the antigen of interest, but is otherwise replication deficient. This vaccine's antigen is the SARS-CoV-2 spike surface glycoprotein with a leading tissue plasminogen activator (TPA) signal sequence. The sequence of the spike protein is codon-optimised from the SARS CoV-2 genome sequence accession MN908947. ChAdOx1 is a non-enveloped virus, and the spike antigen is not present in the viral vector capsid, but is expressed on host cell membranes following viral gene translation. Two doses of $5x10^{10}$ viral particles in 0.5ml were delivered intramuscularly at an interval of 4-12 weeks.

1.12.2 BNT162b2 / tozinameran / Comirnaty (Pfizer/BioNTech) – shorthand BNT

This vaccine is a lipid nanoparticle-formulated, nucleoside-modified mRNA vaccine that encodes trimerised SARS-CoV-2 spike glycoprotein that was stabilised with two proline substitutions. Two doses of 30 µg in 0.3ml are delivered at an interval of 3 weeks.

1.12.3 mRNA-1273 / Spikevax (Moderna) – shorthand Mod

Similar to BNT, this is an mRNA lipid nanoparticle vaccine, whose spike code is diprolinated at positions 986 and 987 at the top of the central helix in the S2 subunit. The lipid nanoparticle is proprietary, but is composed of four lipids in a fixed ratio. Two doses of 100 µg in 0.5ml are delivered intramuscularly at an interval of 4 weeks.

1.12.4 NVX-CoV2373 / Nuvaxovid (Novavax) – shorthand NVX

This vaccine can variably be described as a virus like particle or an adjuvanted protein vaccine. It is constructed from full length spike protein trimers with substitutions to limit protease cleavage, improve thermal stability and optimise expression. The recombinant genes, expressing diprolinated spike protein, are cloned into a baculovirus vector before being transferred into Sf9 cells (from the insect Spodoptera frugiperda). These cells then produce the protein which is extracted, purified and assembled onto a lipid nanoparticle. It is co-formulated with a proprietary saponin based adjuvant, Matrix-M1. Two doses of 5 µg protein with 50 µg Matrix-M1 adjuvant in 0.5ml are delivered intramuscularly at an interval of 3 weeks.

1.13 Vaccine programme logistics

Roll out of these vaccines started quickly in the UK. However, it was evident, even before vaccine roll out started, that the demand for efficacious vaccines was going to outstrip manufacturing capacity. In addition to this, the logistical complexity of national and international roll out of mass immunisation programmes meant that it would be difficult to ensure that two doses of the same vaccine were delivered to the same individual at the manufacturer licensed interval.

The UK government, with advice from the Joint Committee on Vaccination and Immunisation (JCVI), alongside other national immunisation committees made a decision to prolong the interval between first and second doses for all vaccine recipients in the UK from 4 weeks to 8-12 weeks (86). This was supported by inferences from other areas of vaccinology as well as modelling to show that this strategy might prevent the largest number of deaths. Non-randomised post-hoc trial analyses from the ChAdOx1 nCoV-19 randomised efficacy trial also supported this decision, albeit a few months later (87).

1.14 Vaccine equality

Finally, although richer nations vaccinated large portions of their population with homologous schedules fairly rapidly, low and middle income countries (LMICs) did not gain access to large quantities of approved vaccine very quickly or reliably. The vaccines that they did have access to were frequently those that were, at least initially, viewed as less efficacious. This made it incredibly difficult to plan out national immunisation policies.

1.15 Heterologous priming vaccination

There was no information or guidance available as to how to use vaccines from different manufacturers in combination and there was no previous experience with mixing different vaccine platforms of this nature. A more complete review of heterologous vaccination schedules follows in Chapter 3 . The term heterologous vaccination has been variably used to describe vaccine doses that differ only by vector (eg viral vectored vs mRNA lipid nanoparticle), that differ only by route of administration (intramuscular vs intranasal) or that differ only by antigen delivered. In this thesis, the term heterologous vaccine priming schedule, indicates that the vaccine administered as the second dose is not the same one used for the first dose (88).

1.16 National Age-specific Restrictions

Roll out of national immunisation programmes across many countries, including Sweden, Denmark, France and Germany was further complicated by the introduction of age-specific restrictions on administration of certain vaccines due to the emergence of rare adverse events (89–91). These included vaccine-induced immune thrombocytopaenic thrombosis (VITT), a rare syndrome most commonly resulting in cerebral venous thrombosis soon after administration of the first dose of adenovirus-vectored SARS-CoV2 vaccination (92); as well as myocarditis in younger populations

following the second dose of mRNA based vaccines (93). Countries which rapidly changed their vaccination guidelines during vaccine roll out, left large numbers of their population who had received only one dose, by default, to have heterologous immunisation priming schedules .

1.17 Priming Interval

Leading into the pandemic there was evidence from other vaccines that increasing the interval between first and second doses would likely lead to a larger immune response (94). However, the decision on the priming interval or extending the priming interval had to be made considering the availability of vaccine and balanced against maximising protection with a single dose in a larger population. A more complete review of the data regarding priming interval follows in Chapter 4 .

1.18 Knowledge gaps

In order to ease the logistical constraints involved with mass vaccine roll out, it was important to understand whether it was safe and immunologically feasible to give different vaccines for the first two doses of the priming immunisation regimen. It was additionally important to understand how prolonging priming interval would affect the immunogenicity and efficacy of both the homologous and heterologous combinations of priming vaccine schedules. This information would inform vaccine policy in the UK, in countries who had introduced age-specific restrictions on immunisation, and in low and middle income countries (LMICs), whose economic buying power of vaccines meant they were disadvantaged in receiving sufficient timely vaccine and, finally, would also inform immunisation choices for patients who had suffered unacceptable adverse reactions after their first vaccine dose.

1.19 Aims & Objectives

With the backing of the Deputy Chief Medical Office of England, Prof Jonathan Van Tam and the UK Vaccine Taskforce, we therefore set about to gauge the immunogenicity and reactogenicity of homologous and heterologous schedules at shorter and longer priming intervals. We designed and set up two single-blind, randomised, non-inferiority immunogenicity trials – Com-COV (henceforth referred to as Com-COV1 in this thesis) and Com-COV2 to answer these questions. The vaccines that made up the homologous and heterologous schedules within these trials were determined by which vaccines were going to be available to the UK population in a time-frame relevant to a rapid immunisation roll out.

The target recruitment populations of both trials were those most at risk of severe COVID-19, with the knowledge currently available at that time (adults aged 50 years and over with no or mild-moderate well-controlled co-morbidities).

Com-COV1 was the original trial and tested each permutation of the ChAd and BNT vaccines at two different priming intervals (4 weeks and 12 weeks):

Table 1 *– Com-COV1 schedules*

First Dose	Second Dose	Interval
ChAd	ChAd	4 weeks
ChAd	BNT	4 weeks
BNT	BNT	4 weeks
BNT	ChAd	4 weeks
ChAd	ChAd	12 weeks
ChAd	BNT	12 weeks
BNT	BNT	12 weeks
BNT	ChAd	12 weeks

With news from the UK Vaccine Taskforce that further vaccine options would become available to the UK population – Spikevax (mRNA-1273 manufactured by Moderna) and Nuvaxovid (NVX-CoV2373 manufactured by Novavax) – further information regarding their use in heterologous schedules was required.

Since Nuvaxovid had not yet received emergency authorisation, it was not feasible to add these vaccines to Com-COV1 as a platform study, but instead a separate study was required: Com-COV2. Given the rapid nature of the roll out of SARS-CoV2 vaccination and the fact that Com-COV2 was conceived two months after Com-COV1, the design of the trial necessarily had to be different. A similar population of participants was recruited, but this time, each participant would already have received their first vaccine dose as part of the national immunisation programme (BNT or ChAd). The first dose received in the community determined the vaccine options to be given in the trial. All participants had a priming interval of 8-12 weeks:

Table 2 *– Com-COV2 schedules*

Community First Dose	Second Dose	Type of schedule
ChAd	ChAd	Homologous
	Mod	Heterologous
	NVX	Heterologous
BNT	BNT	Homologous
	Mod	Heterologous
	NVX	Heterologous

The aims of both the Com-COV1 and Com-COV2 trials were to provide absolute and comparative data on safety, reactogenicity and immunogenicity for heterologous vs homologous schedules.

In order to describe the mucosal immune response and to correlate this with the serum immune response, I secured additional funding and permission to add mucosal sampling and exploratory immunology assays to a subset of participants without delaying the pragmatic and urgent end-points of the trial.

Finally, aside from the practical, policy-informing endpoints, these trials also provided a unique situation to explore the immunological differences between vaccine platforms. Never before have there been so many diverse platforms (mRNA lipid nanoparticle, adenovirus vectored, adjuvanted protein subunit) using the identical or near identical antigen to produce an immune response. The data and lessons learned from these studies provide valuable information to prepare for the next pandemic as well as to optimise routine vaccine schedules using non-homologous doses.

Chapter 2 General Methodologies

In this chapter, I outline the methodologies of the clinical trials Com-COV1 and Com-COV2, the analyses of whose samples and data are presented in this thesis. Where there is duplication or similarity of methodology between the trials, I have amalgamated these sections together, specifying where the trials diverge in nature.

Also included in this chapter are the methodologies for sample processing/extraction and the assays performed by contracted clinical research organisations and academic collaborators.

Finally, the methodologies for sample collection, processing and immunological assaying are presented for the analysis of mucosal immune responses.

2.1 Trial registrations

2.1.1 Com-COV (Com-COV1)

Study Title: A single-blind, randomised, phase II UK multi-centre study to determine reactogenicity and immunogenicity of heterologous prime/boost COVID-19 vaccine schedules (**Table 3**).

Table 3 *– Com-COV1 trial registration information*

Short Title: Comparing COVID-19 Vaccine Schedule Combinations (Com-COV)
Ethics Ref: 21/SC/0022 (South Berkshire Research Ethics Committee)
IRAS Project ID: 291055
ISRCTN: 69254139
EudraCT Number: 2020-005085-33
OVG Study Number: OVG 2020/03
Latest protocol date and version no: V10.3 20-October-2022

2.1.2 Com-COV2

Study Title: A single-blind, randomised, phase II UK multi-centre study to determine reactogenicity and immunogenicity of heterologous prime/boost COVID-19 vaccine schedules – Stage 2 (**Table 4**).

Table 4 *– Com-COV2 trial registration information*

Short Title: Comparing COVID-19 Vaccine Schedule Combinations – Stage 2 (Com-COV2)
Ethics Ref: 21/SC/0119 (South Berkshire Research Ethics Committee)
IRAS Project ID: 297443
ISRCTN Number: 27841311
EudraCT Number: 2021-001275-16
OVG Study Number: OVG 2021/01
Latest protocol date and version no: 20-October-2022 V7.2

2.2 Rationale & Aims

Given the high global demand of efficacious vaccine and limited initial vaccine stock, coupled with uncertainty regarding the expansion of vaccine production capabilities, we looked to ascertain whether flexibility of national immunisation schedules could be attained through the use of heterologous schedules or through varying priming intervals. This would allow any immunisation programme, but especially those in LMICs, whose access to timely vaccine stock was even less certain than for wealthier countries, to plan for greater depth in their immunisation programmes, which would make them more robust to supply shocks.

The questions we sought to answer for both Com-COV1 and Com-COV2 were:

1) Is the use of heterologous schedules safe (with the limitations that a study of this size may not pick up rare adverse reactions)
2) Are heterologous schedules tolerable in terms of reactogenicity?

3) Are heterologous schedules immunologically non-inferior to their respective homologous schedules in terms of peak anti-SARS-CoV2 spike IgG (28 days post-second dose).

And for Com-COV1 only:

4) Does a longer priming interval (12 weeks between first and second doses) produce a higher level of immunogenicity to the shorter interval (4 weeks between first and second doses)?
5) Does advice for prophylactic vs reactive paracetamol impact on reactogenicity or immunogenicity following the second dose of the 12-week study arms.

2.3 Study design

Both Com-COV1 and Com-COV2 were participant-blinded, randomised, controlled, non-inferiority immunogenicity studies. Both trials were powered to answer the primary questions comparing humoral responses between homologous and heterologous schedules for participants who received either ChAdOx1 nCoV-19 (ChAd) or BNT162b2 (BNT) as their first dose. Com-COV1 included ChAd and BNT as potential heterologous second doses, whereas, Com-COV2 included mRNA-1273 (Mod) and NVX-CoV2373 (NVX) as potential heterologous second doses. They were not designed to identify the schedule producing the highest antibody responses, but rather to establish whether heterologous priming vaccine regimens were safe and immunologically viable alternatives. Com-COV1 participants were blinded only to the vaccines received, but not to the interval due to the practical challenges this presented. Both studies recruited separately into two cohorts, by personal preference: A general cohort, and a smaller immunology cohort, which had more frequent visits and blood sampling for additional assays after each study vaccination better to understand the immunological responses.

The immunology cohort in the Com-COV1 study contained arms only from the 4-week interval schedules.

The decision to choose immunogenicity rather than efficacy as the primary endpoint was based on pragmatic considerations to limit the cost and size of the trials and hugely increase the speed of delivery to ensure results could be delivered in a timeframe suitable to inform policy. Additionally, it is almost impossible to conduct a non-inferiority efficacy trial comparing ChAd and BNT, considering the high vaccine efficacies reported in their respective phase III trials. At the time, there was sufficient internal data from the Oxford Vaccine Group, that was later published (95), to justify using anti-SARS-CoV2 spike IgG as an immune endpoint that would likely be correlated with protection. This was supported by data shared during the COVAX workshop in February 2021, just prior to the start of recruitment of the Com-COV1 trial (96).

2.3.1 Study arms

The vaccines which were included as investigational medicinal products (IMPs) were determined by those which had already received or were deemed likely to receive emergency use authorisation under regulation 174, and which the Department of Health and Social Care had access to. The decision as to which combinations of vaccine schedule were included was additionally based on which schedules were most likely to have been deployed in the UK given the timings of vaccine stock acquisition:

2.3.1.1 Com-COV1

Total n=820 as per **Table 5**.

Table 5 *– Com-COV1 schedules*

First Dose	Second Dose	Interval	N (Immunology cohort)	N (General cohort)	Visits
ChAd	ChAd	4 weeks	25	90	D0, D28, D56, D112, D182, D364*
ChAd	BNT	4 weeks	25	90	
BNT	BNT	4 weeks	25	90	
BNT	ChAd	4 weeks	25	90	
ChAd	ChAd	12 weeks	-	90	D0, D56, D84, D112, D182,D364*
ChAd	BNT	12 weeks	-	90	
BNT	BNT	12 weeks	-	90	
BNT	ChAd	12 weeks	-	90	

**D364 visits are relabelled D255 as visits were expedited due to national vaccine roll out*

2.3.1.2 Com-COV2

Total n=1050 as per **Table 6**.

Table 6 *– Com-COV2 schedules*

Community First Dose	Second Dose	Type of schedule	N Immunology cohort	N General cohort	Visits
ChAd	ChAd	Homologous	25	150	D0, D28, D56, D112, D196
	Mod	Heterologous	25	150	
	NVX	Heterologous	25	150	
BNT	BNT	Homologous	25	150	
	Mod	Heterologous	25	150	
	NVX	Heterologous	25	150	

2.4 Target recruitment population

To guide immunisation policy, both Com-COV1 and Com-COV2 recruited participants who were viewed by the Joint committee on vaccination and immunisation (JCVI) to be at increased risk of severe SARS-CoV2 infection: Adults aged 50 years and over with no or mild-moderate, well-controlled co-morbidity.

Com-COV1 recruited vaccine-naïve and SARS-CoV2 infection naïve participants in February 2021 and administered both doses of vaccine under the auspices of the trial at a time during the national vaccine roll out when there were sufficient numbers of the public who had not yet received their first vaccine dose.

Com-COV2 started only two months later, but the speed of the national vaccine roll out meant that recruitment of unvaccinated volunteers was more challenging. In order to mitigate the risk of slow recruitment in the target population, a pragmatic decision was made for Com-COV2 to recruit participants who had already received their first doses of either BNT or ChAd in the community as part of the national programme.

Com-COV2 was designed to answer the same heterologous vs homologous question in two different populations – those primed with ChAd and those primed with BNT (Com-COV2 therefore acted as two trials under one study). Recruitment was stratified by prime vaccination with all participants enrolled within 56-84 days of their first dose. In order to meet these targets during a period of rapid UK national immunisation programme roll out, demographics between the ‘ChAd participants’ and ‘BNT participants’ were not matched. The split of ChAd vs BNT prime doses varied between sites. Additionally, due to logistical constraints only five out of nine sites collected lithium heparin samples for cellular immune response analysis.

Further details of recruitment are available in the study protocols (97).

2.5 Objectives

2.5.1 Primary objectives

1. Non-inferiority of immunogenicity of heterologous vs homologous schedules in baseline COVID seronegative participants, as measured by anti-SARS-CoV2 spike IgG 28 days post-second dose:
    a. Com-COV1 primary outcome: Anti-SARS-CoV2 spike IgG 28 days post-second dose in 4-week schedules only
    b. Com-COV2 primary outcome: Anti-SARS-CoV2 spike IgG 28 days post-second dose

2.5.2 Secondary objectives

1. Safety of heterologous schedules, as measured by Serious adverse events (SAEs), adverse events of special interest (AESI) and changes from baseline laboratory measures (**Table 9**)
2. Tolerability as measured by solicited and unsolicited adverse reactions and medically attended adverse events (**Table 9**)
3. Evaluation of effect of baseline SARS-CoV2 seropositivity on immunogenicity and reactogenicity
4. Non-inferiority of immunogenicity of heterologous vs homologous schedules across all intervals (in Com-COV1 only) in baseline SARS-CoV2 seronegative participants as measured by anti-SARS-CoV2 spike IgG 28 days post-second dose (day 56 for 4-week interval schedules and day 112 for 8-week interval schedules)
5. Kinetics of humoral and cellular immune responses across all timepoints

2.5.3 Exploratory objectives

1. Characterisation of immune responses in participants who developed SARS-CoV2 infection during the trial
2. Characterisation of mucosal immune responses in a subgroup of participants
3. Assessment of effect of advice for prophylactic vs reactive paracetamol at the point of the second dose in the 12-week schedules on immunogenicity and reactogenicity (Com-COV1, protocol amendment)

Table 7 *– Com-COV1 visit schedule with sampling protocol*

Com-COV1												
Cohort		0	7	14	28	35	42	56	84	112	182	364
Immunology (4-week)	Anti-SARS-CoV2 spike IgG	x	x	x	x	x		x		x	x	x
	Neutralising Antibody Pseudotyped-virus neutralising Antibody Anti-Nucleocapsid IgG	x		x	x			x		x	x	x
	Interferon-γ T-cell ELISpot	x		x	x		x	x		x	x	x
	Laboratory safety markers	x			x	x		x				
General (4-week)	Anti-SARS-CoV2 spike IgG	x			x			x			x	x
	Neutralising Antibody Pseudotyped-virus neutralising Antibody Anti-Nucleocapsid IgG	x			x			x			x	x
	Interferon-γ T-cell ELISpot	x			x			x			x	x
	Laboratory safety markers	x			x			x				
General (12-week)	Anti-SARS-CoV2 spike IgG	x						x	x	x	x	x
	Neutralising Antibody Pseudotyped-virus neutralising Antibody Anti-Nucleocapsid IgG	x						x	x	x	x	x
	Interferon-γ T-cell ELISpot	x						x	x	x	x	x
	Laboratory safety markers	x							x	x		

***Table 8** – Com-COV2 visit schedule with sampling protocol*

Com-COV2								
Cohort		0	7	14	28	56	112	294
Immunology	Anti-SARS-CoV2 spike IgG	x	x		x	x	x	x
	Neutralising Antibody Pseudotyped-virus neutralising Antibody Anti-N IgG	x			x	x	x	x
	Interferon-γ T-cell ELISpot	x		x	x	x	x	x
	Laboratory safety markers	x	x		x			
General	Anti-SARS-CoV2 spike IgG	x			x	x	x	x
	Neutralising Antibody Pseudotyped-virus neutralising Antibody Anti-N IgG	x			x	x	x	x
	Interferon-γ T-cell ELISpot*	x			x	x	x	x
	Laboratory safety markers	x			x			

**Due to logistical constraints, lithium heparin tubes for PBMCs were collected only at 5 sites in approximately 60% of participants of all trial participants across both cohorts*

Clinical adverse events were captured via individual self-reported e-diaries as well as through interim history taking at routine study visits.

***Table 9** – Safety monitoring schedule for both Com-COV1 & Com-COV2*

Safety & Reactogenicity monitoring	
Solicited Reactions (Local & Systemic)	Recorded for 7 days after each immunisation
Unsolicited Reactions	Recorded for 28 days after each immunisation
Medically attended adverse events	Recorded for 3 months post second dose
Adverse Events of Special Interest Serious Adverse Events	Trial duration

2.6 Sites

In order to deliver these trials in a policy relevant time-frame, the studies were conducted across multiple sites to ensure rapid recruitment and delivery: Oxford, Southampton, Bristol, Birmingham, St George's University London (SGUL), Guys & St Thomas' Hospital, London (GSTT), University College London Hospitals (UCLH), Hull, Liverpool, Nottingham, Newcastle and Sheffield.

2.7 Sample size calculation

The Com-COV1 sample size calculation was performed by the study statistician Prof Xinxue Liu and verified by UKHSA statistician, Prof Nick Andrews. This was based on the primary analysis of non-inferiority comparisons within each group of 4-week schedule vaccines in Com-COV1: ChAd/BNT (heterologous ChAd) compared to ChAd/ChAd (homologous ChAd), and BNT/ChAd (heterologous BNT) compared to BNT/BNT (homologous BNT). Immunology and general cohorts would be combined for this. The following assumptions were made for the calculation:

- Non-inferiority margin: Chosen as a 0.63 fold difference in the geometric mean concentrations (GMCs) between the homologous and heterologous arms, equivalent to a -0.2 absolute difference of GMC on a $\log_{10}$ scale. This margin was chosen pragmatically to be close to the WHO-defined criterion of 0·67 for licensing new vaccines, whilst still allowing rapid study delivery (98)
- The standard deviation (SD) of GMC on a $\log_{10}$ scale was 0.4 based on data available at the time from the COV001/COV002 trials (ChAd efficacy trials) in participants aged 56-69 (n=29) receiving ChAd/ChAd at an interval of 4 weeks
- The true difference in GMCs on a $\log_{10}$ scale between homologous and heterologous schedules is 0

These assumptions estimated that 86 baseline seronegative participants per each of the 4-week schedule arms were required to achieve 90% power at a one-sided 2.5% significance level. Given the high levels of SARS-CoV2 transmission, we estimated 25% would be excluded from the primary analysis due to baseline seropositivity or loss to follow up. This increased the arm size to 115. Of these 115, we enrolled 25 participants in the immunology cohort. The immunogenicity cohort sample size of 25 was chosen without sample size calculation and made pragmatically based on logistical constraints as its purpose would be to generate, rather than test, hypotheses.

The sample size of 90 per arm in the 12-week boosted arms of the general cohort was made pragmatically to simplify study design and randomisation by keeping this recruitment number the same across all arms of the general cohort.

2.8 Trial Procedures

2.8.1 Recruitment

Given the large media attention at the time given to the pandemic, recruitment for COVID-19 vaccine trials, in general, and the Com-COV1 & Com-COV2 trials in particular, was rapid via a bespoke website with a large number of participants being alerted to the study by email through the NIHR COVID-19 vaccine volunteer database.

2.8.2 Screening & Consent

Given the social distancing and infection control requirements present in the UK at the time of recruitment, we introduced a recruitment website with a two-stage online pre-screening process. The first stage assessed exclusion criteria. If still eligible, participants would be able to report their medical history as free text and give permission for the trial team to contact the volunteer and their GP if either was required. Volunteers for whom further clarification of eligibility was required could then be screened by telephone by the

trial team. If there were no indications for telephone screening, then the participant could be invited directly to in-person screening.

This staged process reduced the amount of face-to-face time required, which was beneficial from an infection prevention and control point of view. It also reduced the number of volunteers who failed in-person screening. This process, in combination with a video recording of a summary participant information sheet which accompanied the written participant information sheet, also allowed further standardisation of the information conveyed to participants. Overall, this led to a more stream-lined and efficient process, which minimised unnecessary contact at a time when social distancing precautions were being enforced. Consent was taken verbally in person and documented on paper.

2.8.3 COVID-19 Pathway (C19P)

SARS-CoV2 infection diagnosis did not form a clinical efficacy endpoint of this trial and so we symptomatic participants were not diagnosed under the auspices of the trial. Instead, participants were asked to notify the trial of symptomatic or asymptomatic SARS-CoV2 infection by any recognised test (ie LFA or NAAT test). Participants were then reviewed by telephone and then in person for safety on the C19P. Further immunological samples were taken at this point.

2.8.4 Blinding & Randomisation

2.8.4.1 Com-COV1

Computer-generated randomisation lists were prepared and stratified by study site, and cohort (general/immunology) by the study statistician. Participants were given the option to enter the immunology cohort as this was a greater time commitment. Randomisation was 1:1:1:1 within the immunology cohort with a block size of four and 1:1:1:1:1:1:1:1 in the general cohort with a block size of eight, each cohort being stratified by site.

Following an interim analysis of reactogenicity in the 4-week schedules, a protocol amendment was submitted to investigate how advice for 24 hours of prophylactic paracetamol vs advice for 24 hours of reactive paracetamol given at the point of second immunisation in the 12-week arms only, would affect reactogenicity and immunogenicity. Another computer-generated randomisation list was made and participants randomised 1:1 at the time of the second dose visit, using block randomisation (block sizes 2 or 4). Randomisation was stratified by study site and vaccine schedule.

2.8.4.2 Com-COV2

Computer-generated randomisation lists were prepared and stratified by study site, cohort and prime community vaccine. Randomisation was 1:1:1 with block sizes of three and six, stratified by prime vaccine, cohort and study site.

2.8.5 Unblinding

Initial unblinding was planned for the end of the study with early unblinding reserved for individuals whose clinical management might be changed through unblinding. However, due to the rapidly changing regulations at the time of the trials in relation to social distancing, but particularly international travel, after discussion with the Trial Steering Committee (TSC), we made provision through an amendment to allow mass unblinding after the last participant in each trial had been seen 28 days post second dose, provided unblinding would mitigate any disadvantage conferred upon participants by virtue of being in the trial.

2.9 Safety & Reactogenicity

2.9.1 Solicited Symptoms

Reactogenicity was measured via self-reported daily e-diaries following on from each vaccination whose links were sent to the participant via email on the day of vaccination. Solicited symptoms were asked about each day for 7 days following each vaccination.

Table 10 *– Local & Systemic e-diary solicited adverse events*

Local solicited adverse reactions	**Systemic solicited adverse reactions**
Pain	Measured temperature
Pruritus	Feverishness
Warmth	Chills
Erythema	Headache
Swelling	Malaise
Induration	Fatigue
	Arthralgia
	Myalgia
	Nausea
	Vomiting
	Diarrhoea

2.9.2 Unsolicited symptoms

Unsolicited symptom diaries were open for 28 days following each vaccination, allowing participants to enter free text regarding the nature of any adverse event not listed amongst the solicited AEs. Diaries allowing the recording of adverse events that required in-person or virtual medical consultation or assessment (medically attended adverse events) were available for up to 3 months following each vaccination. All unsolicited and medically attended AEs were reviewed by the study team and were transferred to the clinical safety database.

2.9.3 Severity Assessment

Severity assessment of solicited and unsolicited adverse events via self-reporting were made using the following tables, further details regarding local solicited symptoms are available in the trial protocol (97).

Table 11 *– Adverse Event clinical grading criteria*

Grade	Description
1	Mild: Transient or mild discomfort (< 48 hours); No interference with activity; No medical intervention/therapy required
2	Moderate: Mild to moderate limitation in activity – some assistance may be needed; no or minimal medical intervention/therapy required
3	Severe: Marked limitation in activity, some assistance usually required; medical intervention/therapy required.
4	Potentially Life-threatening: Requires assessment in A&E or hospitalisation

2.9.4 AESI

These were adverse events identified as being of particular relevance to the IMPs. The inclusion of adverse events in this list was guided by the most up to date advice of the Brighton Collaboration (99) at the time of protocol writing.

Table 12 *– Adverse Events of Special Interest (AESI)*

Immunologic	Anaphylaxis	
Neurological	Isolated anosmia/ageusia* Guillain-Barre Syndrome Acute disseminated encephalomyelitis (ADEM) Aseptic meningitis	Meningoencephalitis Peripheral facial nerve palsy Generalised convulsion Myelitis
Haematological	Thrombosis** Stroke Thrombocytopaenia*** Eosinophilia****	Coagulation disorder (includes coagulopathy, thrombosis, thromboembolism, internal/external bleed and stroke)
Cardiac	Acute cardiovascular injury (includes myocarditis, pericarditis, arrhythmias, heart failure, infarction)	
Dermatological	Chilblain-like lesions Single organ cutaneous vasculitis	Erythema multiforme Alopecia
Gastrointestinal	Acute liver injury †† †	Appendicitis
Respiratory	ARDS††	
Renal	Acute kidney injury	
Other	COVID-19 disease†	SARS-CoV2 positivity (validated test)

*In the absence of COVID-19
** Excluding superficial thrombophlebitis (including line-associated)
**** Used as a marker of a skewed Th2 responses
† In particular, any occurrence of suspected vaccine associated enhanced disease (VAED) as defined by the most recent Brighton Collaboration Case Definition
†† In the absence of an infective aetiology (including COVID-19)
††† As defined in Hy's Law

2.9.5 Serious Adverse Events

A serious adverse event (SAE) is any adverse event with definition in **Table 13**:

Table 13 *– SAE definitions*

Results in death
Is life-threatening
Requires inpatient hospitalisation or prolongation of existing hospitalisation
Results in persistent or significant disability/incapacity
Consists of a congenital anomaly or birth defect*
Is an 'other important medical event', when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above

2.9.6 Causality Assessment

A medically qualified doctor assessed the causality of adverse events to vaccine administration, except for solicited adverse events which were assumed, by default, to have been related to vaccination.

***Table 14** – Causality assessment criteria*

Relationship	Description
No relationship	No temporal relationship to study product *and* Alternate aetiology (clinical state, environmental or other interventions); *and* Does not follow known pattern of response to study product
Unlikely	Unlikely temporal relationship to study product *and* Alternate aetiology likely (clinical state, environmental or other interventions) *and* Does not follow known typical or plausible pattern of response to study product.
Possible	Reasonable temporal relationship to study product; *or* Event not readily produced by clinical state, environmental or other interventions; *or* Similar pattern of response to that seen with other vaccines
Probable	Reasonable temporal relationship to study product; *and* Event not readily produced by clinical state, environment, or other interventions *or* Known pattern of response seen with other vaccines
Definite	Reasonable temporal relationship to study product; *and* Event not readily produced by clinical state, environment, or other interventions; *and* Known pattern of response seen with other vaccines

2.9.7 Laboratory Adverse Events

"Safety bloods" included Haematology (Full blood count [FBC]) and Biochemistry (Liver function tests [LFTs] and Renal Function/Urea & Electrolyte [U&Es]) tests were reviewed within 24 hours by a study clinician. A modified Food & Drug administration (FDA) grading table was used to assess the severity of derangement of blood test results.

Table 15 *– Laboratory normal ranges and adverse event grading criteria*

Test		Units	Lab range	Grade 1	Grade 2	Grade 3	Grade 4
Haematology							
Haemoglobin Absolute	Male	g/l	130-170	115-125	100-114	85-99	<85
Haemoglobin Absolute	Female	g/l	120-150	105-113	90-104	80-89	<80
Haemoglobin change from baseline		g/l	n/a	10-15	16-20	21-50	>50
White Blood Cells	Elevated	x 10^9/L	11.00	11.50-15.00	15.01-20.00	20.01-25.00	>25.00
White Blood Cells	Low	x 10^9/L	4.00	2.50-3.50	1.50-2.49	1.00-1.49	<1.00
Platelets	Low	x 10^9/L	150-400	125-140	100-124	25-99	<25
Neutrophils	Low	x 10^9/L	2.00-7.00	1.50-1.99	1.00-1.49	0.50-0.99	<0.50
Lymphocytes	Low	x 10^9/L	1.00-4.00	0.75-0.99	0.50-0.74	0.25-0.49	<0.25
Eosinophils	Elevated	x 10^9/L	0.02-0.50	0.65-1.50	1.51-5.00	>5.00	Hyper-eosinophilia
Biochemistry							
Sodium	Elevated	mmol/L	145	146-147	148-149	150-155	>155
Sodium	Low	mmol/L	135	132-134	130-131	125-129	<125
Potassium	Elevated	mmol/L	5.0	5.1-5.2	5.3-5.4	5.5-6.5	>6.5
Potassium	Low	mmol/L	3.5	3.2-3.3	3.1	2.5-3.0	<2.5
Urea	Elevated	mmol/L	2.5-7.4	8.2-9.3	9.4-11.0	>11.0	Requires dialysis
Creatinine	Elevated	μmol/L	49-104	1.1-1.5 x ULN 114-156	>1.5-3.0 x ULN 157-312	>3.0 x ULN >312	Requires dialysis
Bilirubin	Elevated Normal LFTs	μmol/L	0-21	1.1-1.5 x ULN 23-32	>1.5-2 x ULN 33-42	>2-3 x ULN 43-63	>3 x ULN >63
Bilirubin	Elevated Abnormal LFTs	μmol/L	0-21	1.1-1.25 x ULN 23-26	>1.25-1.5 x ULN 27-32	>1.5-1.75 x ULN 33-37	>1.75 x ULN >37
ALT	Elevated	IU/L	10-45	1.1-2.5 x ULN 49-112	>2.5-5 x ULN 113-225	>5-10 x ULN 226-450	>10 x ULN >450
ALP	Elevated	IU/L	30-130	1.1-2 x ULN 143-260	>2-3 x ULN 261-390	>3-10 x ULN 391-1300	>10 x ULN >1300
Albumin	Low	g/L	32-50	28-31	25-27	<25	-
CRP	Elevated	mg/L	0-10	11-30	31-100	101-200	>200

2.9.8 24-hour safety monitoring

Participants were reviewed for safety at each of their in-person appointments. There was also daily monitoring of the symptom diaries. Participants had access to a 24-hour on call clinician via a mobile phone number.

2.9.9 Data Safety Monitoring Board (DSMB)

A single DSMB was convened for both trials to evaluate the frequency of adverse events, safety and immunogenicity data to be able to give informed recommendation concerning the conduct, continuation or modification of the study for safety reasons to the TSC

2.9.10 Trial Steering Committee (TSC)

A trial steering committee was convened to oversee the study and advise the senior trial investigators (who made up the study management committee) on key issues of study conduct including, importantly, on any safety concerns that may be raised by the DSMB.

2.10 Database and EDC

The REDCap electronic data capture system (EDC) version 10.6.13 was used as the primary data source with trial staff directly entering data into the EDC. Participant-entered electronic diaries were also hosted on REDCap and reviewed electronically by study clinicians.

2.11 Statistical Analysis

2.11.1 Serostatus & Mucostatus definitions

"Seronegative " – Participants whose baseline serum anti-nucleocapsid IgG was below the pre-pandemic threshold and who did not show immunological evidence of seroconversion, nor virological evidence of infection during the trial.

"Seropositive " – Participants whose serum anti-nucleocapsid IgG was above the pre-pandemic threshold and who did not show immunological evidence of seroconversion, nor virological evidence of infection during the trial.

"Seroconversion" – Participants with at least a two-fold increase in serum anti-nucleocapsid IgG, between two timepoints, with the second value being above the pre-pandemic threshold.

"Muconegative population" – Participants whose mucosal anti-nucleocapsid IgA was below the pre-pandemic threshold and who did not show immunological evidence of mucoconversion during the trial, nor virological evidence of infection during the trial

"Mucopositive population" – Participants whose mucosal anti-nucleocapsid IgA was above the pre-pandemic threshold and who did not show immunological evidence of mucoconversion, nor virological evidence of infection during the trial

"Mucoconversion" – Participants with at least a two-fold increase in mucosal anti-nucleocapsid IgA, between two timepoints, with the second value being above the pre-pandemic threshold.

"Seromucostatus" – A combined marker of serostatus and mucostatus, although seropositive/muconegative participants and seropositive/mucopositive participants were regarded to be the same, as the former cannot occur in isolation, but instead most liklely

represents a waned mucosal response. Therefore, only three categories were deemed to exist: Seronegative/Muconegative, Seronegative/Mucopositive and Seropositive)

2.11.2 aGMR

Serum anti-SARS-CoV2 spike IgG was transformed by taking the base 10 logarithm to render a normal distribution. Geometric mean concentrations (GMC) and 95% confidence intervals (CI) were then calculated per vaccine schedule by the binomial exact method and the antilogarithm taken of these to convert them back.

Adjusted geometric mean ratios (aGMRs) between comparison groups were evaluated by taking the antilogarithm of the difference between the geometric mean of the log10 transformed serum anti-SARS-CoV2 spike IgG in the heterologous group and the GMC in the reference homologous group, after adjusting for Age, Sex, BMI, Ethnicity, baseline serum anti-SARS-CoV2 spike IgG, cohort, site and actual number of days between second vaccination and primary outcome blood draw, in multivariate linear regression models.

aGMRs of “persistence timepoint : peak timepoint” ratios were used as a measure of wane and were calculated using the same adjustment covariates in the multiple regression with the exception of ‘actual number of days between second vaccination and primary outcome blood draw’.

Pseudotype virus neutralisation and “Pseudotype virus neutralisation : anti-SARS-CoV2 spike IgG ” ratio analyses used the same covariates as in the heterologous/homologous analysis, but for the interval analysis excluded BMI, Ethnicity and ‘actual number of days between second vaccination and primary outcome blood draw’.

Com-COV2 analyses additionally had interval as an adjustment factor, but excluded ethnicity.

Com-COV1 interval analyses for immunogenicity and reactogenicity additionally had paracetamol sub-study randomisation as an additional adjustment factor in a sensitivity analysis.

2.11.3 Significance

The basis of the threshold for statistical significance of non-inferiority is as described in (Section 2.7). In brief aGMRs with one-sided 97.5% confidence intervals are reported separately to adjust for multiple testing because two primary comparisons were made: One for participants primed with ChAd and one for those primed with BNT. One-sided 98.75% CI were used in Com-COV2 as four primary comparisons were made. The criterion for non-inferiority of heterologous boost compared with homologous boost was for the lower limit of the 97.5% CI (Com-COV1) or the 98.75% CI (Com-COV2) of the aGMR to be greater than 0·63.

2.11.4 Analysis Populations

2.11.4.1 Heterologous/Homologous analysis

Analysis populations for immunogenicity end-points included the immunology cohorts, as there was still an independent randomisation of the 100 immunology cohort participants to each of the four 4-week homologous/heterologous schedules in Com-COV1 and to all six of the schedules in Com-COV2.

The trial-defined principal non-inferiority analysis for Com-COV1 was conducted in the per-protocol population which included only seronegative participants in 4-week interval schedules and excluded participants whose Day 56 visit (peak antibody response) was out of window or missing, as well as participants who had developed confirmed SARS-CoV2 infection prior to this primary endpoint visit. Similarly for Com-COV2, the per protocol population included only seronegative participants, but excluded participants whose Day 28 visit (peak antibody response) was out of window or missing, or

participants who had developed confirmed SARS-CoV2 infection prior to this primary endpoint visit.

The published primary outcome per protocol analyses (100,101) did not exclude participants who had serum anti-nucleocapsid IgG evidence of SARS-CoV2 infection in the absence of a confirmed virological diagnosis. These participants were excluded in the per protocol analyses presented in this thesis.

The per-protocol population analyses were deemed more likely to give conservative estimates of non-inferiority and so were chosen as the primary outcomes.

All other immunogenicity analyses used a modified intention-to-treat population, where participants whose visits were out-of-window were not excluded. Baseline seropositive participants were excluded. Participants who developed virological evidence or immunological evidence of infection over the course of the trial remained part of the mITT population, up until the point of infection/seroconversion.

When analysing the primary endpoint using this population, it allows a conservative estimation for a superiority comparison, as well as a sensitivity analysis for the non-inferiority comparison. These populations were chosen based on guidance from the European Medicines Agency (102).

2.11.4.2 Com-COV1 Interval analysis

The mITT analysis population, here, follows the same conditions described for the heterologous/homologous mITT analysis, however, it excludes the immunology cohort, as this cohort contained 4-week interval schedules only.

2.11.5 Primary Immunological Analysis

The primary objectives of both Com-COV1 and Com-COV2 were the non-inferiority analyses of heterologous schedules against homologous schedules. The immune

measure for this comparison was serum anti-SARS-CoV2 spike IgG, 28 days post second dose. For Com-COV1 this is D56 for the 4 week schedules (the 12-week schedules' comparisons did not form part of the primary objective) and for Com-COV2 this is D28. Confidence intervals, rather than p-values, are presented for both primary and secondary analyses. This is in keeping with guidance on statistical reporting of outcomes (103). Forest plots are used graphically to display the aGMRs and confidence intervals.

2.11.6 Secondary Immunological Analyses

Secondary immunological comparisons were made for serum anti-SARS-CoV2 spike IgG titres at non-primary-endpoint timepoints, T-cell ELISpot frequencies and pseudotype virus neutralising titres at all timepoints. An additional measure of the ratio of pseudotype virus neutralising titre against total binding anti-SARS-CoV2 spike IgG was calculated as a measure of relative neutralising capability of the induced antibody response.

2.11.7 Kinetics

Kinetics graphs were produced using the modified intention to treat population with participants who showed virological or serological evidence of SARS-CoV2 infection included up until the last point that they showed no evidence of infection after which their results were censored.

2.11.8 Reactogenicity analysis

The reactogenicity population is a different modified-intention-to-treat population, which included out of window visits, as well as both seronegative and seropositive participants. Participants showing virological or immunological evidence of SARS-CoV2 infection prior to 7 days following the second dose, were excluded.

Reactogenicity is shown descriptively using stacked horizontal bar charts. Further analyses comparing schedules, doses, intervals and serostatus were conducted:

1) Frequency of reactogenicity – A binary variable of the symptom being present vs not present, modelled using logistic regression adjusting for Sex, Age, BMI and serostatus.
2) Severity of reactogenicity – A binary variable of a grade 2 symptom or above being present vs a grade 1 symptom being present, excluding participants without any symptom, modelled using logistic regression without further adjustment.
3) Duration of moderate-severe reactogenicity – A count variable of the number of days spent at grade 2 or above modelled using linear regression adjusting for Sex, Age, BMI and serostatus.

2.11.9 Safety analysis

The safety population consisted of all participants who had received at least one dose of any vaccine. Descriptive tables and summary statistics are displayed including total numbers of events, classifications and proportions of participants who suffered adverse events, times of onset of adverse event, enumeration of grading and causality categories.

2.12 Immunological assays for primary & secondary endpoints

2.12.1 Preparation of serum aliquots

Blood samples for serum were taken using serum tubes with silica particles to trigger coagulation. They were processed the same day, but if this was not possible, they were stored overnight at 4°C and processed within 24 hours of venepuncture. They were centrifuged in a pre-cooled (4°C) centrifuge at 3000g for 15 minutes. Serum was then aliquoted out into 10 x 500µL cryovials and stored at -80 °C.

2.12.2 Extraction of peripheral blood mononuclear cells (PBMCs)

Blood samples for cellular assays were taken using lithium heparin tubes. They were transported to Oxford Immunotec, overnight at room temperature and processed within 32 hours of venepuncture. 25µL of Tcell Xtend™ reagent was then added per mL of whole blood and incubated for 20 mins at room temperature. The PBMC fraction was then isolated using FICOLL density gradient centrifugation. At this point, samples were run 'fresh' on the T-spot Discovery™ SARS-CoV2 ELISpot assay with excess PBMCs being frozen down in aliquots of 0.5-1mL using Dimethyl sulfoxide (DMSO) and foetal bovine serum (FBS) as the cryopreservatives and stored at -80 °C.

2.12.2.1 T-Cell Xtend™

T-cell Xtend™ is a proprietary reagent from Oxford Immunotec. The principle is that it acts as an aid in the separation of lymphocytes from whole blood. T-cells isolated from whole blood collected in lithium heparin tubes that are not processed within 8 hours show reduced responses to antigenic stimulation in interferon gamma ELISpot assays. This is due, in part, to the presence of other cell types in the sample. T-cell Xtend reduces the proportion of other cells in the sample by using a bi-specific antibody, one half of which is directed against cell surface markers on selected lymphocytes, which have been

identified to be inhibitory to the T-cell ELISpot. The other half of these bi-specific antibodies binds to red blood cells, changing the density of these inhibitory immune cell types, so that during FICOLL separation, they remain in the red blood cell layer, away from the PBMC layer.

2.12.3 Nexelis – Serum anti-SARS-CoV2 spike IgG ELISA

Sera were analysed at Nexelis, (Laval, Canada) to determine anti-SARS-CoV2 spike IgG concentrations by ELISA (reported as ELISA laboratory unit (ELU)/mL). To convert these results to the WHO international standard of BAU/mL, the following formula may be used: Result (BAU/mL) = Result (ELU/mL) / 7.9815

This classical indirect ELISA uses SARS-CoV-2 pre-spike recombinant antigen adsorbed onto a 96-well microplate. Following incubation, the microplate is washed to remove unbound antigen and blocked to prevent non-specific binding. A standard dilution series, controls and sample dilutions are incubated in the wells of the coated microplate. Following incubation, the microplate is washed to remove unbound primary antibodies. Primary antibodies are detected with the addition of the secondary anti-human IgG antibody conjugated to peroxidase. After incubation, the microplate is washed to remove unbound secondary antibodies. The peroxidase substrate solution, tetramethylbenzidine (TMB), is added to the microplate and a colored product is developed which is proportional to the amount of anti-SARS-CoV-2 pre-spike IgG present in the serum sample. 2N H_2SO_4 is then added to stop the colorimetric reaction. The absorbance of each well is measured using a microplate spectrophotometer reader at a specific wavelength (450/620 nm). The intensity of the optical density is proportional to the concentration of the anti-SARS-CoV-2 pre-spike IgG antibodies present in each sample.

2.12.4 Nexelis – Pseudotype virus neutralisation assay

The SARS-CoV-2 pseudotype virus neutralizing assay (PNA) is a surrogate virus neutralization assay that detects neutralizing antibodies targeting the viral spike RBD (receptor-binding domain). Pseudotyped virus particles are made from a modified vesicular stomatitis virus (VSVΔG) backbone which displays the SARS-CoV2 spike glycoprotein, from which the last 19 amino acids of the cytoplasmic tail have been removed. The pseudoparticles contain a Luciferase reporter used for detection.

Heat-inactivated serum samples are prepared as a series of seven two-fold serial dilutions in a 96-well transfer plate. The SARS-CoV-2 pseudovirus is added sequentially to the serum dilutions at a target working dilution (to obtain a minimum of 100,000 RLU/well [relative luminescence units]) and incubated at 37°C with 5% CO_2 supplementation for 60 minutes. Serum-virus complexes are then transferred onto plates, previously seeded overnight with Vero E6 cells, and incubated at 37°C with 5% CO_2 supplementation for 20 hours.

Following incubation, the luciferase substrate is added to the cells in order to assess the level of luminescence per well. Reading is then performed on a luminescence plate reader. The intensity of the luminescence is quantified in RLU and is inversely proportional to the level of neutralizing antibodies present in the serum. The neutralizing titer of a serum sample is calculated as the reciprocal serum dilution corresponding to the 50% neutralization antibody titer (NT_{50}) for that sample (104).

Of note, Nexelis changed their pseudotype virus neutralization assay part way through the study, moving from their ‘qualified’ assay to their ‘verified assay’. Where there were results from both assays, only the verified assay result was analysed. However, the samples for which there were paired qualified and verified assay results allowed estimation of the conversion factor from qualified to verified assays, which was close to one.

To convert these results to IU/mL, the following formula may be used: Result (IU/mL) = Result (NT_{50} titre) / 1.872

2.12.5 Oxford Immunotec – T-cell ELISpot

Interferon-gamma secreting T-cells specific to overlapping whole spike protein epitopes based on the Wuhan-Hu-1 sequence (YP_009724390.1) were detected using a modified T-SPOT-Discovery test performed at Oxford Immunotec (Abingdon, UK) within 32 hours of venepuncture, using the addition of T-Cell Xtend reagent. T cell frequencies were reported as spot forming cells (SFC) per 250,000 PBMCs with a lower limit of detection of one in 250,000 PBMCs, and these results multiplied by four to express frequencies per 10^6 PBMCs. Each sample was run in 8 wells:

Table 16 *– Eight T cell ELISpot conditions per PBMC sample*

Unstimulated control
Overlapping epitopes of Spike protein N terminus (S1 domain)
Overlapping epitopes of Spike protein C terminus (S2 domain)
Overlapping epitopes of Nucleocapsid protein (N)
Overlapping epitopes of Membrane protein (M)
Overlapping epitopes of full Spike protein with epitopes that were cross-reactive with seasonal coronaviruses removed
Overlapping epitopes of full Spike protein (S)
Positive control (PHA)

50μL of each panel solution was added to each of the 8 wells per sample. 100μL of the final cell suspension containing 250,000 PBMCs for each sample was then added to each well. This was kept in a humidified incubator at 37°C with 5% CO_2 for 18 hours. Cell culture medium was then discarded and washed four times with 200μL DPBS. Each well was then incubated at 4°C with 50μL of 1:200 dilution of conjugate reagent in DPBS. Four further DPBS washes were performed. 50μL of substrate solution was added per well and incubated for 7 minutes at room temperature. The wells were then washed with

deionised water. Plates were then dried for 4 hours in a well ventilated oven at 37°C before being read with each distinct dark blue spot on the membrane of each well contributing to the count.

2.12.6 Public Health England (now UKHSA) – Serum anti-nucleocapsid IgG

Serum aliquots were analysed at Porton Down, Public Health England, by ECLIA (Cobas platform, Roche Diagnostics) to determine serum anti-SARS-CoV-2 nucleocapsid IgG status (reported as negative if below a cut off index of 1.0).

2.13 Data Cleaning of Clinical Research Organisation Delivered Results

Data was received from each of the commercial laboratories or collaborators and was reconciled against the samples sent. Samples which had invalid or missing results were queried with the laboratory until this was resolved with retesting or was confirmed as missing with no possibility to retest. Laboratories were blinded as to the study allocation and so datasets were only merged with the main trial database after cleaning and receipt of data.

2.14 Rationale for immunological assays

Serum binding anti-SARS-CoV2 spike IgG was the primary outcome and has been shown to be a reasonable correlate of protection against symptomatic infection (105). Neutralisation and pseudotype virus neutralisation assays provided more detailed information on the functionality of these antibodies. IFNγ T-cell ELISpot is a well-recognised first-line assay to describe the cellular response. Since all vaccines being tested in these trials contained only the spike protein as their immunogen, serum anti-nucleocapsid IgG was able to act as a marker of natural infection throughout the trial.

2.15 Rationale for laboratory partners

It was apparent at the outset of these trials, that academic laboratories alone would have insufficient capacity to manage the sample volumes planned in a timeframe that would allow delivery of meaningful policy-relevant results. The decision was made to contract out this work to commercial clinical research organisations. There was coordination with various bodies including the National Institute for Health Research (NIHR) and Coalition for Epidemic Preparedness Innovations (CEPI) to try to standardise assays across several different trials in order to make results directly comparable. This resulted in Oxford Immunotec becoming the chosen laboratory for ELISpot, Nexelis for spike ELISA & pseudotype neutralisation and PHE for nucleocapsid ELISA. PHE had also been contracted to provide microneutralisation data on this trial, however, due to capacity issues, the results of this were incomplete and therefore a local collaboration with Prof Gavin Screaton's academic group at the University of Oxford, was set up.

2.16 Mucosal immunology

2.16.1 Sample types

2.16.1.1 Serum

Serum used in this work was collected and processed as previously described.

2.16.1.2 Mucosal Lining Fluid

There are several possible sampling techniques to acquire mucosal lining fluid (MLF) each with their own advantages and disadvantages. These include brushings, washes, biopsy, saliva and synthetic absorbable matrix (SAM^{TM})-swab devices. SAM^{TM}-swabs were chosen on balance as a simple, rapid, non-invasive way of MLF sampling, although these devices are more expensive. There is evidence that SAM-strips may provide more reproducible results than nasopharyngeal aspiration or swabbing (106). The Nasosorption^{TM} FX-i device (7mm width) [HMD 798-34] manufactured by Mucosal Diagnostics, Hunt Developments UK, was chosen for use, as it is the largest size available and intended for adult usage with an estimated absorptive capacity of 100μL of water. Work done in a paediatric population, endeavoured to quantify the mucosal sampling volume by SAM-strip, by measuring the pre and post sampling weight of the SAM-strip, assuming a mucosal lining fluid sample density equivalent to water (1g/cm^3) (107). On average, sampling appeared to result in 10μL of mucosal lining fluid, which is well within the maximum absorptive capacity, making it unlikely that absorption would be hindered by SAM-strip saturation.

2.16.2 Assay selection

The Mesoscale Diagnostics' (MSD) 10-spot V-PLEX COVID-19 Coronavirus Panel 3 96-well plate was used. The multiplex nature of the assay allowed simultaneous analysis of seasonal coronavirus responses, which were of potential interest (**Table 17**).

Table 17 *– MSD ECLIA multiplex spot antigens*

Spot Number	Antigen
1	SARS-CoV2 Spike
2	HCoV-NL63 Spike
3	SARS-CoV2 Nucleocapsid
4	SARS-CoV1 Spike
5	BSA
6	MERS-CoV Spike
7	HCoV-HKU1 Spike
8	HCoV-OC43 Spike
9	HCov-229E Spike
10	SARS-CoV2 S1 RBD

The MSD ECLIA assay works in a similar way to a standard enzyme-linked immunosorbent assay (ELISA) with the exception that the final reporter step is not an enzyme degrading a substrate with a colorimetric output. Instead, the quantified output is measured by the light emitted from the MSD SULFO-TAG which emits light, once a current is passed through the bottom of the plate in the plate reader.

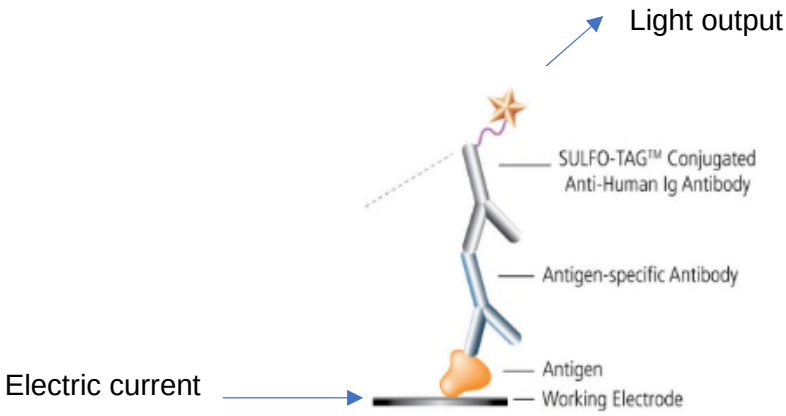

Figure 2 *– Cartoon graphic of MSD ECLIA mechanism, Modified from MSD V-PLEX® COVID-19 Serology Kits* (108)

2.16.3 General Materials

Table 18 *– General laboratory equipment*

Materials	**Company/Code #**
Corning® Costar® Spin-X® centrifuge tube filters	Sigma CLS9301-1000EA
Costar® 2.0 mL Snap Cap Microcentrifuge Tube, Polypropylene, Nonsterile	Sigma CLS3213-1000EA
Multispot 96-well, 10-spot plate. V-PLEX COVID-19 Coronavirus Panel 3 Kit. Storage 2-8ºC	Mesoscale Diagnostics (MSD) K15399U-2 (IgG kit) K15401U-2 (IgA kit)
Microplate Adhesive Film	MSD
MESO QuickPlex AQ 120MM plate reader with Methodical Mind™ TeamLink reading software	MSD

2.16.4 Reagents

Table 19 *– Reagents*

Reagent	**Company/Code #**
Deionized water	From Milli-Q
Chemgene. Storage Room Temperature (RT)	StarLabs XTM309
99.5% Ethanol (RT)	Sigma-Aldrich E7023
Virkon powder or tablets. Storage RT	VWR International 148-0202
Tween detergent. Storage RT	Sigma-Aldrich P1754
Dulbecco's Phosphate Buffered Saline (DPBS) (RT)	Sigma D8537
Sodium Chloride (NaCl). (RT)	Sigma S9888
Bovine Serum Albumin (BSA). (RT)	Sigma A7906-100G
Protease cocktail inhibitor, 20 tablets. Storage 2-8ºC	Sigma 11873580001
SULFO-TAG Anti-Human Antibody (200X) 200µL Storage 2-8ºC. Protect from light IgG (mouse mAb against IgG Fab segment) IgA (mouse mAb against human IgA)	 MSD, D21ADF-3 MSD, D21ADE-3
Diluent 100, 1000ml. Storage 2-8ºC	MSD, R50AA-3
Blocker A. 250mL. (RT)	MSD, R93BA-2
MSD Phosphate Buffer (5X). 50mL. (RT)	MSD, R93SA-2
MSD GOLD™ Read Buffer B. 90mL. (RT)	MSD, R60AM-2
Reference Standard 1. 1mL. (pooled convalescent donor serum) Storage ≤-70ºC	MSD, C00ADK-2 Lot number: A00V0004
Serology Control Pack 1. (pooled convalescent donor serum) Storage ≤-70ºC Serology Control 1.1. 1mL Serology Control 1.2. 1mL Serology Control 1.3. 1mL	MSD, C4381-1 Lot numbers: A00C0731 A00C0732 A00C0733

2.16.4.1 MLF Extraction buffer

1.5g NaCl was added to 0.2g dry BSA and two tablets of protease cocktail inhibitor in a sterile container. These were stirred and dissolved in 100ml DPBS using a magnetic flea and stirrer until clear. The solution (sufficient to elute 200 samples) was then filtered through a 0.22µm vacuum filter into a sterile reagent bottle. The extraction buffer was then stored at 4^{o}C and discarded after 1 month if unused.

2.16.4.2 PBS + 0.25% Tween / Wash for MSD plates

2.5mL of Tween detergent was added to 1L of PBS and stored at room temperature after complete mixing. Unused solution was discarded after 6 months.

2.16.4.3 70% Ethanol

300mL of distilled water was added to 700 mL 99.5% ethanol and stored at room temperature.

2.16.4.4 Blocker A solution

50g MSD Blocker A dry powder (stored at room temperature) was added to 200mL deionised water. This was then mixed until dissolved leaving a clear pale yellow solution with no visible flakes. 50mL MSD Phosphate Buffer (5X) (stored at room temperature) was then added to this solution and thoroughly mixed before vacuum filtering through a 0.22µm filter into a sterile container. The solution was stored at 4^{o}C and unused solution discarded after 5 weeks.

2.16.4.5 Diluent

MSD Diluent 100 used as supplied by the manufacturer and stored at 4^{o}C.

2.16.4.6 Controls

MSD Serology Controls were prepared by MSD from pooled donor serum and had been previously calibrated for all nine antigens on the plate. They were supplied at working concentration and did not require dilution. They were stable for 5 years when frozen at -80ºC, but were discarded after a maximum of five freeze-thaw cycles. Tubes were vortexed before use.

2.16.4.7 Reference Standard

The reference standard was prepared by MSD from pooled donor serum and had been previously calibrated for all nine antigens on the plate. Dilution series as defined in Section 2.16.6.4.2. Reference standards are stable for 5 years when frozen at -80ºC, but were discarded after a maximum of five freeze-thaw cycles. Tubes were vortexed before use.

2.16.4.8 SULFO-TAG Anti-Human IgG/IgA Antibody Solution

Detection antibody was provided as 200X stock and stored at 4ºC. Working solution was 1X and stored at 4ºC. 30µL of SULFO-TAG Anti-Human Ig antibody was added to 5,970µL of Diluent 100 in a 15mL Falcon tube. 200x and 1x tubes were protected from light. Tubes were vortexed before use.

2.16.4.9 MSD GOLD™ Read Buffer

Read buffer was supplied at working concentration. Stored at room temperature.

2.16.5 Safety

All handling and processing of SAM™-swabs was undertaken in a class II Microbiological safety cabinet in a containment level 2 (CL2) laboratory for participants without evidence of SARS-CoV2 infection concurrent with the time of sampling.

2.16.6 Methods

2.16.6.1 Populations

For each 12-week schedule, 25 participants were selected over two sites (Southampton and Oxford) for logistical ease and were consented for mucosal fluid sampling. In the 4-week schedules, participants who were enrolled to the immunology cohort also had mucosal sampling conducted, but these were not routinely analysed in this thesis. 4-week schedule participants who had virological evidence of infection or serological evidence of infection (by serum anti-nucleocapsid IgG ECLISA, Roche, UKHSA) were, however, additionally selected for analysis.

An additional set of 36 SAM-strips from participants in the PITCH study (Protective Immunity from T cells in Healthcare Workers) were also analysed, chosen, as they had no baseline immunological, virological or clinical evidence of prior SARS-CoV2 infection, had not been vaccinated, but had had baseline mucosal samples taken.

2.16.6.2 Sample Collection

SAM^{TM}-swabs were removed from their foil packaging just prior to use. The participant's head was gently tilted backwards and after examination of the nostril, the applicator attached to the SAM^{TM}-swab itself was removed with a twist from the attached cryogenic tube and gently inserted horizontally into one of the nostrils of the participant up to the hilt of the applicator, ensuring that the SAM^{TM}-swab itself lay flat against the inferior turbinate of the nose. The participant was then instructed gently to apply pressure to the side of their nostril and to keep the device in place for 60 seconds. Pressure from the finger was then released and the device removed and re-screwed into its original cryogenic tube. The SAM^{TM}-swab was then labelled and immediately placed into a 4^{o}C cool bag before being taken as soon as possible for storage in a -80^{o}C freezer.

2.16.6.3 Mucosal lining fluid extraction

Samples were taken from the freezer and thawed on wet ice. Each SAM™-swab was cut just below the end of the applicator, at the second indented line of the SAM™-swab, allowing the SAM™-swab to fall back into its own cryotube. Scissors were decontaminated with 70% ethanol between samples. 500µL of MLF extraction buffer was pipetted into each cryotube. Each cryotube was then twisted closed and vortexed for 30 seconds before incubating for 30 minutes on wet ice. During this incubation, 2ml Eppendorf tubes, each with a filterless spin-x chamber, were prepared and the eluent of each SAM™-swab transferred in. The cut SAM™-swab itself was also inserted into the spin-x chamber using blunt forceps, which were cleaned with 70% ethanol in between samples. The spin-x Eppendorfs containing the supernatants were then microcentrifuged for 10 minutes at 13,000 rpm (17,900 x g) at 4°C. After centrifugation, the spin-x chamber and residual SAM-strip were discarded, and the remaining supernatant vortexed in the Eppendorf for 5 seconds. The eluent was then transferred into two fresh tubes as ~250µL aliquots, labelled and then immediately frozen at -80°C at least overnight before assaying.

2.16.6.4 MSD assay

Plates were removed from 4°C storage and allowed to warm to room temperature before removing from their packaging. Samples, MSD reference standards and MSD controls were removed from the -80°C freezer and thawed on wet ice. To minimise risk of damage to bottom of MSD wells, samples and reference standards were prepared in Eppendorf tubes before being transferred to the MSD plate. Care was taken not to let the MSD plates dry out between steps. Extreme care was taken not to allow any bubbles to remain in any of the wells at any stage.

2.16.6.4.1 Blocking

150µL of Blocker A solution was added to each well and the plate sealed with an adhesive strip for a 30 minutes incubation at room temperature (20^{o}C) shaking at 700rpm. During this stage samples, controls and reference standard were prepared (Sections 2.16.6.4.2, 2.16.6.4.3).

At the end of this blocking step, the plate was washed three times with 150µL PBS-Tween per well followed by a single wash of DPBS to reduce frothing/bubbling. In between each wash, the plate was tapped upside down to remove as much fluid as possible.

2.16.6.4.2 Reference standard preparation

2.16.6.4.2.1 IgG reference standard

A set of serial dilutions were prepared (**Figure 3**). 180µL of MSD diluent was added to the first tube (CAL-01), and 150µL was added to tubes CAL-02 to CAL-08. The MSD Reference Standard 1 was vortexed before 20µL was added to CAL-01. This tube was vortexed and 50µL of CAL-01 was transferred to CAL-02, which was then vortexed. This process was repeated through until CAL-07 with final tube volumes being 150µL. CAL-08 was kept as diluent only, for the negative control. The 150µL was sufficient for 2 x 50 µL replicate wells on the MSD plates from which an average reading would be obtained.

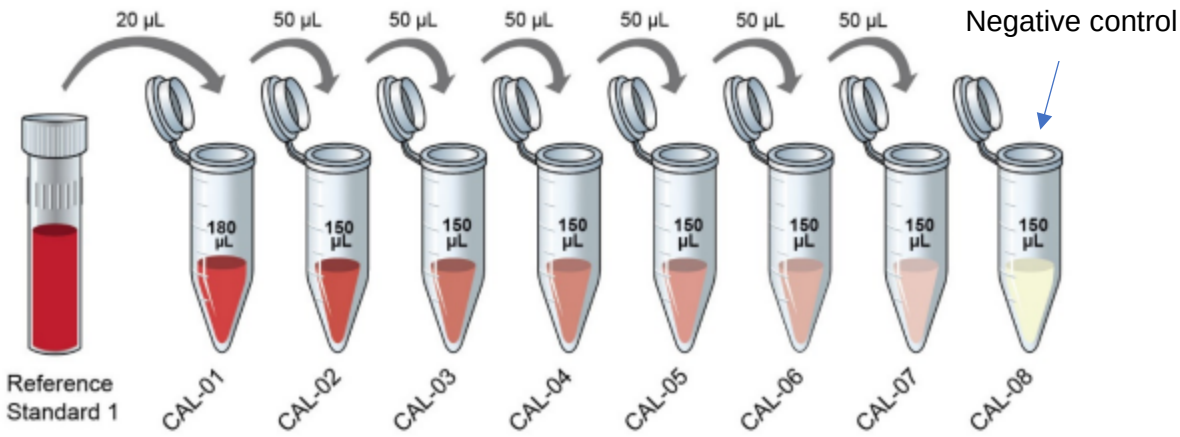

Figure 3 *– Reference standard dilution series for IgG assay Modified graphical representation from MSD V-PLEX® COVID-19 Serology Kits* (108)

2.16.6.4.2.2 IgA reference standard

A similar process was carried out to prepare the IgA standard (**Figure 4**), except the CAL-01 tube remained empty whilst 150µL MSD diluent was added to CAL-02 to CAL-08. The MSD Reference standard 1 was vortexed and 200µL was added to CAL-01. CAL-01 was then vortexed and 50µL transferred to CAL-02, which was then vortexed. This process was repeated for CAL-03 to CAL-07. The CAL-08 tube remained as diluent only, acting as a negative control.

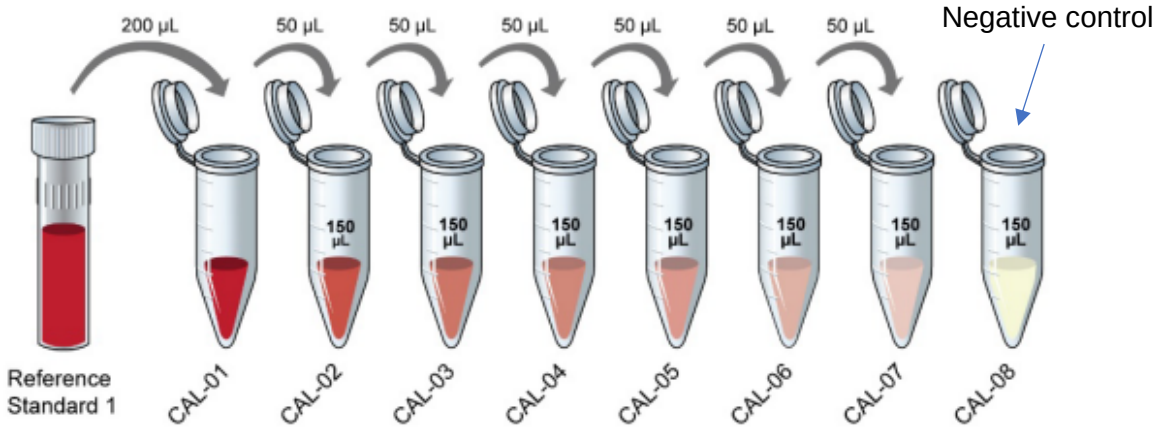

Figure 4 *– Reference standard dilution series for IgA assay. Modified graphical representation from MSD V-PLEX® COVID-19 Serology Kits* (108)

2.16.6.4.3 Sample Preparation

2.16.6.4.3.1 Extracted mucosal samples

Following on from previous work, the optimal starting concentration for a SAM^{TM}-swab eluted with 500µL extraction buffer was determined to be 1:50 for IgG for all timepoints in seropositive participants, but only for post-vaccination timepoints in seronegative participants. For the pre-prime timepoint (Day 0) in seronegative participants, a 1:5 dilution was found to be optimal. For the IgA assay, 1:50 was found to be optimal for all timepoints regardless of serostatus. The final range of dilutions used for the mucosal samples were 1:5 – 1:50 – 1:500, with volumes shown in (**Table 20**). If sample readouts were out of range they were repeated at the next appropriate dilution. If sample readouts were still out of range at the extremes of this dilution series, values were imputed as the LLOQ/2 (lower limit of quantification) or ULOQ (upper limit of quantification) as appropriate.

Table 20 *– Mucosal sample dilutions*

Simple Dilution factor	Volume sample	Volume MSD Diluent 100	Notes
1:5	30µL	120µL	Sufficient for one plate only
1:50	5µL	245µL	Sufficient for two plates
1:500	3µL	1497µL	Sufficient for two plates

2.16.6.4.3.2 Serum samples

The optimal starting dilution for serum samples for detection of specific IgA was determined to be 1:1667 for all samples at all timepoints. This dilution was also used for the detection of specific IgG in pre-prime (D0) samples from seronegative participants. For the detection of specific IgG at all other timepoints in both seronegative and seropositive pariticpants, a starting dilution of 1:10,000 was used. The final range of dilutions used for serum samples were 1:167 – 1:1667 – 1:10,000 – 1:100,000, with volumes shown in (**Table 21**). If sample readouts were out of range they were repeated at the next appropriate dilution. If sample readouts were still out of range at the extremes of this dilution series, values were imputed as the LLOQ/2 (lower limit of quantification) or ULOQ (upper limit of quantification) as appropriate.

Table 21 *– Serum sample dilutions*

Simple Dilution factor	1st Dilution		2nd Dilution	
	Volume sample	Volume MSD Diluent 100	Volume sample	Volume MSD Diluent 100
1:167	3µL	497µL	-	-
1:1667	3µL	497µL	60µL	540µL
1:10,000	3µL	497µL	10µL	590µL
1:100,000	3µL	497µL	3µL	1797µL

2.16.6.4.3.3 Plating out and Initial incubation

Table 22 *– MSD Plate layout*

	1/2	3/4	5/6	7/8	9/10	11/12
A	CAL-01	Sample 1	Sample 9	Sample 17	Sample 25	Control 1
B	CAL-02	Sample 2	Sample 10	Sample 18	Sample 26	Control 2
C	CAL-03	Sample 3	Sample 11	Sample 19	Sample 27	Control 3
D	CAL-04	Sample 4	Sample 12	Sample 20	Sample 28	Sample 33
E	CAL-05	Sample 5	Sample 13	Sample 21	Sample 29	Sample 34
F	CAL-06	Sample 6	Sample 14	Sample 22	Sample 30	Sample 35
G	CAL-07	Sample 7	Sample 15	Sample 23	Sample 31	Sample 36
H	CAL-08 (Blank)	Sample 8	Sample 16	Sample 24	Sample 32	Sample 37

The reference standard curve, controls and each samples were all added to the plate (50µL/well) in duplicate as shown in **Table 22**.

All of the dilution tubes for the standards and samples were vortexed for 5 seconds before adding 50µL to each replicate well of the IgG and IgA plates following the plate template. The control vials were vortexed and 50µL added directly to the MSD plate wells. The plates were then covered with an adhesive plate seal, removed from the Class II MSC, and left to incubate at room temperature, shaking at 700rpm for 2 hours. After this the plates were then returned to the Class II MSC and the contents of the plate gently emptied into 2% virkon. Each well was then washed with at least 150µL/well of PBS-Tween. After this initial wash, the plate was then taken out of the Class II MSC where a further two washes of 150µL/well of PBS-Tween were conducted followed by an additional wash of DPBS .

2.16.6.4.4 Antibody detection

The detection antibody (SULFO-TAG Anti-Human Ig antibody) was then added to the plates (50µL of the prepared detection antibody/well). The plate was covered with an adhesive plate seal and then covered again with tin foil to protect from light and then left to incubate at room temperature, shaking at 700rpm for 1 hour. After this each well was washed three times with 150µL/well of PBS-Tween followed by an additional wash of DPBS.

2.16.6.4.5 Plate Reading

Finally, 150µL of MSD Gold read buffer was added to each well. No adhesive plate seal was used at this point. The plate was then loaded into the MSD plate reader.

2.16.6.5 Analysis

2.16.6.5.1 Analysis Population

Non-randomised selection of 25 participants per each of the 12-week schedules (all the 12-week participants recruited from Oxford and Southampton). Those who showed virological or immunological evidence of infection were excluded from kinetics, heat plot and forest plot analyses.

Those with virological or immunological evidence of infection were analysed separately alongside 4-week participants who also demonstrated evidence of infection, who had been originally identified by serum anti-nucleocapsid IgG seroconversion or through virologic testing.

2.16.6.5.2 Quality control

The output from the MSD plate reading software (Methodical Mind) was reviewed within the MSD desktop software (DISCOVERY WORKBENCH 4.0 for Windows 7). For each plate, each of the nine standard curves (one per antigen) were reviewed to assess their sigmoid shape and to see if any calibration points had an excessively wide confidence interval. Calibration curves used to calculate antibody concentrations were established by the software fitting the signals from the mean signal of duplicate calibrators to a 4-parameter logistic (or sigmoidal dose-response) model with a $1/Y^2$ weighting.

2.16.6.5.2.1 Actual concentrations

Antibody unit concentrations in controls and diluted samples are determined from their signals by backfitting to the calibration curve. The actual concentration of antibody for each sample, was automatically calculated by the software by multiplying the assay output by the ‘overall dilution’ factor.

The ‘overall dilution’ factor for serum is the ‘simple dilution’ factor used for the assay.

However, mucosal lining fluid is not a typical uniform sample. There is no way to know precisely what volume of sample was absorbed by the SAM-strip at the time of sampling.

From previously published data (107), we estimated 10μL MLF was absorbed per SAM-swab, which was eluted into a 500μL elution buffer, this results in an 'elution factor' of approximately 1:50. To work out the 'overall dilution' factor, this 'elution factor' is then multiplied by the 'simple dilution' factor (for example a starting simple dilution of 1:50 in **Table 20**), resulting in an overall dilution factor of 1:50 * 1:50 = 1:2,500. This overall dilution factor was then used to calculate the mucosal antibody titre for semi-quantitative comparisons against the serum titre.

2.16.6.5.2.2 Quality Control – Reference Standard

Data was then exported to a custom made Microsoft Excel Quality Check spreadsheet which I designed. Within the spreadsheet, the reproducibility of each dilution within the standard curve was reviewed to ensure the coefficient of variation (CV) was less than 30%. If the CV for one dilution was greater than 30%, the raw signal values were reviewed and if one of the duplicates suffered from a clear pipetting error in the context of the dilution series, this was excluded. The accuracy of each of the reference standard dilutions was reviewed (% Recovery) with a threshold of acceptability of ±30%. If there were up to two clearly erroneous points within the dilution series these were excluded.

2.16.6.5.2.3 Quality Control – Controls

Control values were then reviewed. The antigen assay was accepted as 'successful' if both the CV ≤30% and the %recovery ≤30% for at least two out of three of the control samples. If an antigen assay failed, its results were excluded from further analysis.

2.16.6.5.2.4 Quality Control – Samples

Each assay's results were reviewed and excluded if the CV >30%. The sample was then repeated. If any samples had a signal that was above the reference standard curve then the sample was repeated with a greater dilution factor or imputed as per **Table 20** or **Table 21**. If any samples had a signal that was below the reference standard curve then the sample was repeated with a lesser dilution factor or imputed.

Since this is a multiplex assay, if a sample was repeated at a different dilution because one of the 9-plex results was out of range, the other eight antigens were automatically repeated as a matter of course. Where this resulted in more than one valid result for each sample, the geometric mean concentration of these valid results was taken as the true result for analysis.

Data was then exported to Stata BE 17, where it was cleaned further, analysed and visualised.

2.16.6.5.3 Pre-pandemic threshold setting

2.16.6.5.3.1 Serum

A set of 28 participants of comparable age to the Com-COV1 cohort of participants, who had enrolled in an RSV vaccine study in 2016, were selected from the Oxford Vaccine Centre Biobank. Their 2016 study baseline serum sample (pre-RSV-vaccine candidate administration) was tested to establish pre-pandemic baseline antibody levels.

2.16.6.5.3.2 Mucosal

A set of 25 participants, who had SAM-strips taken in pre-COVID mucosal studies were analysed on the same MSD platform, by a separate research group at Imperial College London. The samples were analysed on the "Coronavirus 2" plate rather than the "Coronavirus 3" plate. The only difference being that the MERS-CoV spike antigen on

the Coronavirus 3 plate was replaced by the SARS-CoV2 NTD (non-transcriptional domain) antigen. The raw data for this experiment was shared by a research group based at Imperial College London and this was used to establish pre-pandemic mucosal baselines.

2.16.6.5.4 Statistical analyses & Data presentation methods

Individual level data are presented longitudinally over time. Vaccine schedule group averages are presented as the GMC ± 95% CI for each timepoint over time. Seronegative and seropositive participants are presented separated by colour on graphs. Participants who show immunological or virological evidence of SARS-CoV2 infection were included in their appropriate baseline group (seronegative or seropositive) depending on their baseline status, up until the timepoint prior to infection, after which they were removed from later timepoints. Results are presented in quadruplicate – serum IgG, serum IgA, mucosal IgG and mucosal IgA.

Individual fold changes are calculated as the raw peak titre (day 112) divided by the raw baseline titre (day 0). The GMCs ± 95% CI per vaccine schedule group are displayed.

Multivariate linear regressions are used to predict immunological responses with age, sex, BMI, Serostatus, site, actual number of days between blood draw and enrolment and vaccine schedule included as covariates.

Correlation plots use Pearson's correlation coefficients with Bonferroni corrected p-value thresholds to assess statistical significance. Bonferroni correction was made for the total number of comparisons made in this specific analysis. In this case 3024 comparisons were made reducing the significance threshold to 0.0000165.

The correlations are between gradients of logarithmically transformed antibody titres between timepoints for different immune measures, and are displayed on heat plots displaying the correlation coefficient.

Forest plots are used to demonstrate adjusted geometric mean ratios comparing geometric mean concentrations and their 95% confidence intervals between comparator groups.

Chapter 3 Heterologous COVID-19 vaccine schedules

3.1 Background

3.1.1 Pre-COVID-19

Heterologous vaccination – the use of different vaccines to act as first and second or subsequent doses of a vaccine schedule – has been investigated since at least the 1980s (109). Researchers attempted to improve the observed weak immune response to a recombinant vaccinia virus vectored HIV vaccine expressing the gp160 envelope protein delivered via scarification with an additional vaccine, via one of four different methods including homologous vaccination and a protein subunit vaccine also delivered by scarification. Limitations of the study included small participant numbers and the non-randomised nature of the study, however, the outcome was still promising particularly for the use of heterologous regimes eliciting an anamnestic humoral and cellular response lasting for over one year after the original vaccination.

Heterologous vaccination was further studied in 1991 in mice (110), when an HIV-1 gp160 protein subunit vaccine and a virally vectored vaccine (live recombinant vaccinia virus expressing HIV-1 envelope glycoproteins) were administered in both homologous and heterologous schedules. The combination of a virally vectored vaccine, followed by a protein subunit vaccine gave the highest serum binding IgG response and was the only schedule to produce an antibody response with neutralising activity. Another HIV study in non-human primates (111) demonstrated that a priming schedule of four DNA vaccines followed by two protein boosters successfully induced both B and T-cell responses, but did not demonstrate clinical protection against SIV (simian immunodeficiency virus) challenge.

HIV is not the only pathogen against which heterologous vaccination has been investigated. In 2004, a group investigated homologous vs heterologous vaccination of

mice using a DNA vaccine encoding the *Mycobacterium tuberculosis* protein ESAT6 and a protein subunit vaccine of ESAT6 in Incomplete Freund's adjuvant (IFA) (112). A qualitative difference in immune responses was demonstrated between the differently vectored vaccines, with DNA immunisation inducing a Th1-polarised response, whilst adjuvanted protein induced a Th2-dominant response. In those who received a DNA-prime followed by protein boost, the boosted response had a larger IFNγ (Th1) response, but also had significant boosting of both antibodies and Th-cells. This suggested that it was the priming vaccine that was important in determining the qualitative nature of the immune response. However, the converse schedule (protein followed by DNA vaccine) was not reported to allow a more complete comparison. Another study of candidate TB vaccines in 2013 suggested that heterologous vaccination (IM BCG followed by an IM MVA vectored vaccine) might increase the recall potential of memory CD4 T-cells (113).

Finally, a mouse study of H5N1 avian influenza in 2013 has suggested that the immune response following a heterologous adenovirus-vectored / virus-like particle regimen produces a greater and broader neutralising and binding antibody response than either homologous regimen (114).

These studies give cautious optimism that heterologous vaccination, when administered 'optimally', might be able to be more immunogenic with broader humoral and cellular responses, and perhaps also with longer lasting immunity..

The pre-COVID literature up until this point is caveated. Adequately powered studies in humans had not yet been conducted and pre-clinical or small scale human studies had not had the opportunity to delineate the role of many key factors such as the effect of dose or order of vaccines on immune response.

However, in 2020, a heterologous priming vaccine schedule to protect against Ebola received marketing authorisation from the European Medicines Agency (EMA) for the first time. It consisted of a human adenovirus vectored first dose (Ad26. ZEBOV,

Zabdeno) and a modified vaccinia Ankara virus second dose (MVA-BN-Filo, Mvabea) manufactured by Janssen Vaccines (115–117). Protection against Ebola in humans was inferred from animal studies. Although it was the Ad26 first dose, MVA second dose schedule that was taken forward to licensure, it is interesting to note that MVA-Ad26 produced a similar, if not larger antibody response when compared to Ad26-MVA, however there were two important differences between these two permutations: From a humoral point of view, Ad26-MVA produced a significant antibody response after prime, which was inferred to offer at least partial protection after one dose, whilst first dose MVA-Ad26 did not. Secondly Ad26-MVA had a significantly larger initial CD4 and CD8 response, in comparison to MVA-Ad26, as measured by intracellular cytokine staining, although IFNγ responses by ELISpot were comparable. This difference reduced over time, suggesting that in the long term there may be little to choose between these two heterologous regimens.

3.1.2 COVID-19

At the time of licensure of both the Oxford/AstraZeneca and Pfizer/BioNTech COVID-19 vaccines at the end of 2020, there was already concern about vaccine availability and future supply. Early safety signals such as VITT (vaccine induced thrombocytopaenic thrombosis) associated with virally vectored vaccines (118) as well as myocarditis and pericarditis associated with mRNA lipid nanoparticle vaccines had led to a number of different countries adopting a variety of complicated, age-specific and frequently changing immunisation programme restrictions. Many of these restrictions came into being after significant swathes of each country's population had received their first vaccine dose, causing these people, by default, to require a heterologous vaccine dose to complete their priming schedule.

At the time, there was no direct evidence regarding heterologous COVID priming vaccine schedules to support their administration and there was significant concern expressed

in the general media about the adoption of such strategies (119). UK National Immunisation guidance (The Green Book) (120) suggested a cautious approach to heterologous vaccination, where it was recognised that in cases where the first dose was unknown or a second vaccine from the same manufacturer was unavailable, the balance of risk was that receiving a heterologous second dose was, overall a safer option, than receiving no second vaccine dose at all.

There followed a number of studies that aimed to assess viability of heterologous schedules, amongst which Com-COV1 and Com-COV2 were the only randomised control trials with active comparator arms. These other studies included a randomised control trial in Spain (121), whose comparator arm to BNT/ChAd was no administration of a second dose. This showed a 36-fold difference in antibody titre 14 days post-second dose when compared to those who did not receive a second dose. The reactogenicity profile was acceptable. This trial gave confidence that heterologous COVID vaccination was viable, but with no active comparator arm, inference was limited.

A non-randomised cohort study in Germany used a neutralisation surrogate assay (a competitive binding antibody assay against recombinant angiotensin converting enzyme 2 [ACE2]) to compare the homologous ChAd/ChAd and BNT/BNT schedules against the heterologous ChAd/BNT (122). ChAd/BNT induced the largest humoral responses. Notable caveats were its non-randomised nature and differences in the ages of participants receiving each schedule as populations were recruited in different settings (BNT/BNT median 38 years, ChAd/BNT median 47 years, ChAd/ChAd median 57 years). A second study from Germany showed similar results again (123) and a third study from Sweden revealed comparable results this time with mRNA-1273 (Spikevax, Moderna) rather than BNT (124).

Further cohort studies from Germany (125,126) analysed both humoral and cellular responses between homologous ChAd/ChAd, homologous mRNA vaccines (including

both Mod and BNT), and heterologous ChAd/mRNA vaccine. Homologous mRNA schedules and ChAd/mRNA schedules produced significantly larger binding and neutralising humoral responses than ChAd/ChAd. T-cell responses against whole spike protein were also greater in these two schedules. Of particular note, was the frequency of CD8 T cells which were greatest in the heterologous schedule suggesting a qualitative difference between vaccine platforms. Additionally, there was a signal that the frequency of Th1 polyfunctional spike-specific T-cells was proportionally higher in viral-vector containing regimens. The relevance of this is not entirely clear currently. Polyfunctional CD8 T-cells have been associated with better control of HIV (127) and it is possible that they are correlated with vaccine-elicited protection in trials with HIV, HBV, HCV, CMV and Influenza (128), but the mechanisms by which they may mediate this remain unclear.

Aside from the observational studies discussed, there was also one purpose-designed heterologous SARS-CoV2 vaccine – Gamaleya: Sputnik V (Gam-COVID-Vac). This was an Ad26 vectored first dose followed by an Ad5 vectored second dose (129,130). There was a robust humoral and cellular response along with an estimated efficacy against symptomatic confirmed SARS-CoV2 infection of 73%, however a lack of head-to-head comparison studies and a lack of standardisation of the assays used in this trial make it difficult to make quantitative comparisons with other COVID vaccines. The T cell response (IFNγ ELISpot), much like homologous ChAd/ChAd, was not well boosted by a second virally vectored dose (131).

3.2 Introduction – COVID-19 vaccination schedules

With the emergency authorisation of several different vaccines with different delivery platforms, demand for immunisation was high and outstripped initial manufacturing capabilities. There were immense logistical difficulties delivering national immunisation programmes at speed, particularly getting two doses of the same vaccine to the same

person within the manufacturer-recommended timeframe set in a background of uncertainty as to the continuity of each vaccine's supply.

It was therefore important to gauge how suitable it may be to deliver different vaccines for first and second doses. Here I present separately, the results of the comparisons of heterologous schedules to homologous schedules for the Com-COV1 and Com-COV2 trials.

Additionally, alongside each set of comparisons of homologous and heterologous schedules, I show an exploratory subgroup analysis investigating the effect of baseline serostatus on absolute immunological titres.

3.3 Hypotheses

1) Heterologous schedules are non-inferior to their relative homologous schedule (28 days post second dose binding anti-SARS-CoV2 spike IgG antibody)
2) Heterologous schedules are non-inferior to their relative homologous schedule (28 days post second dose frequencies of IFNγ-spot forming cells (SFCs)). Notably, the true T-cell peak is likely to have been at 14 days post second dose rather than 28 days, but this was not measured in the majority of participants at this timepoint due to logistical constraints.
3) Heterologous schedules are similarly reactogenic to their relative homologous schedules and safe.

3.4 Results

3.4.1 Recruitment & Baseline Demographics

3.4.1.1 Com-COV1

975 participants were screened via a one to two stage telephone ± in-person screening process across eight sites in England. 145 were deemed ineligible by inclusion/exclusion criteria. Of the remaining 830, 100 were allocated to the immunology cohort based on participant preference, at three of the eight sites only, to simplify trial delivery logistics. Randomisation was then to one of the four 4-week interval study arms. The remaining 730 were assigned to the general cohort across all eight sites and were randomised to one of the eight arms, which included four 4-week interval schedules and four 12-week interval schedules (**Figure 5**).

Detailed baseline demographic information is displayed in **Table 23**

The mean age of participants was 58, 44% (366/829) were female and 56% (463/829) were male. 24% (196/829) were of a self-described non-white ethnicity, whilst the remaining 76% (633/829) were of a white ethnicity. 33% (275/829) had at least one respiratory or cardiovascular comorbidity or diabetes, whilst 67% (554/829) were non-comorbid. Groups were well-balanced between all arms.

We aimed to recruit a UK-representative population. We therefore ensured that Com-COV1 had 24% non-white participants, in keeping with the UK population 2021 census data (132). Additionally, we wanted to ensure that the recruited population was representative of a population more at risk of severe SARS-CoV2 infection: One third of participants had diabetes, cardiovascular, or comorbidity.

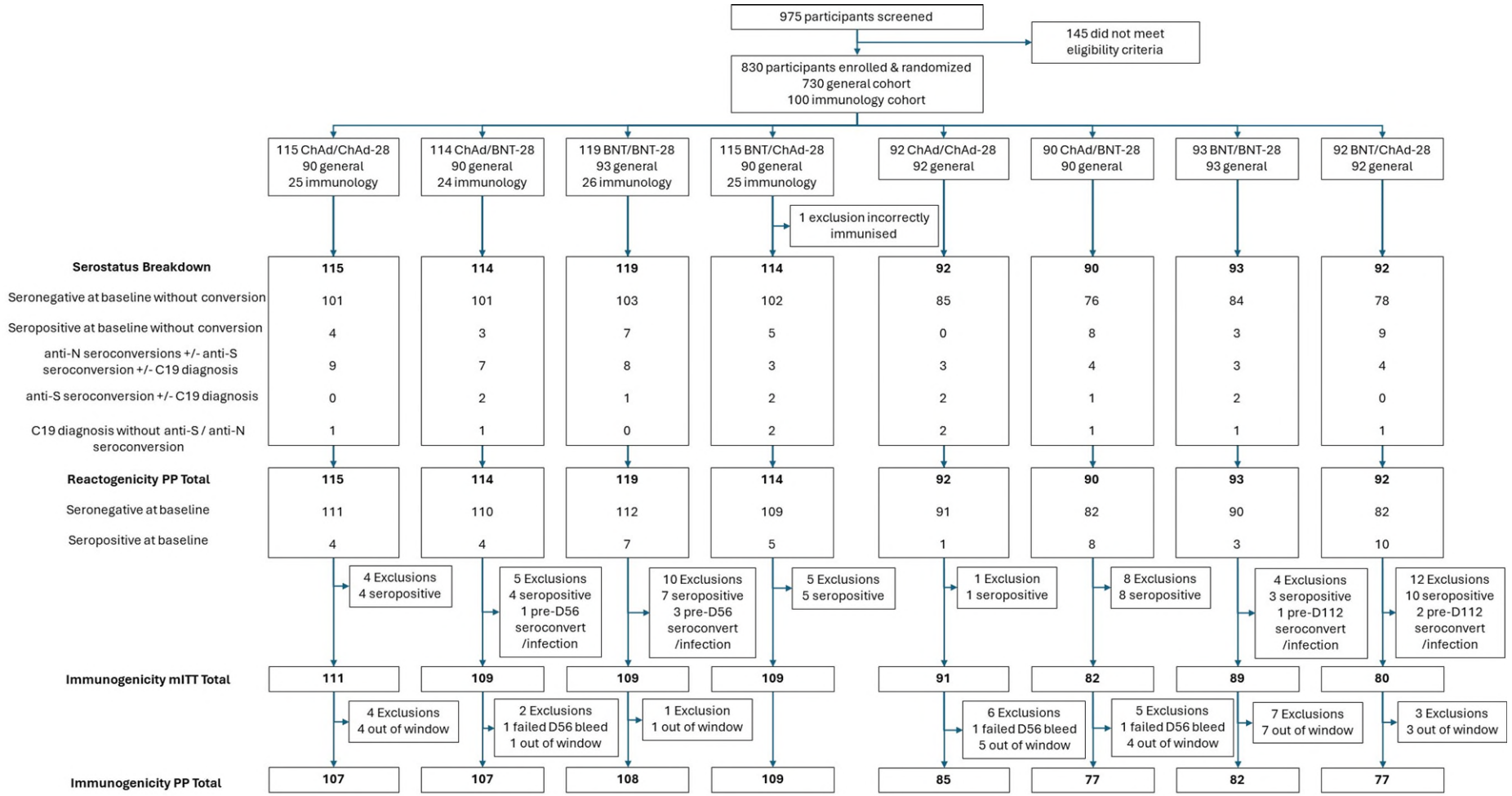

Figure 5 *– Com-COV1 CONSORT Diagram. Seronegative: <1.0 anti-N IgG at baseline and for study duration. Seropositive: ≥1.0 anti-N IgG at baseline but with no evidence of anti-N seroconversion or anti-S seroconversion at non-vaccination timepoints nor any virological evidence of infection by PCR or LFA. "Anti-N seroconversions +/- anti-S seroconversion +/- C19 diagnosis": ≥2-fold increase in anti-N IgG at any timepoint with the second titre ≥1.0 regardless of anti-S IgG, virological diagnosis or baseline serostatus. "Anti-S seroconversion +/- C19 diagnosis": No evidence of anti-N seroconversion, with ≥2-fold increase in anti-S IgG at a timepoint with no expected increase in titre due to vaccination, regardless of virological diagnosis or baseline serostatus. "C19 diagnosis without anti-S / anti-N seroconversion": No evidence of anti-S or anti-N seroconversion, but virological evidence of infection, regardless of baseline serostatus*

Table 23 *– Demographics of Com-COV1 participants*

	ChAd/ChAd-28 N=115	ChAd-BNT-28 N=114	BNT-BNT-28 N=119	BNT-ChAd-28 N=114	ChAd-ChAd-84 N=92	ChAd-BNT-84 N=90	BNT-BNT-84 N=93	BNT-ChAd-84 N=92	Total N=829
Age (years)									
Mean (SD)	57.7 (4.8)	58.1 (4.7)	58.0 (4.9)	57.4 (4.6)	57.5 (5.0)	58.6 (4.3)	58.1 (4.6)	58.1 (4.7)	57.9 (4.7)
Sex									
Female	51 (44%)	49 (43%)	61 (51%)	50 (44%)	42 (46%)	36 (40%)	44 (47%)	33 (36%)	366 (44%)
Male	64 (56%)	65 (57%)	58 (49%)	64 (56%)	50 (54%)	54 (60%)	49 (53%)	59 (64%)	463 (56%)
Ethnicity									
White	87 (76%)	82 (72%)	93 (78%)	83 (73%)	71 (77%)	72 (80%)	72 (77%)	73 (79%)	633 (76%)
Non-White	28 (24%)	32 (28%)	26 (22%)	31 (27%)	21 (23%)	18 (20%)	21 (23%)	19 (21%)	196 (24%)
Black	1 (1%)	1 (1%)	2 (2%)	2 (2%)	3 (3%)	1 (1%)	2 (2%)	1 (1%)	13 (2%)
Asian	19 (17%)	20 (18%)	12 (10%)	13 (11%)	8 (9%)	11 (12%)	9 (10%)	11 (12%)	103 (12%)
Mixed	8 (7%)	9 (8%)	10 (8%)	13 (11%)	7 (8%)	5 (6%)	7 (8%)	4 (4%)	63 (8%)
Other	0 (0%)	2 (3%)	2 (3%)	3 (3%)	3 (3%)	1 (1%)	3 (3%)	3 (3%)	17 (2%)
Comorbidity									
Non-comorbid	68 (59%)	79 (69%)	79 (66%)	75 (66%)	65 (71%)	60 (67%)	65 (70%)	63 (68%)	554 (67%)
Comorbid	47 (41%)	35 (31%)	40 (34%)	39 (34%)	27 (29%)	30 (33%)	28 (30%)	29 (32%)	275 (33%)
Cardiovascular	26 (23%)	22 (19%)	28 (24%)	27 (24%)	18 (20%)	21 (23%)	16 (17%)	20 (22%)	178 (21%)
Respiratory	21 (18%)	17 (15%)	17 (14%)	16 (14%)	7 (8%)	10 (11%)	14 (15%)	10 (11%)	112 (14%)
Diabetes	13 (11%)	9 (8%)	2 (2%)	3 (3%)	2 (2%)	1 (1%)	1 (1%)	4 (4%)	35 (4%)
BMI									
Mean (SD)	27.4 (4.9)	27.1 (4.8)	26.9 (4.8)	27.5 (4.0)	28.1 (4.3)	26.8 (4.5)	26.9 (4.6)	27.2 (5.1)	27.2 (4.6)

Percentages may sum to more than 100% due to rounding or due to having more than one co-morbidity. Immunology & General cohorts combined

3.4.1.1.1 Sub-study

The introduction of an ‘advice for paracetamol’ sub-study as a late amendment (as described in 2.8.4.1) potentially impacts on the analyses and is discussed later.

3.4.1.2 Com-COV2

1,174 participants were screened. Of these, 22 were excluded due to inclusion/exclusion criteria. A further 80 did not complete the telephone screening process. Of the remaining 1072, 151 were allocated into the immunology cohort, based on participant preference, but only at four of the nine sites to simplify trial logistics. As only five out of nine sites collected lithium heparin samples for cellular immune response analysis, only 59% of participants across both the general and immunology cohorts have T-cell ELISpot data (**Table 24**).

Detailed baseline demographic information is displayed in **Table 25**. The mean age of participants was 63, 42% (450/1070) were female and 58% were male (620/1070). 7% (77/1070) were of a self-described non-white ethnicity, whilst the remaining 93% (993/1070) were of a white ethnicity. 42% (451/1070) had at least one respiratory or cardiovascular comorbidity or diabetes, whilst 58% (619/1070) were non-comorbid. Interval between first and second doses was 8-12 weeks as one of the inclusion criteria, with the average interval being 67 days (9.5 weeks). This did not vary significantly by site, prime vaccination or arm.

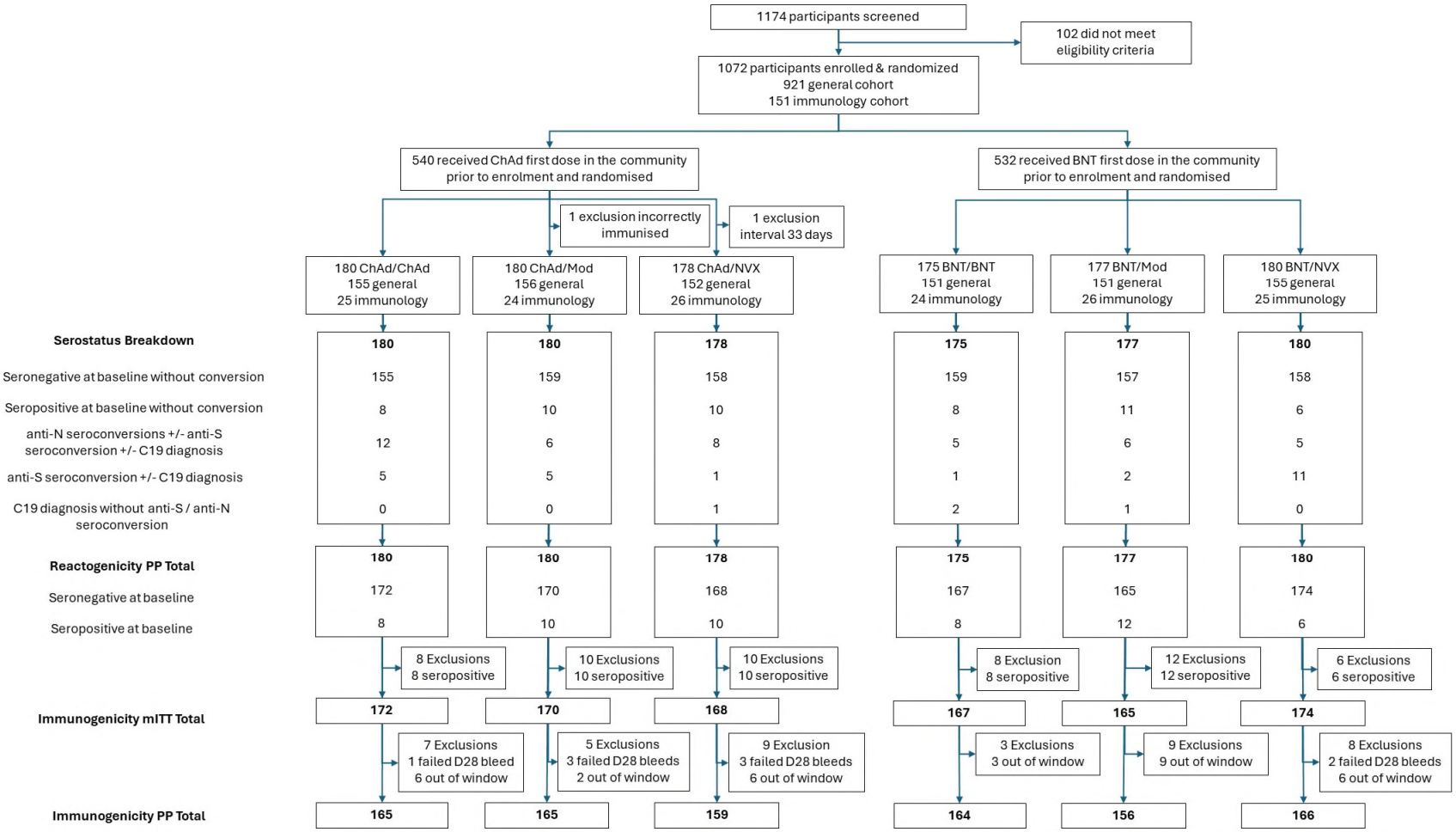

Figure 6 *– Com-COV2 CONSORT Diagram . Seronegative: <1.0 anti-N IgG at baseline and for study duration. Seropositive: ≥1.0 anti-N IgG at baseline but with no evidence of further anti-N increases or anti-S increases at non-vaccination timepoints nor any virological evidence of infection by PCR or LFA. "Anti-N seroconversions +/- anti-S seroconversion +/- C19 diagnosis": ≥2-fold increase in anti-N IgG at any timepoint with the second titre ≥1.0 regardless of anti-S IgG, virological diagnosis or baseline serostatus. "Anti-S seroconversion +/- C19 diagnosis": No evidence of anti-N seroconversion, with ≥2-fold increase in anti-S IgG at a timepoint with no expected increase in titre due to vaccination, regardless of virological diagnosis or baseline serostatus. "C19 diagnosis without anti-S / anti-N seroconversion": No evidence of anti-S or anti-N seroconversion, but virological evidence of infection, regardless of baseline serostatus*

Table 24 *– Prime vaccines and PBMC collection by site*

	Birmingham	GSTT	Hull	Liverpool	Newcastle	Nottingham	SGUL	Sheffield	UCLH	Total
Tcell Site	Yes	Yes	Yes	No	No	No	Yes	No	Yes	-
ChAdOx1	51 (50%)	113 (63%)	72 (63%)	49 (45%)	58 (47%)	0 (0%)	26 (52%)	75 (74%)	94 (50%)	538 (50%)
Interval	65.2	68.0	65.5	64.5	65.3	-	68.3	66.4	66.0	66.2
BNT	50 (50%)	67 (37%)	42 (37%)	60 (55%)	65 (53%)	104 (100%)	24 (48%)	27 (26%)	93 (50%)	532 (50%)
Interval	71.2	67.4	66.6	66.4	65.2	68.4	67.8	66.7	63.4	66.8
Total	101 (100%)	180 (100%)	114 (100%)	109 (100%)	123 (100%)	104 (100%)	50 (100%)	102 (100%)	187 (100%)	1070 (100%)

Table 25 *– Demographics of Com-COV2 participants*

	ChAd/ChAd N=180	ChAd/Mod N=180	ChAd/NVX N=178	BNT/BNT N=175	BNT/Mod N=177	BNT/NVX N=180	Total N=1070
Age (years)							
Mean (SD)	63.1 (5.5)	63.4 (5.5)	63.2 (5.8)	61.9 (5.4)	62.1 (5.9)	62.3 (5.6)	62.7 (5.6)
Sex							
Female	87 (48%)	80 (44%)	73 (41%)	80 (46%)	68 (38%)	62 (34%)	450 (42%)
Male	93 (52%)	100 (56%)	105 (59%)	95 (54%)	109 (62%)	118 (66%)	620 (58%)
Ethnicity							
White	169 (94%)	159 (88%)	161 (90%)	166 (95%)	166 (94%)	172 (96%)	993 (93%)
Non-White	11 (6%)	21 (12%)	17 (10%)	9 (5%)	11 (6%)	8 (4%)	77 (7%)
Black	1 (1%)	1 (1%)	3 (2%)	3 (2%)	2 (1%)	3 (2%)	13 (1%)
Asian	4 (2%)	11 (6%)	9 (5%)	3 (2%)	6 (3%)	2 (1%)	35 (3%)
Mixed	3 (2%)	7 (4%)	3 (2%)	1 (1%)	1 (1%)	2 (1%)	17 (2%)
Other	3 (2%)	2 (1%)	2 (1%)	2 (1%)	2 (1%)	1 (1%)	12 (1%)
Comorbidity							
Non-comorbid	116 (64%)	110 (61%)	118 (66%)	82 (47%)	98 (55%)	95 (53%)	619 (58%)
Comorbid	64 (36%)	70 (39%)	60 (34%)	93 (53%)	79 (45%)	85 (47%)	451 (42%)
Cardiovascular	49 (27%)	55 (31%)	40 (22%)	63 (36%)	46 (26%)	57 (32%)	310 (29%)
Respiratory	15 (8%)	18 (10%)	19 (11%)	30 (17%)	34 (19%)	31 (17%)	147 (14%)
Diabetes	9 (5%)	10 (6%)	14 (8%)	22 (13%)	21 (12%)	24 (13%)	100 (9%)
BMI							
Mean (SD)	26.5 (4.8)	26.4 (4.6)	26.8 (5.1)	27.5 (5.1)	27.8 (5.8)	28.8 (6.6)	27.3 (5.4)
Interval (days)							
Mean (SD)	65.9 (6.7)	66.2 (6.6)	66.6 (6.6)	66.6 (6.8)	66.8 (6.7)	67.0 (6.7)	66.5 (6.7)

Percentages may sum to more than 100% due to rounding or due to having more than one co-morbidity. Combined Immunology & General cohorts

Participants recruited were randomised separately according to their community-received first dose vaccines. There were some population differences between the ChAd-primed and BNT-primed populations. The BNT-primed population had a slightly higher proportion of males, were slightly more comorbid and had a slightly higher BMI, overall reflecting a more at risk population.

There were also some differences between the Com-COV1 and Com-COV2 populations, in part because Com-COV2 recruitment was logistically more challenging. Overall, Com-COV2 participants were older and more comorbid, however the distribution of sexes between the trials was similar. Com-COV2 recruitment was less ethnically diverse, with only 7% being ethnically non-white, however, a greater proportion had comorbidity (44%), which was in keeping with a more at-risk population being targeted for early immunisation by the national immunisation roll-out.

3.4.2 Immunogenicity

3.4.2.1 Com-COV1

3.4.2.1.1 Binding Anti-SARS-CoV2 spike IgG

In the per protocol analysis, seronegative participants receiving 4-week (28-day) interval heterologous ChAd/BNT (ChAd/BNT-28), on average, produced 9.2 (97.5% CI: 7.4, 11.6) times as much serum binding anti-SARS-CoV2 spike IgG as those receiving 4-week interval homologous ChAd/ChAd (ChAd/ChAd-28), meeting the non-inferiority criterion. Participants receiving 4-week interval heterologous BNT/ChAd (BNT-ChAd-28), on average, produced 0.5 (97.5% CI: 0.4, 0.6) times as much serum binding anti-SARS-CoV2 spike IgG antibody as those receiving 4-week interval homologous BNT/BNT (BNT/BNT-28), failing to meet non-inferiority (**Figure 7**).

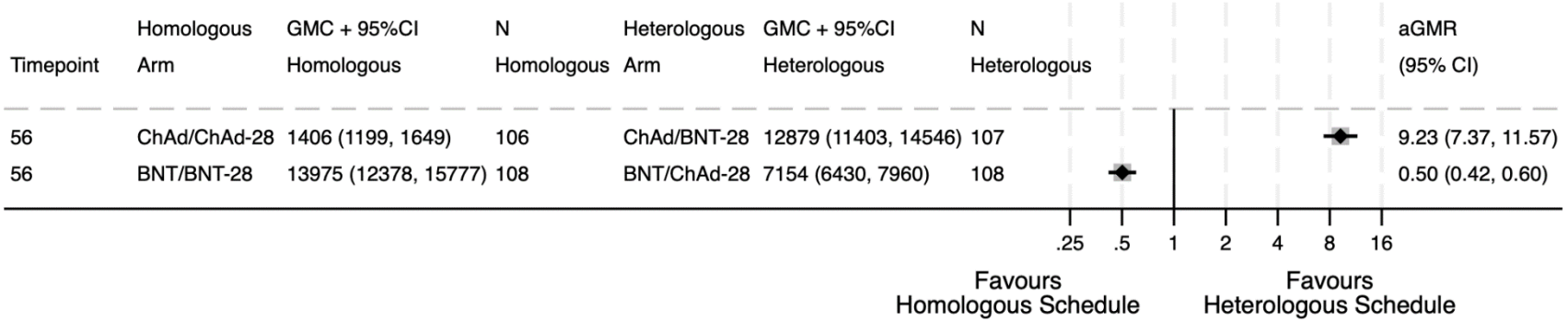

Figure 7 *– Com-COV1 Per protocol analysis. Forest plot comparing D56 peak (28 days post second dose) anti-SARS-CoV2 spike IgG between homologous and heterologous 4-week interval schedules. . Left sided columns represent the raw, unadjusted GMC ± 97.5% CI. The forest plot represents the right sided aGMR derived from a multivariate linear regression*

In the mITT analysis (**Figure 8**), seronegative participants receiving heterologous BNT/ChAd-28, on average, produced 0.5 (95% CI: 0.4, 0.6) times as much serum binding anti-SARS-CoV2 spike IgG, 28 days post-second dose, as those receiving homologous BNT/BNT-28, reaffirming the failure of non-inferiority. In the 12-week (84-day) interval schedules, the serum binding anti-SARS-CoV2 spike IgG response of heterologous BNT/ChAd-84 was similarly 0.6 (95% CI: 0.5, 0.7) times that of homologous BNT/BNT-84.

Conversely seronegative participants receiving heterologous ChAd/BNT-28, on average, produced 9.4 (95% CI: 7.7, 11.5) times as much serum binding anti-SARS-CoV2 spike IgG, 28 days post-second dose, as those receiving homologous ChAd/ChAd-28. In the 12-week interval heterologous/homologous comparison, although the difference in serum binding anti-SARS-CoV2 spike IgG response reduced in comparison to the 4-week interval comparison, the serum binding anti-SARS-CoV2 spike IgG response of heterologous ChAd/BNT-84 was still 5.3 (95% CI: 4.1, 6.8) times that of homologous ChAd/ChAd-84.

At five months post-second dose, the difference in serum binding anti-SARS-CoV2 spike IgG titres between BNT/ChAd-84 and BNT/BNT-84 was maintained, GMR 0.6 (95% CI: 0.5, 0.8). However, for all other heterologous/homologous comparisons at both intervals, the difference between schedules was reduced: The aGMR between BNT/ChAd-28 and BNT/BNT-28 schedules got closer to one indicating a reduction in magnitude of difference from 0.5 (95% CI: 0.4, 0.6) to 0.6 (95% CI: 0.5, 0.8). The aGMR between ChAd/BNT-28 and ChAd/ChAd-28 reduced from 9.4 (95% CI 7.7, 11.5) to 6.8 (95% CI: 5.5, 7.4). The aGMR between ChAd/BNT-84 and ChAd/ChAd-84 reduced from 5.3 (95% CI: 4.1, 6.8) to 3.5 (95% CI: 2.5, 4.8) (**Figure 9**).

The ratio of antibody level at five months vs one month post-second dose (as the reference), was calculated as a measure of antibody wane. This ratio or fold-change was

then compared between heterologous and homologous schedules (ie a ratio of ratios). There was no statistical difference between any 12-week homologous/heterologous comparison. However, ChAd/ChAd-28 waned more slowly than ChAd/BNT-28, with fold changes of 0.30 (95% CI: 0.24, 0.38) and 0.18 (95% CI: 0.16, 0.20) respectively. Conversely the heterologous arm of BNT/ChAd-28 waned more slowly than BNT/BNT-28 with fold changes of 0.26 (95% CI: 0.22, 0.31) and 0.20 (95% CI: 0.18, 0.23) respectively (**Figure 10**).

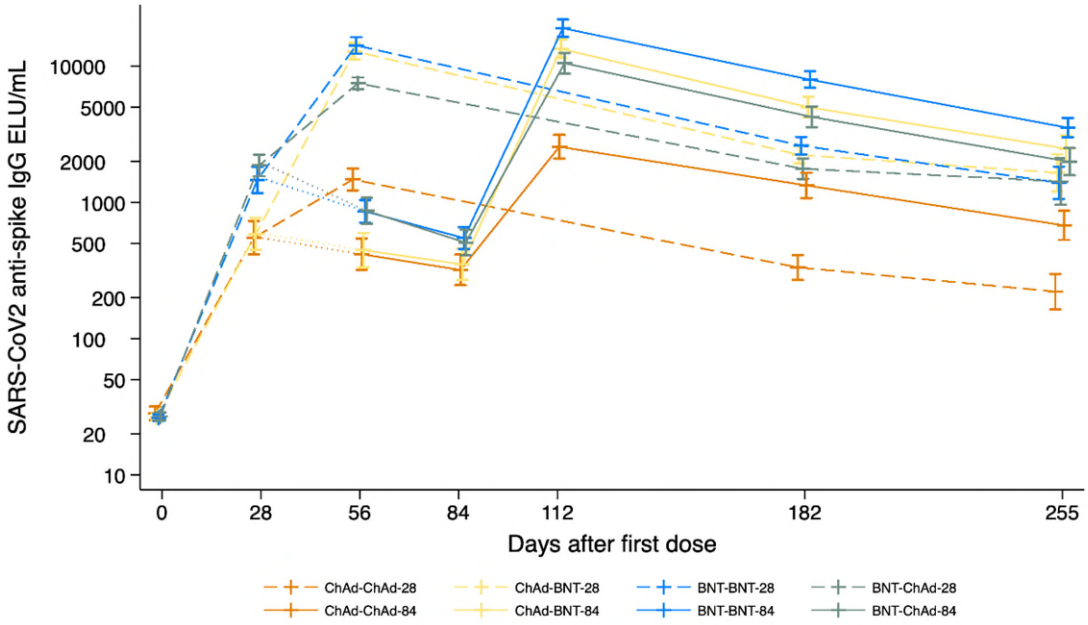

Figure 8 *– Com-COV1 Scatter plot showing kinetics of the anti-SARS-CoV2 spike IgG GMC ± 95% CI. for all eight Com-COV1 schedules. Seronegative participants only. Dashed lines represent 4-week interval schedules, solid lines represent 12-week interval schedules, dotted lines represent an interpolated line for the 12-week interval schedules, as no sample at Day 28 was taken. Orange: Homologous ChAd arms; yellow heterologous ChAd/BNT, blue homologous BNT, and green heterologous BNT/ ChAd*

Timepoint	Homologous Arm	GMC + 95%CI Homologous	N Homologous	Heterologous Arm	GMC + 95%CI Heterologous	N Heterologous	aGMR (95% CI)
56	ChAd/ChAd-28	1392 (1187, 1632)	107	ChAd/BNT-28	12995 (11504, 14680)	108	9.43 (7.74, 11.48)
182	ChAd/ChAd-28	330 (277, 392)	98	ChAd/BNT-28	2204 (1937, 2508)	102	6.82 (5.51, 8.43)
56	BNT/BNT-28	13833 (12246, 15626)	109	BNT/ChAd-28	7154 (6430, 7960)	108	0.50 (0.43, 0.59)
182	BNT/BNT-28	2555 (2250, 2901)	101	BNT/ChAd-28	1693 (1451, 1976)	104	0.64 (0.53, 0.78)
112	ChAd/ChAd-84	2562 (2099, 3126)	90	ChAd/BNT-84	13411 (11436, 15728)	81	5.27 (4.10, 6.77)
255	ChAd/ChAd-84	678 (531, 866)	66	ChAd/BNT-84	2484 (2001, 3085)	60	3.45 (2.47, 4.81)
112	BNT/BNT-84	19011 (16434, 21993)	85	BNT/ChAd-84	10516 (8831, 12523)	78	0.58 (0.47, 0.72)
255	BNT/BNT-84	3535 (2999, 4166)	65	BNT/ChAd-84	1989 (1580, 2504)	56	0.61 (0.46, 0.80)

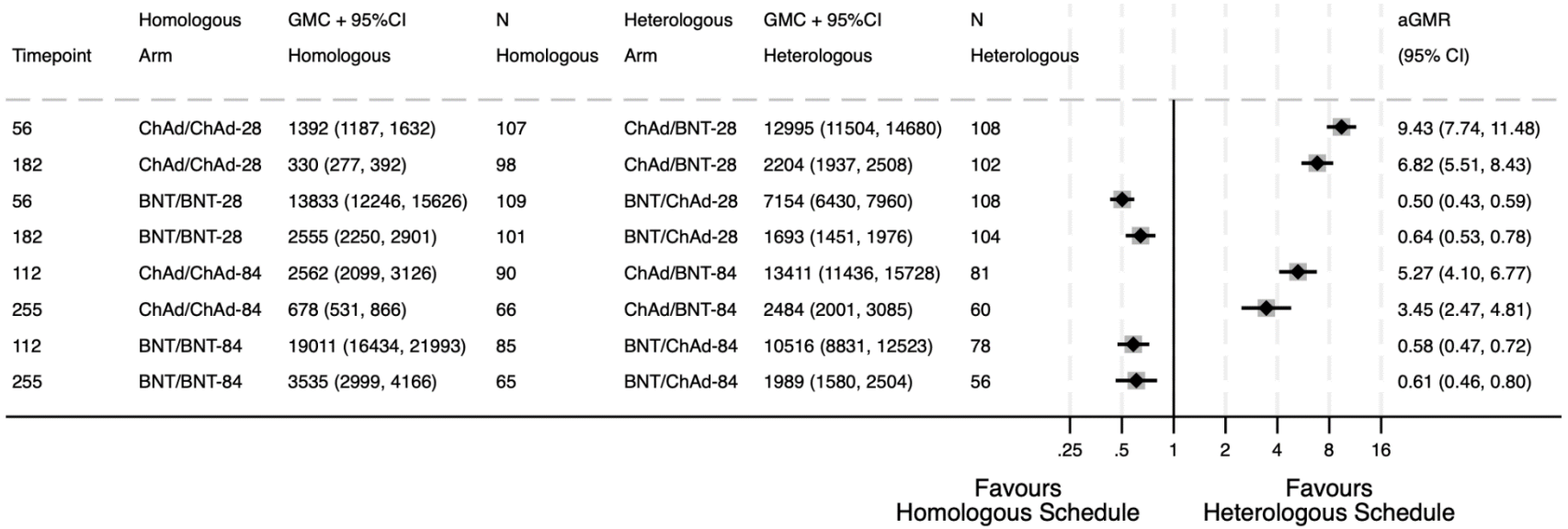

Figure 9 *– Com-COV1 mITT analysis. Forest plot comparing peak (28 days post second dose) and 5-months post second dose anti-SARS-CoV2 spike IgG between homologous and relative heterologous schedules. Peak antibody response for the 4 week schedules was at 56 days, whilst for 12-week this was at 112 days. The 5-month timepoint was at day 182 for 4-week schedules, and at day 255 for the 12-week schedules. Left sided columns represent the raw, unadjusted GMC ± 95% CI. The forest plot represents the right sided aGMR derived from a multivariate linear regression*

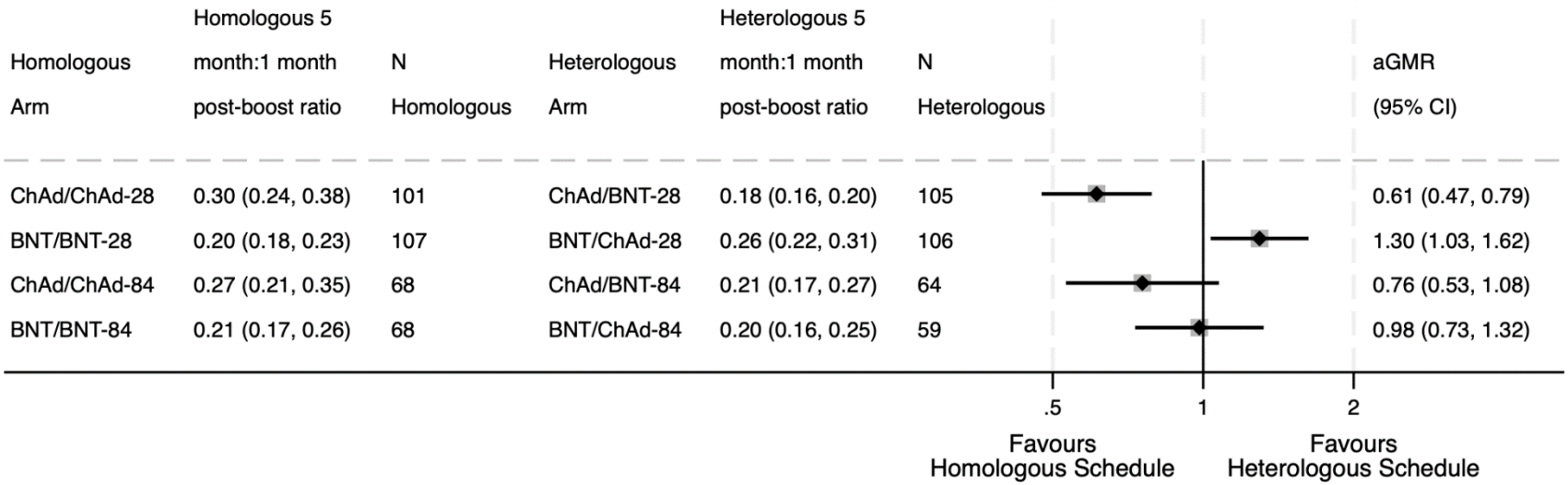

Figure 10 *– Com-COV1 Forest plot comparing rates of anti-SARS-CoV2 spike IgG wane between homologous and relative heterologous schedules. Left sided columns represent the raw, unadjusted GMC ± 95% CI of the ratio of the 5-month antibody level over the peak (1 month) antibody level. The forest plot represents the right sided aGMR derived from a multivariate linear regression*

3.4.2.1.1.1 Serostatus Subgroup Analysis

The number of baseline seropositive participants was small. Statistical analyses comparing immune responses by serostatus are therefore unlikely to be informative. Descriptive displays of responses are presented with circumspect interpretation.

Baseline seropositive participants produced a larger immune response than seronegative participants after the first vaccine dose. Responses to second doses in seropositive participants receiving 4-week schedules appeared not reliably to increase. Where they did increase, this change was small. In 12-week schedules, only ChAd/BNT-84 gave a clear response to the second dose. ChAd second doses in the seropositive population appeared to have very little boosting effect, but may have attenuated wane. Not all schedules had many or, in the case of ChAd/ChAd-84, any, participants to allow further comment (**Figure 11**).

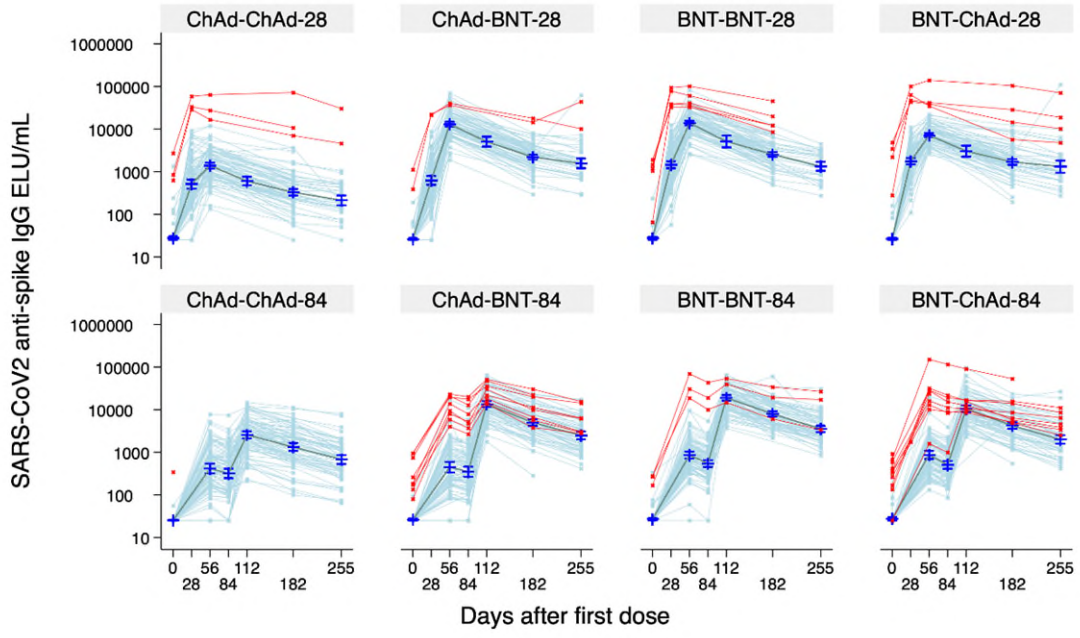

Figure 11 *– Com-COV1 Scatter plot showing the kinetics of anti-SARS-CoV2 spike IgG in seronegative (light blue) and seropositive individuals (red). The GMC ± 95% CI in the seronegative population are shown in dark blue/grey.*

3.4.2.1.2 Pseudotype-virus neutralisation (PNA)

PNA was performed 28-days post-second dose timepoints for the eight arms. As expected, there was hardly any measurable neutralisation activity at baseline and so baseline samples were analysed only for 4-week schedules. 4-week schedules were additionally assayed 28 days post first dose, whereas the 12 week schedules had an intermediate timepoint of 56 days post first dose assayed (**Figure 12**).

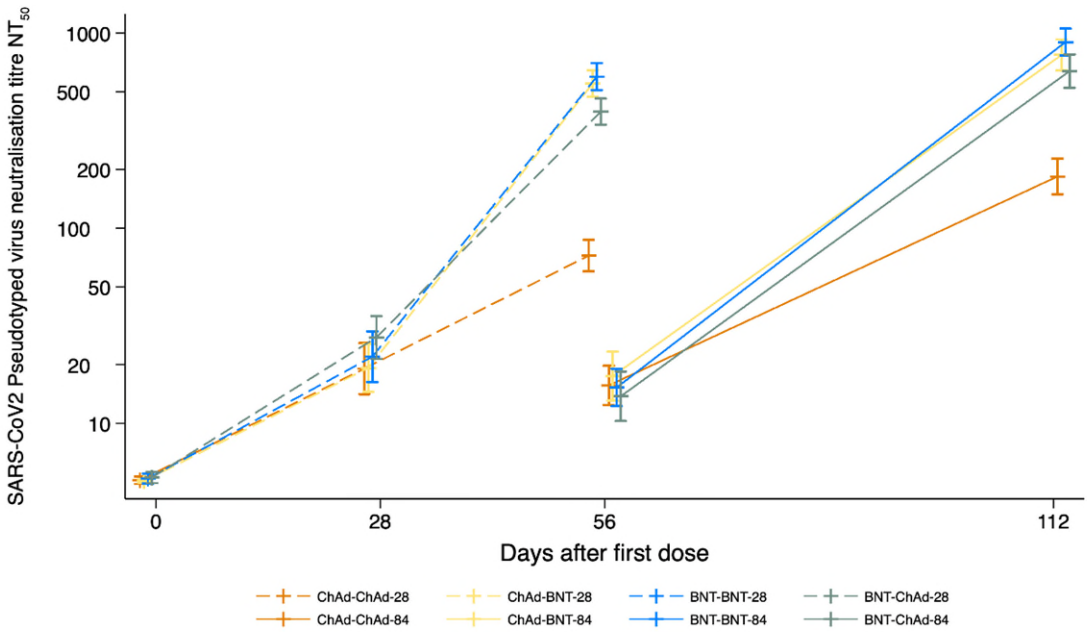

Figure 12 *– Com-COV1 Scatter plot showing the kinetics of the GMC ± 95% CI of pseudotype virus neutralisation for all eight Com-COV1 schedules for seronegative participants only. Dashed lines represent 4-week interval schedules, solid lines represent 12-week interval schedules. Orange represents ChAd/ChAd arms, yellow ChAd/BNT, blue BNT/BNT, and green BNT/ ChAd*

Overall, each homologous/heterologous comparison for PNA is qualitatively similar to its respective anti-SARS-CoV2 spike IgG comparison, although the differences are less in magnitude. The aGMR for ChAd/BNT-28 vs ChAd/ChAd-28 is 7.6 (95% CI: 6.0, 9.8), BNT/ChAd-28 vs BNT/BNT-28 is 0.7 (95% CI: 0.5, 0.8), ChAd/BNT-84 vs ChAd/ChAd-84 is 4.1 (95% CI: 3.1, 5.5) and BNT/ChAd-84 vs BNT/BNT-84 0.8 (95% CI: 0.6, 1.0) (**Figure 13**).

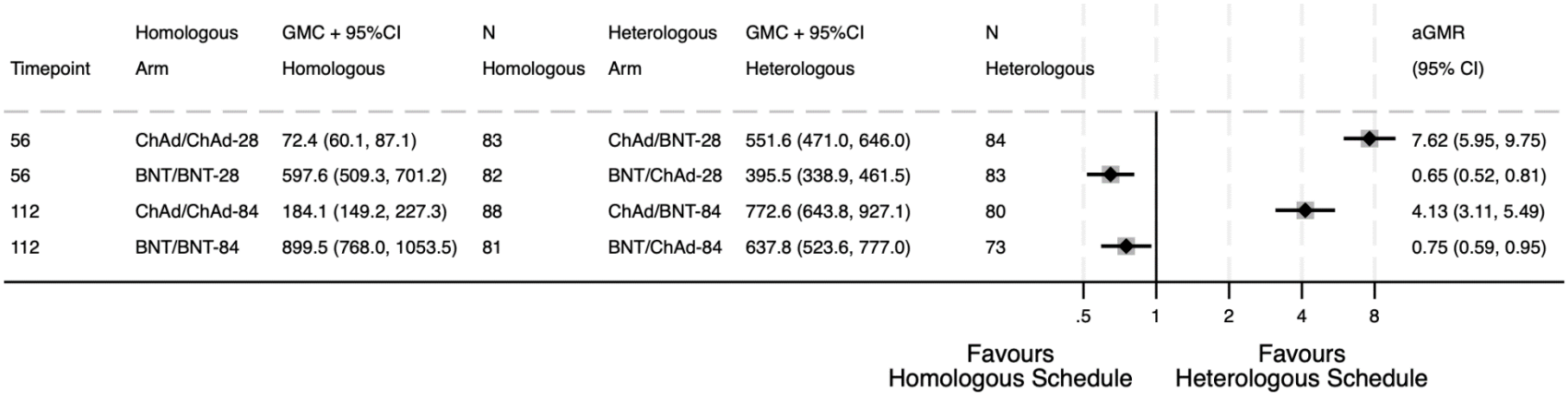

Figure 13 *– Com-COV1 Forest plot comparing peak (28 days post second dose) SARS-CoV2 pseudotype virus neutralisation responses between homologous and relative heterologous schedules. Left sided columns represent the raw, unadjusted GMC ± 95% CI. The forest plot represents the right sided aGMR derived from a multivariate linear regression*

The reduced quantitative differences between schedules for pseudo-typed virus neutralisation assays relative to binding antibody ratios suggests a qualitative difference in immune response between vaccine schedules. In order to investigate this further, the pseudo-typed virus neutralisation titre was divided by the anti-SARS-CoV2 spike binding IgG titre to produce a measure of neutralising antibodies in relation to the total antibody response.

A first dose of ChAd produces a greater proportion of neutralising antibodies than a first dose of BNT, but this difference is largely mitigated by a second dose of vaccine, regardless of type (**Figure 14**).

Schedules with ChAd as a second dose produced proportionally more neutralising antibody after the second dose than schedules with BNT as a second dose. All aGMRs were statistically significant except the ChAd/ChAd-28 vs ChAd/BNT-28 comparison. Despite these difference in the proportion of neutralising response, the absolute neutralising response is still greatest in BNT-boosted schedules (**Figure 15**).

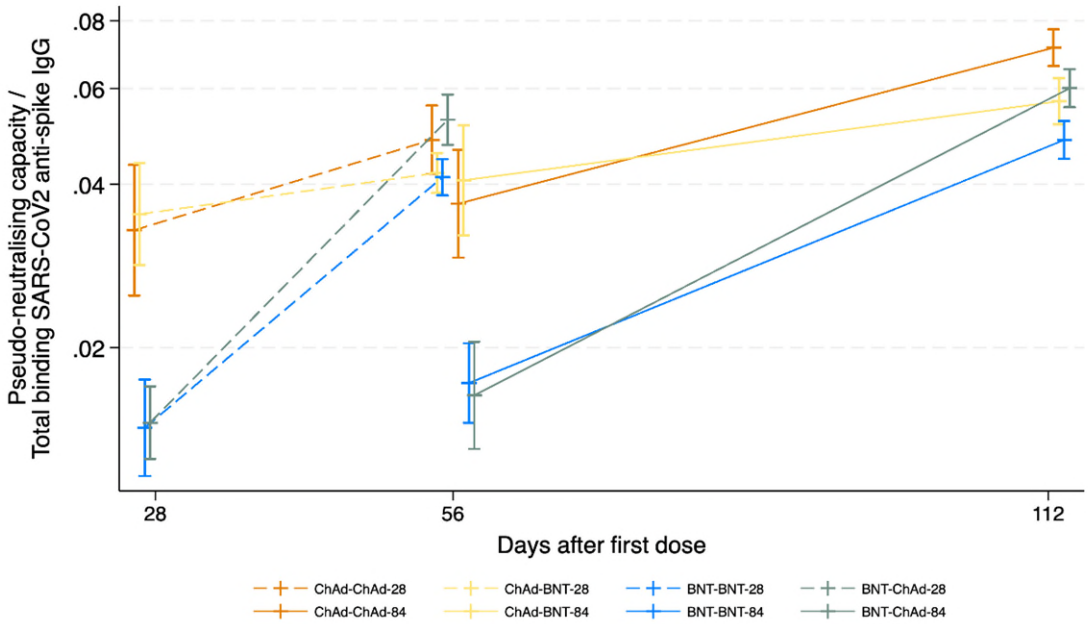

Figure 14 *– Com-COV1 Scatter plot showing the kinetics of the ratio between pseudotype virus neutralisation 50 titre and anti-SARS-CoV2 spike IgG for Com-COV1 schedules for seronegative participants. Dashed lines represent 4-week interval schedules, solid lines represent 12-week interval schedules. Orange: ChAd/ChAd, yellow ChAd/BNT, blue BNT/BNT, green BNT/ ChAd*

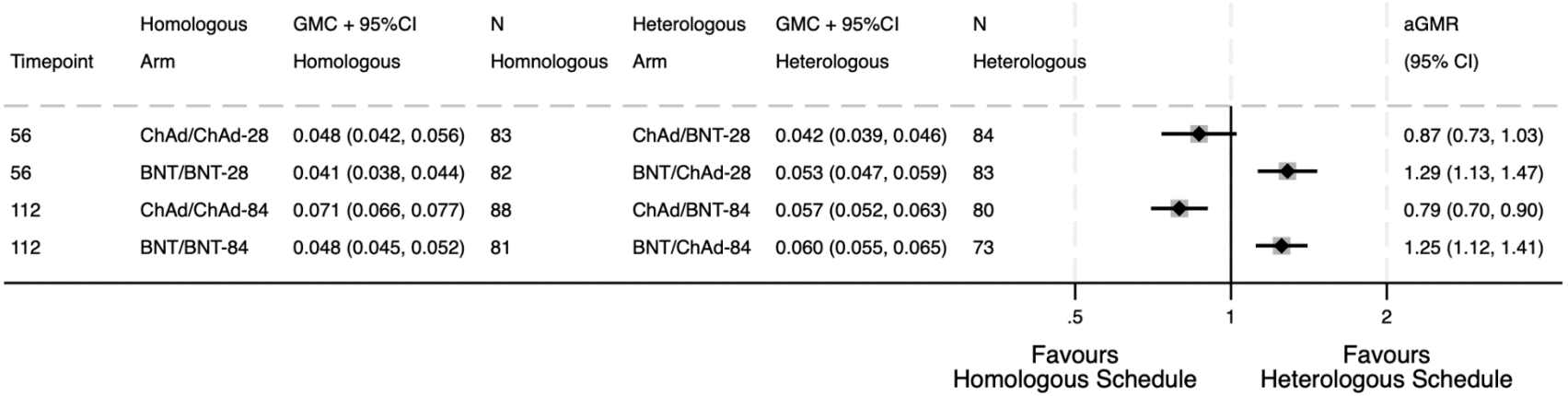

Figure 15 *– Com-COV1 Forest plot comparing the ratio between pseudotype virus neutralisation 50 titre and anti-SARS-CoV2 spike IgG at peak timepoint (28 days post second dose) between homologous and relative heterologous schedules. Left sided columns represent the raw, unadjusted GMC ± 95% CI. The forest plot represents the right sided aGMR derived from a multivariate linear regression.*

3.4.2.1.2.1 Serostatus Subgroup Analysis

Again, low numbers of seropositive participants prevent meaningful statistical comparisons against seronegative participants. However, within these constraints, including limited timepoints being assayed, as with the binding antibody ELISA, seropositive participants produced a larger PNA response after one vaccine dose and seem to reach their maximal response at this point, with relatively little boosting by the second dose. Second doses, largely seemed to mitigate most of the differences between seropositive and seronegative populations seen after one dose. There were no obvious differences between vaccine schedules (**Figure 16**).

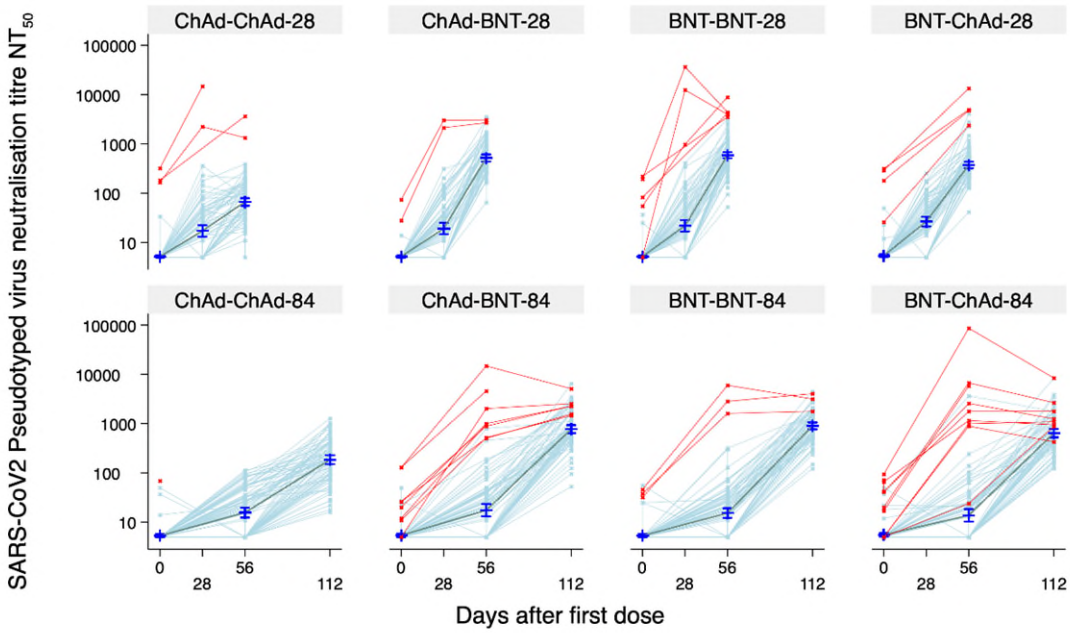

Figure 16 *– Com-COV1 Scatter plot showing the kinetics of the SARS-CoV2 pseudotype virus neutralisation 50 titre in seronegative (light blue) and seropositive individuals (red). The GMC ± 95% CI of seronegative participants are shown in dark blue/grey.*

After a second dose, the relative neutralising capability of each schedule increased for seronegative individuals, but not obviously for seropositive individuals (**Figure 17**).

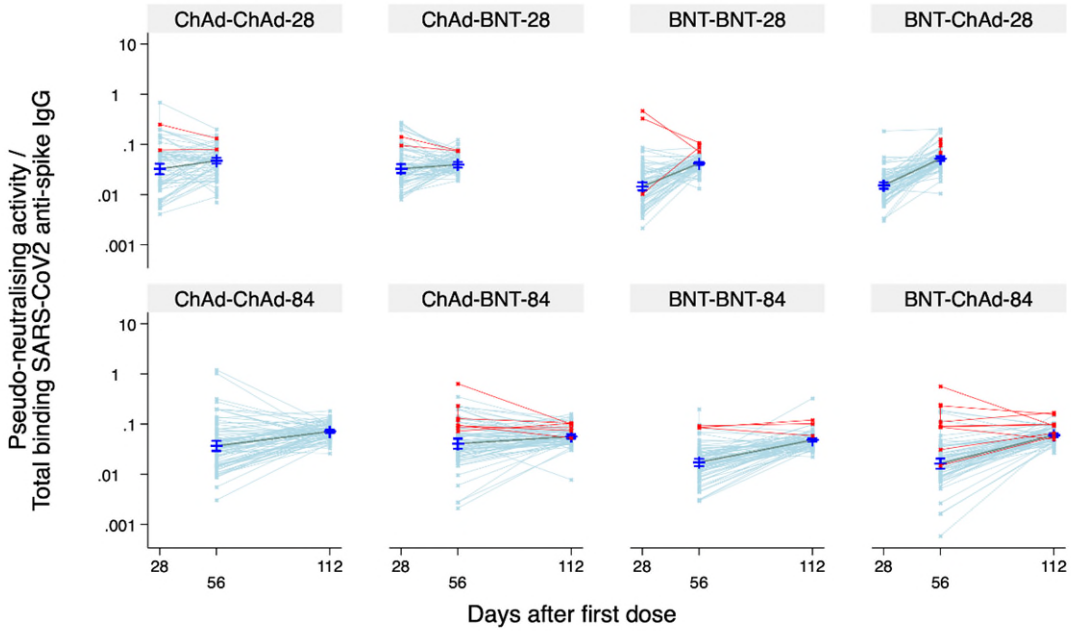

Figure 17 *– Com-COV1 Scatter plot showing kinetics of the ratio between pseudotype virus neutralisation 50 titre and anti-SARS-CoV2 spike IgG for all eight Com-COV1 schedules for seronegative (light blue) and seropositive individuals (red). The GMC ± 95% CI in seronegative participants are shown in dark blue/grey.*

3.4.2.1.3 Spike specific IFNγ secreting T cell response (IFNγ-SFC)

Seronegative participants receiving BNT/ChAd had similar frequencies of spike-specific IFNγ secreting T-cells (IFNγ-SFC) ELISpot to BNT/BNT regardless of interval, at both one and five months post second dose (**Figure 18**). Participants receiving ChAd/BNT had greater frequencies of IFNγ-SFC than ChAd/ChAd with aGMRs of 3.8 (95% CI: 2.9, 5.0) and 3.2 (95% CI: 2.3, 4.4) for both the 4-week and 12-week schedules respectively at one month post second dose. The aGMRs reduced to 2.9 (95% CI: 2.2, 3.8) and 2.9 (95% CI: 2.1, 4.1) for the 4-week and 12-week schedules respectively at five months post-second dose (**Figure 19**).

The five-month to one-month ratios of IFNγ-SFC frequencies, as markers of immunological waning, between homologous/heterologous 12-week schedule comparisons and the BNT/BNT-28 vs BNT/ChAd-28 comparison showed no statistically

significant differences. Only the ChAd/BNT-28 vs ChAd/ChAd-28 comparison demonstrated a difference in this measure indicating a difference in rate of wane, with ChAd/ChAd-28 waning more slowly than the ChAd/BNT-28 (**Figure 20**).

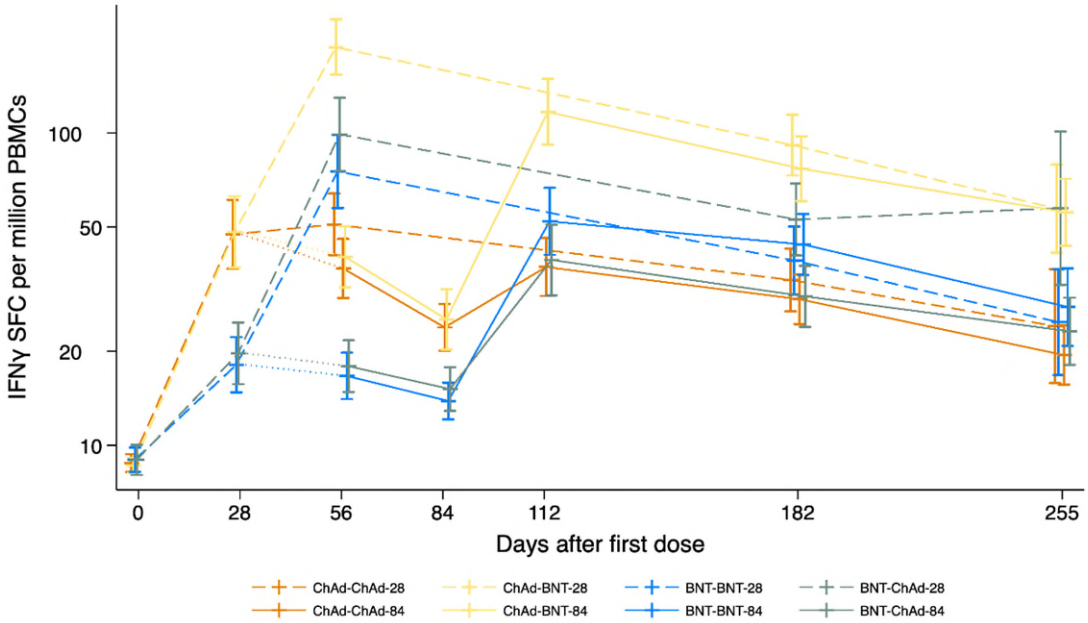

Figure 18 *– Com-COV1 Scatter plot showing kinetics of the GMC ± 95% CI of IFNγ -SFC for all eight Com-COV1 schedules for seronegative participants only. Dashed lines represent 4-week interval schedules, solid lines represent 12-week interval schedules, dotted lines represent an interpolated line for the 12-week interval schedules, as no sample at Day 28 was taken. Orange represents ChAd/ChAd arms, yellow ChAd/BNT, blue BNT/BNT, and green BNT/ ChAd*

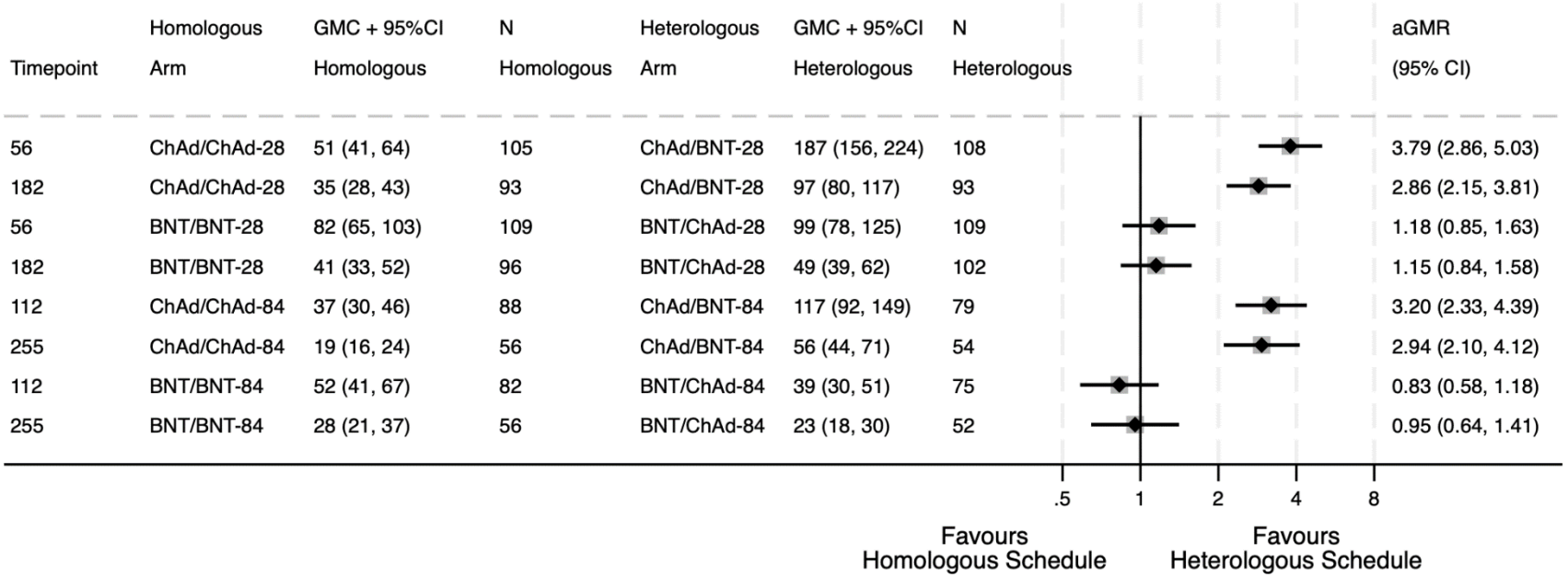

Figure 19 *– Com-COV1 Forest plot comparing peak (28 days post second dose) and 5-months post second dose IFNγ-SFC between homologous and relative heterologous schedules. Peak response for the 4 week schedules was at 56 days, whilst for 12-week schedules this was at 112 days. The 5-month timepoint was at day 182 for 4-week schedules, and at day 255 for the 12-week schedules. Left sided columns represent the raw, unadjusted GMC $\pm$ 95% CI. The forest plot represents the right sided aGMR derived from a multivariate linear regression.*

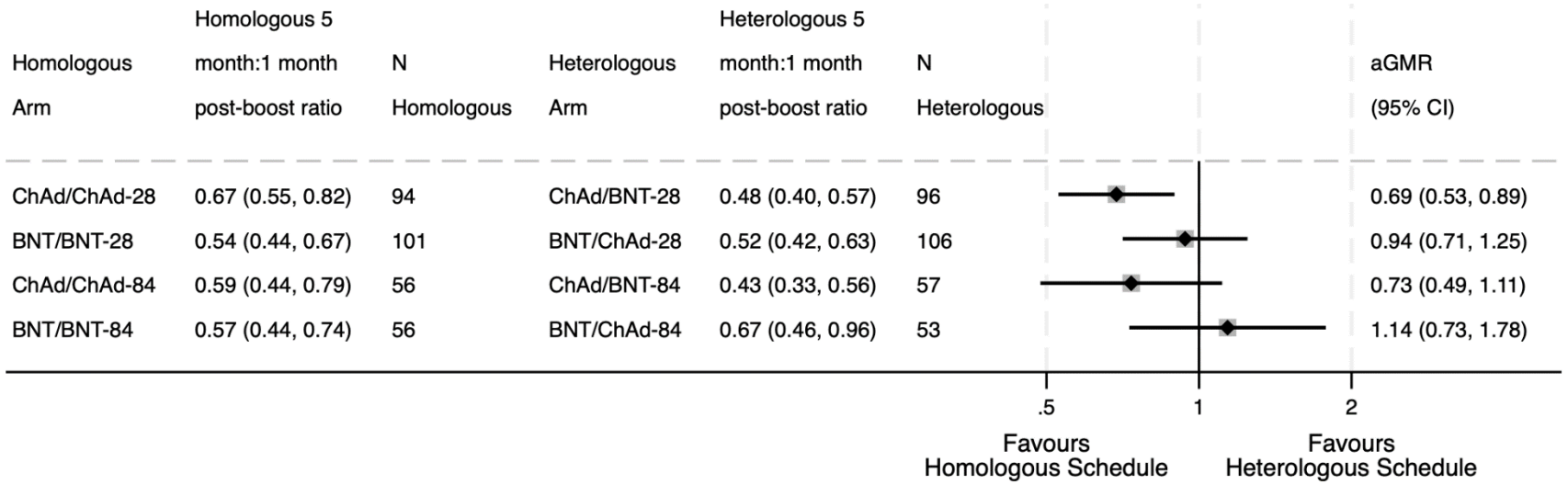

Figure 20 *– Com-COV1 Forest plot comparing rates of IFNγ -SFC wane between homologous and heterologous schedules. Left sided columns represent the raw, unadjusted GMC $\pm$ 95% CI of the ratio of the 5-month antibody level over the peak T-cell response. The forest plot represents the right sided aGMR derived from a multivariate linear regression*

3.4.2.1.3.1 Serostatus Subgroup Analysis

Seropositive cases in general responded more to the first dose of vaccination than seronegative cases did, however, there was a higher degree of variability in this response than there was seen for seropositive anti-SARS-CoV2 spike IgG responses. Second doses of vaccine only partially mitigated the difference between seropositive and seronegative IFNγ-SFC responses (**Figure 21**).

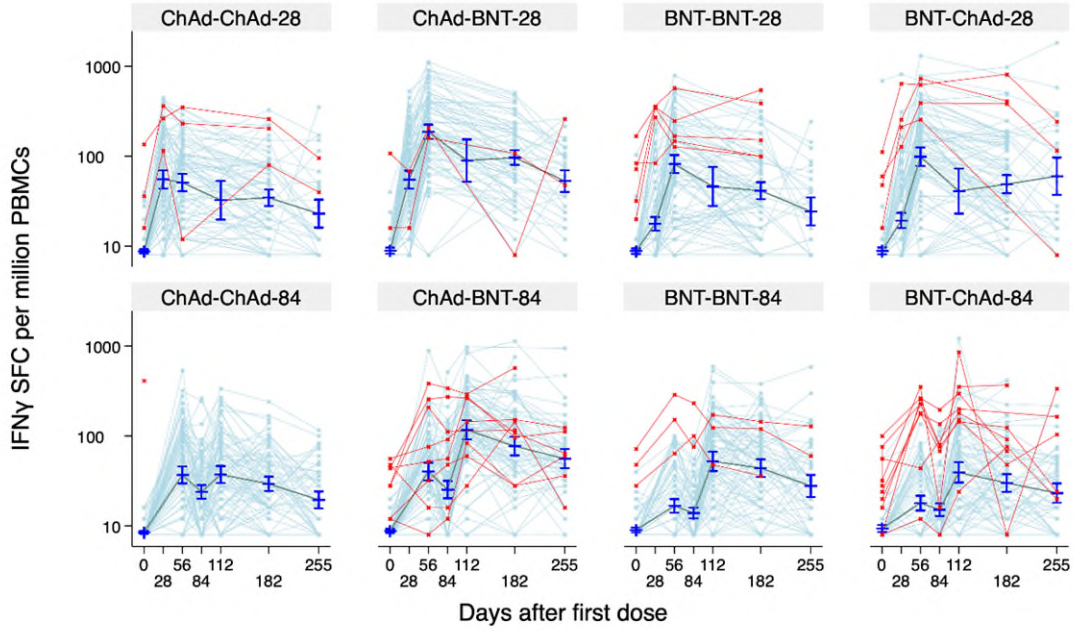

Figure 21 *– Com-COV1 Scatter plot showing the kinetics of IFNγ -SFC in seronegative (light blue) and seropositive individuals (red). The GMC ± 95% CI of seronegative participants are shown in dark blue/grey.*

3.4.2.2 Com-COV2

3.4.2.2.1 Binding Anti-SARS-CoV2 spike IgG

In the per protocol analysis, seronegative participants receiving BNT/NVX, on average, produced 0.6 (97.5% CI: 0.5, 0.7) times as much serum binding anti-SARS-CoV2 spike IgG as those receiving BNT/BNT, failing to meet the non-inferiority criterion. However, those receiving BNT/Mod produced 1.3 (97.5% CI: 1.1, 1.6) times as much antibody as BNT/BNT, meeting the non-inferiority criterion. Both heterologous ChAd-primed schedules met the non-inferiority criterion, when compared against homologous ChAd/ChAd: ChAd/Mod 9.9 (97.5% CI: 8.1, 12.0) and ChAd/NVX 2.9 (97.5% CI: 2.4, 3.6).

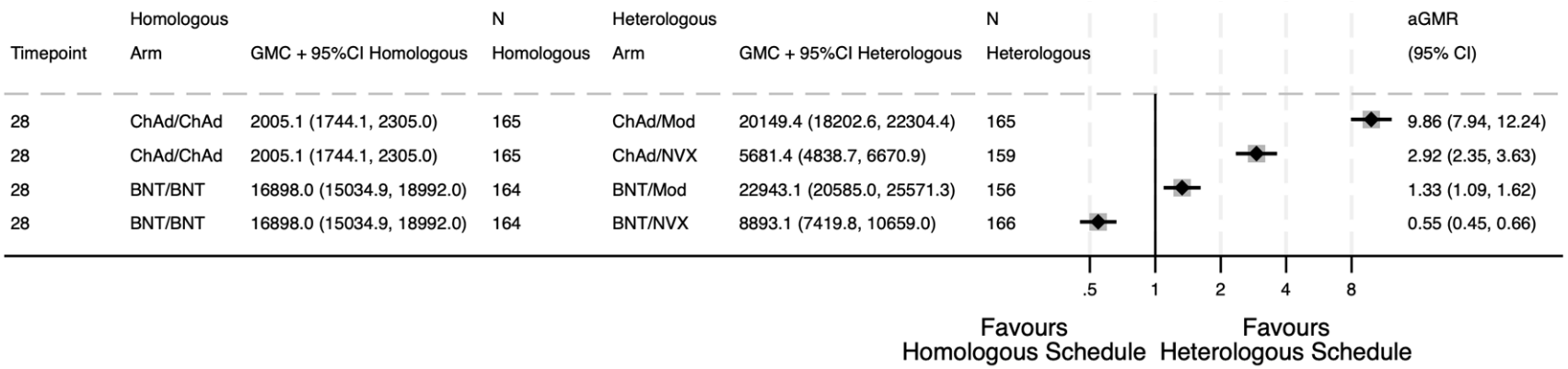

Figure 22 *– Com-COV2 Per protocol analysis. Forest plot comparing peak (28 days post second dose) anti-SARS-CoV2 spike IgG between homologous and relative heterologous schedules.. Left sided columns represent the raw, unadjusted GMC ± 97.5% CI. The forest plot represents the right sided aGMR derived from a multivariate linear regression*

In the mITT population (**Figure 23**), seronegative participants receiving homologous BNT/NVX, on average, produced 0.6 (95% CI: 0.5, 0.6) times as much serum binding anti-SARS-CoV2 spike IgG, 28 days post-second dose, as those receiving BNT/BNT, whereas those receiving BNT/Mod produced 1.3 (95% CI: 1.2, 1.6) times as much as homologous BNT/BNT. Those receiving heterologous ChAd/Mod and ChAd/NVX produced 10.0 (95% CI: 8.4, 11.9) and 2.9 (95% CI: 2.4, 3.3) times as much antibody as homologous ChAd/ChAd. These results were very similar to the per-protocol analysis.

At 5.5 months post-second dose, the differences for BNT-primed schedules had changed very little: 0.6 (95% CI: 0.5, 0.6) to 0.6 (95% CI: 0.5, 0.8) times for BNT/NVX in comparison to BNT/BNT and 1.3 (95% CI: 1.2, 1.6) to 1.6 (95% CI: 1.2, 2.0) for BNT/Mod in comparison to BNT/BNT. There was, similarly, little change for ChAd/NVX in comparison to ChAd/ChAd 2.9 (95% CI: 2.4, 3.3) to 2.3 (95% CI: 1.8, 2.9). There was, however, a greater drop in the difference over time between ChAd/Mod and ChAd/ChAd: 10.0 (95% CI: 8.4, 11.9) to 6.2 (95% CI: 5.0, 7.8) (**Figure 24**).

ChAd/Mod and ChAd/NVX waned more quickly than ChAd/ChAd with 5.5 month/1 month fold changes of 0.18 (95% CI: 0.15, 0.21), 0.24 (95% CI: 0.19, 0.31) and 0.33 (95% CI: 0.26, 0.43) respectively. There were no differences in rates of wane between BNT-primed homologous and heterologous schedules (**Figure 25**).

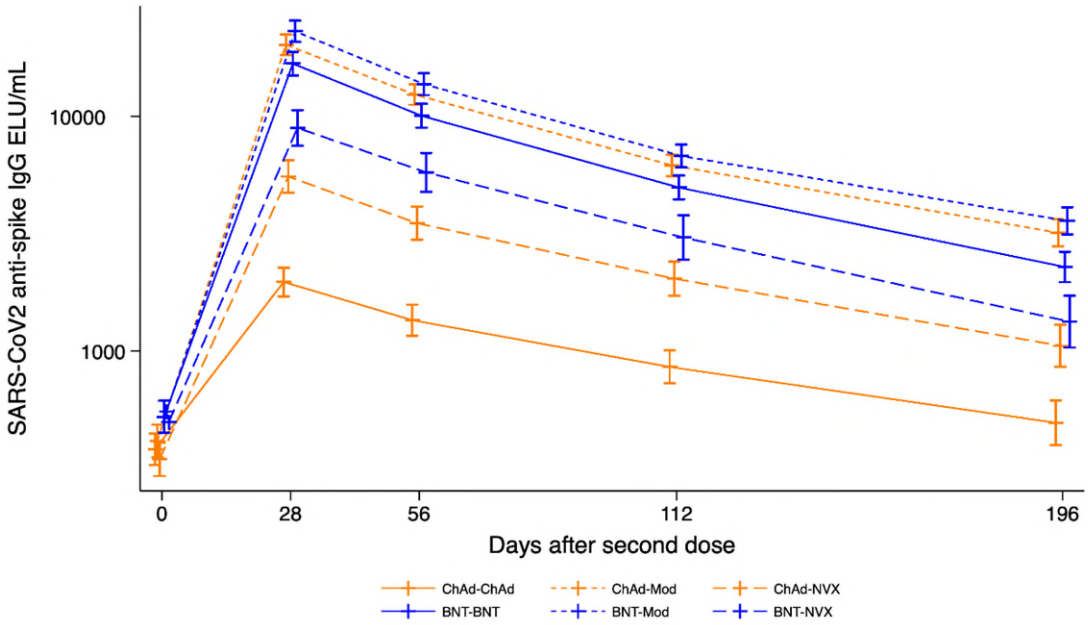

Figure 23 *– Com-COV2 Scatter plot showing the kinetics of anti-SARS-CoV2 spike IgG GMC ± 95% CI for all six Com-COV2 schedules for seronegative participants only. Solid lines represent homologous schedules, short-dashed line represent Mod boosted heterologous schedules and long-dashed lines represent NVX boosted heterologous schedules. Orange represents ChAd primed arms, blue represents BNT primed arms*

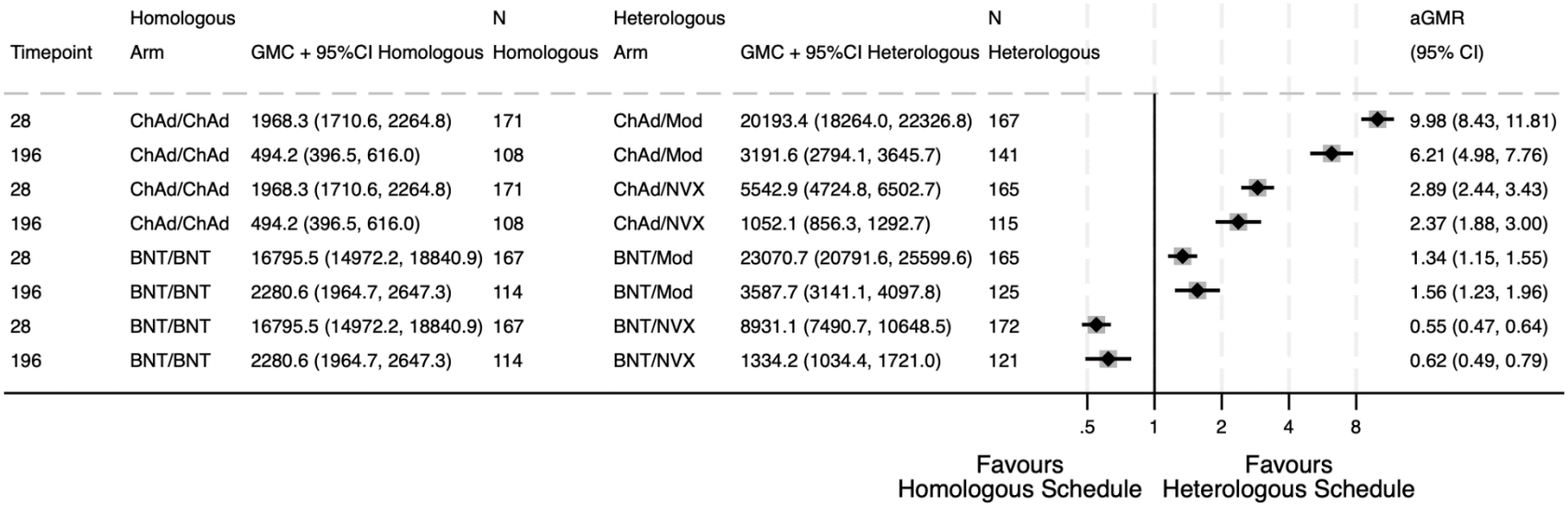

Figure 24 *– Com-COV2 mITT analysis. Forest plot comparing peak (28 days post second dose) and 5.5-months post second dose anti-SARS-CoV2 spike IgG between homologous and heterologous schedules. Left sided columns represent the raw, unadjusted GMC ± 95% CI. The forest plot represents the right sided aGMR derived from a multivariate linear regression*

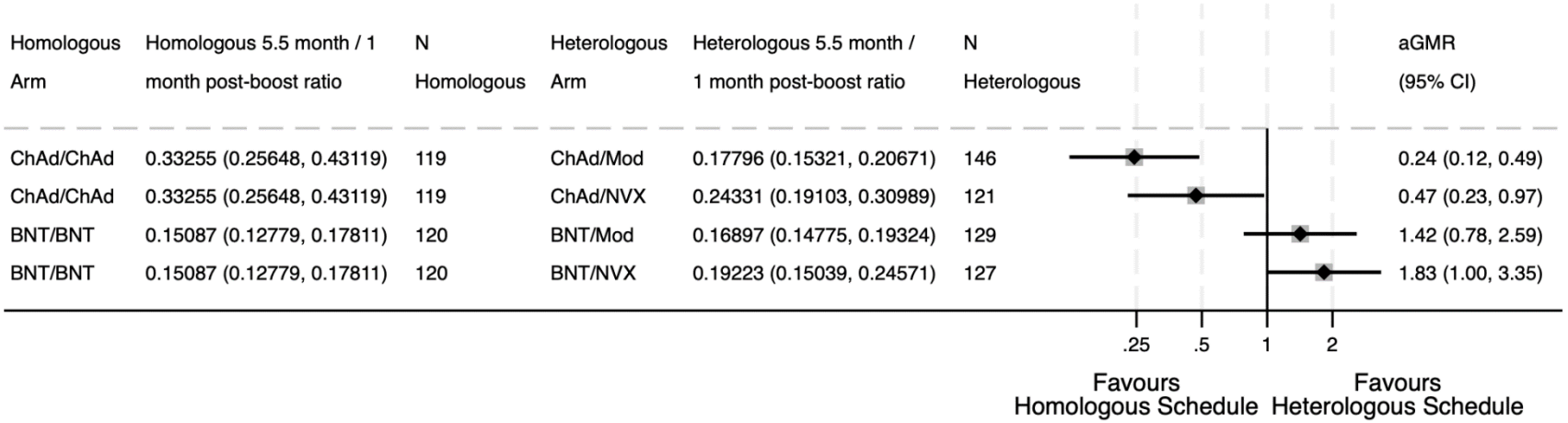

Figure 25 *– Com-COV2 Forest plot comparing rates of anti-SARS-CoV2 spike IgG wane between homologous and relative heterologous schedules. Left sided columns represent the raw, unadjusted GMC ± 95% CI of the ratio of the 5.5-month antibody level over the peak (1 month) antibody level. The forest plot represents the right sided aGMR derived from a multivariate linear regression*

3.4.2.2.1.1 Serostatus Subgroup Analysis

Statistical testing comparing the immune responses according to serostatus is not performed. Instead, descriptive displays of these responses are presented here. Seropositive participants produced larger serum anti-SARS-CoV2 spike IgG responses than seronegative participants at the maximal time point (28 days post-second dose), with some variability between schedules. However, on comparing these post-second dose levels to the pre-second dose baseline, the second dose did not reliably give an increase in antibody response, and where it did – only in mRNA boosted schedules – this increase appeared small (**Figure 26**). However, it again, is plausible that the second dose did lead to an attenuation of wane, although this effect is not discernible from these data.

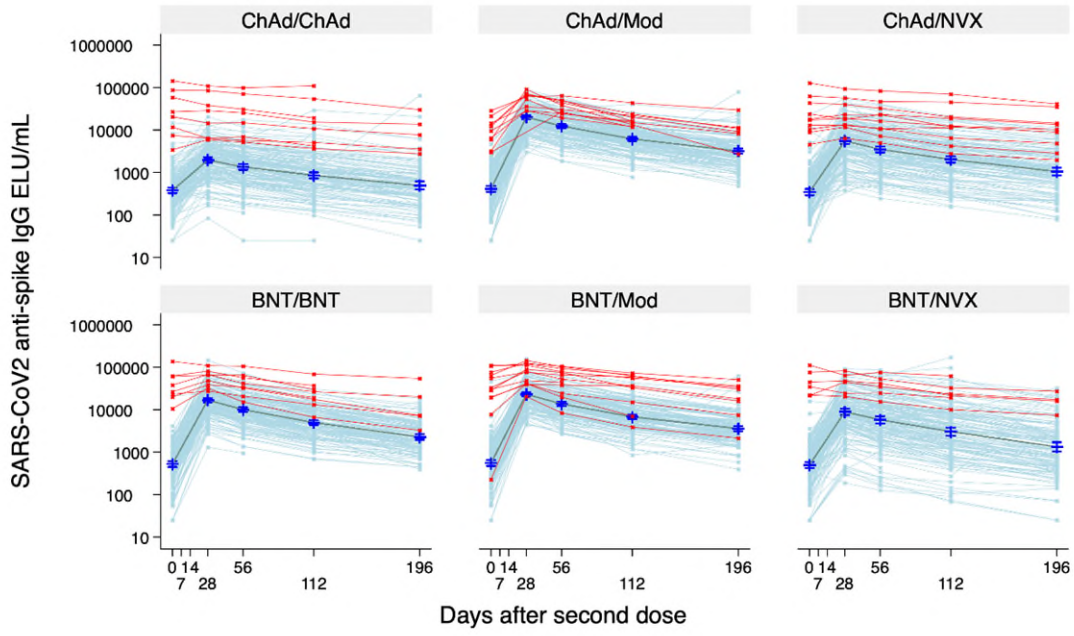

Figure 26 *– Com-COV2 Scatter plot showing the kinetics of anti-SARS-CoV2 spike IgG of seronegative (light blue) and seropositive individuals (red). The GMC ± 95% CI for the seronegative population are shown in dark blue/grey.*

3.4.2.2.2 Pseudotype-virus neutralisation titre (PNA)

PNA were performed only on pre-second dose and maximal (28 days post second dose) timepoints (**Figure 27**). All schedules had a similar increase in titre, with the exception of ChAd/ChAd which had a lower response.

The high ChAd/Mod and ChAd/NVX responses in comparison to ChAd/ChAd are reflected in the larger aGMRs between homologous/heterologous combinations. BNT-primed schedules had very similar titres with BNT/Mod and BNT/NVX achieving 1.4 (95% CI: 1.1, 1.7) and 0.9 (95% CI: 0.8, 1.1) of the BNT/BNT schedule; whereas ChAd-primed schedules revealed aGMRs of 10.0 (95% CI: 8.2, 12.2) and 3.7 (95% CI: 3.1, 4.6) for ChAd/Mod and ChAd/NVX respectively (**Figure 28**).

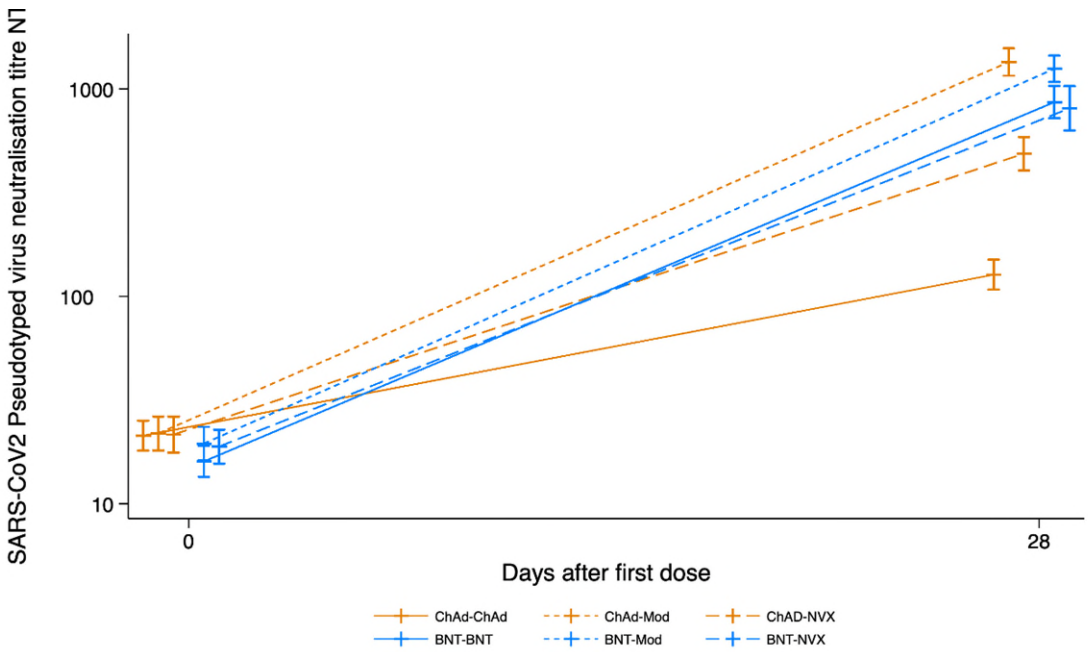

Figure 27 *– Com-COV2 Scatter plot showing the kinetics of the GMC ± 95% CI of pseudotype virus neutralisation titre 50 for all six Com-COV2 schedules for seronegative participants only. Solid lines represent homologous schedules, short-dashed line represent Mod boosted heterologous schedules and long-dashed lines represent NVX boosted heterologous schedules. Orange represents ChAd primed arms, blue represents BNT primed arms*

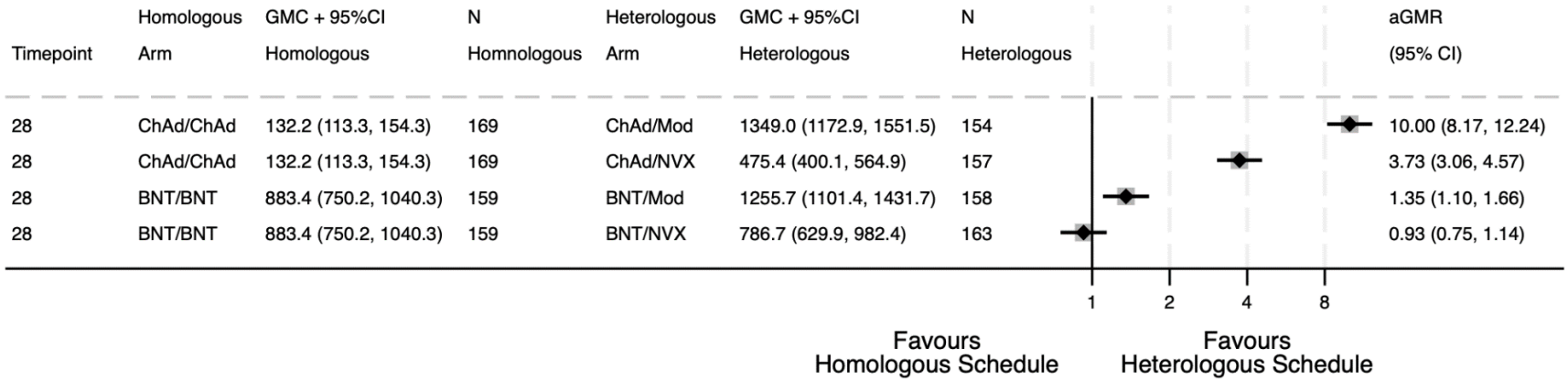

Figure 28 *– Com-COV2 Forest plot comparing pseudotype virus neutralisation 50 titre at peak timepoint (28 days post second dose) between homologous and heterologous schedules. Left sided columns represent the raw, unadjusted GMC ± 95% CI. The forest plot represents the right sided aGMR derived from a multivariate linear regression.*

All schedules had an increase in the proportion of neutralising response with Novavax boosted regimens ending up with the highest proportion of neutralising activity (**Figure 29** & **Figure 30**).

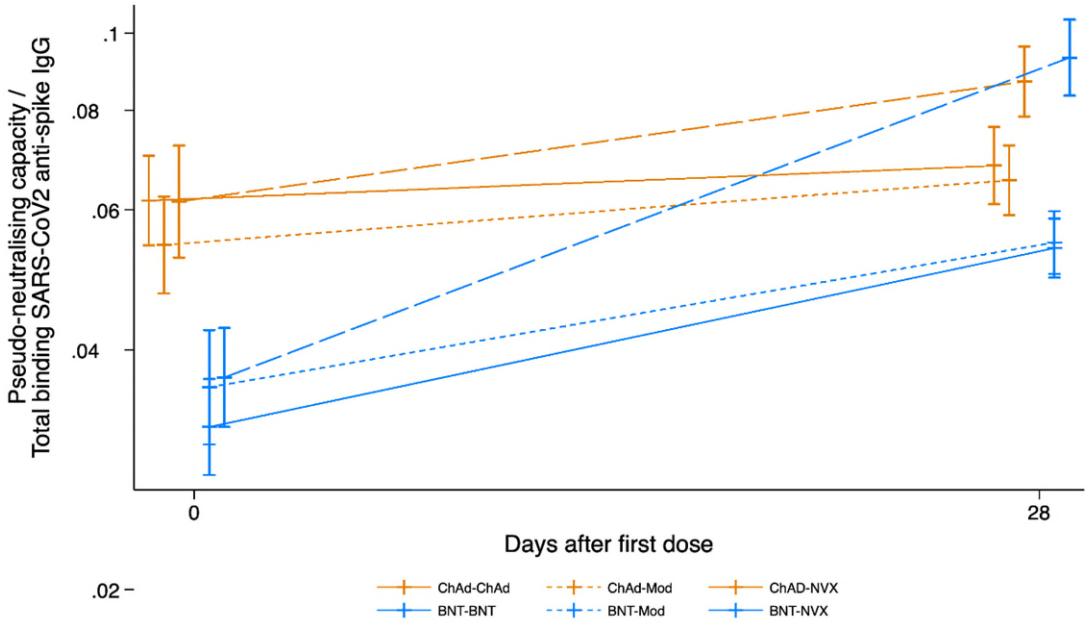

Figure 29 *– Com-COV2 Scatter plot showing the kinetics of the ratio between pseudotype virus neutralisation 50 titre and anti-SARS-CoV2 spike IgG for all six Com-COV2 schedules for seronegative participants only. Solid lines represent homologous schedules, short-dashed line represent Mod boosted heterologous schedules and long-dashed lines represent NVX boosted heterologous schedules. Orange represents ChAd primed arms, blue represents BNT primed arms*

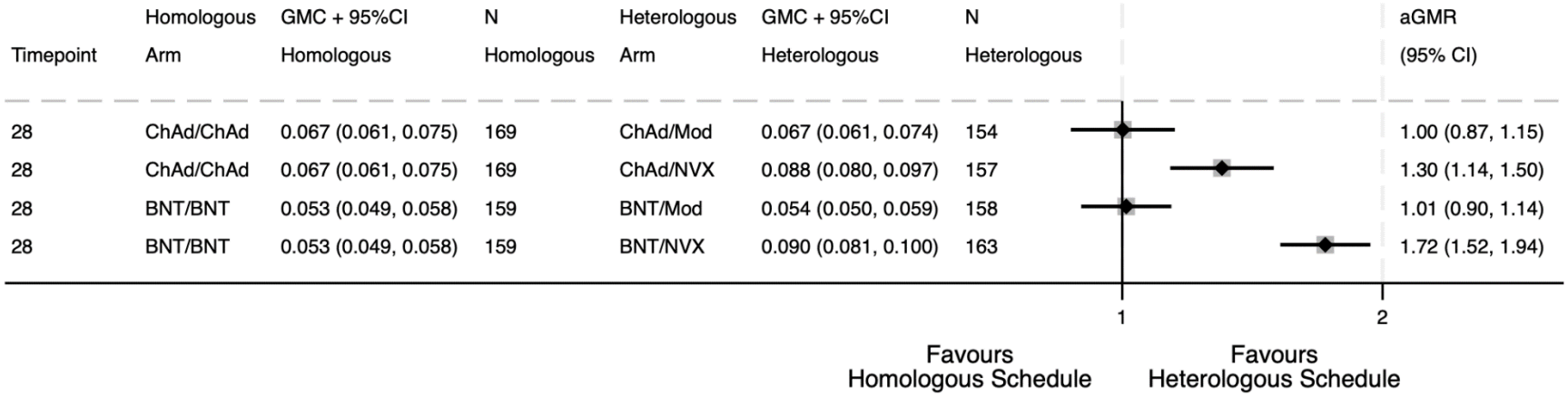

Figure 30 *– Com-COV2 Forest plot comparing the ratio between pseudotype virus neutralisation 50 titre and anti-SARS-CoV2 spike binding IgG at peak timepoint (28 days post second dose) between homologous and relative heterologous schedules. Left sided columns represent the raw, unadjusted GMC ± 95% CI. The forest plot represents the right sided aGMR derived from a multivariate linear regression*

3.4.2.2.2.1 Serostatus Subgroup Analysis

Seropositive participants had a larger neutralisation titre than seronegative participants prior to the second dose regardless of first dose vaccine, but the second dose appeared not to reliably increase the titre, although it may well have attenuated wane. The exception to this was with schedules which had Mod as a second dose, whose effect appeared relatively small (**Figure 31**).

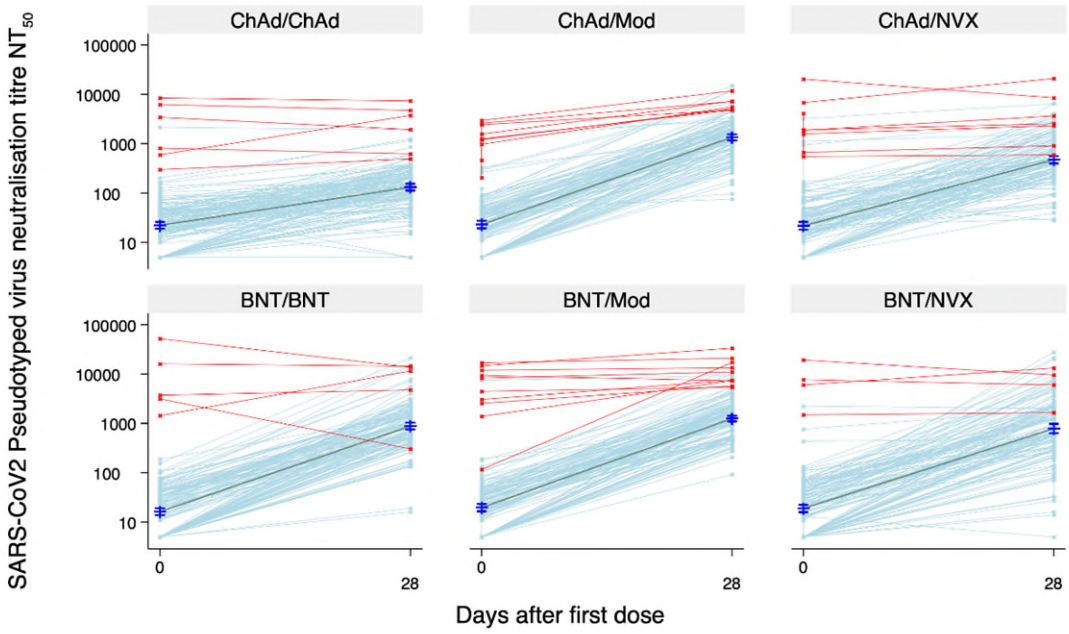

Figure 31 *– Com-COV2 Scatter plot showing the kinetics of pseudotype virus neutralisation titre 50 in seronegative (light blue) and seropositive individuals (red). The GMC ± 95% CI of the seronegative population are shown in dark blue/grey.*

The proportion of neutralising response was slightly higher for seropositive participants than for seronegative participants, but this proportion was not affected by the second dose of vaccination (**Figure 32**).

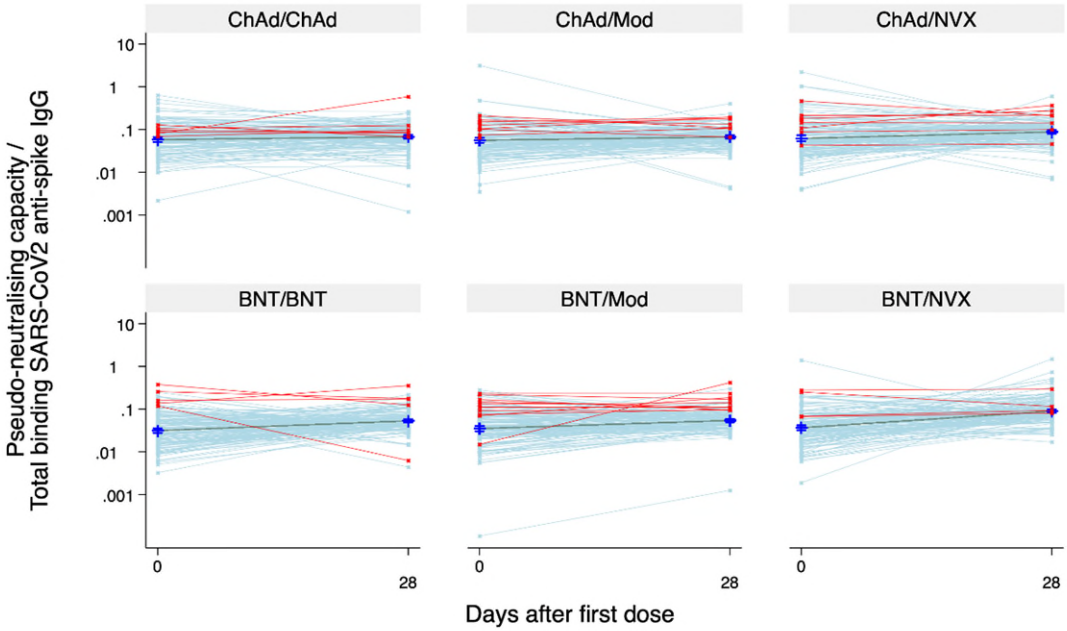

Figure 32 *– Com-COV2 Scatter plot showing the kinetics of the ratio between pseudotype virus neutralisation 50 titre and anti-SARS-CoV2 spike IgG for all six Com-COV2 schedules for seronegative (light blue) and seropositive individuals (red). The GMC ± 95% CI for seronegative participants are shown in dark blue/grey.*

3.4.2.2.3 Spike-specific IFNγ secreting T cell response (IFNγ-SFC)

Seronegative participants receiving BNT/NVX had a slightly lower frequency of IFNγ-SFC than those receiving BNT/BNT: aGMR 0.6 (95% CI: 0.4, 0.9), although this difference was lost by 5.5 months post second dose (**Figure 33**). Participants receiving BNT/Mod had a higher response than those receiving BNT/BNT: aGMR 1.6 (95% CI: 1.1, 2.4), which was maintained 5.5 months after the second dose aGMR 1.7 (95% CI: 1.1, 2.6).

The highest frequencies of IFNγ-SFC were in the heterologous ChAd-primed participants (ChAd/Mod and ChAd/NVX), which had aGMRs of 3.2 (95% CI: 2.3, 4.4) and 4.2 (95% CI: 3.0, 5.8) respectively when compared to ChAd/ChAd. The difference in response remained, but waned to aGMRs of 2.3 (95% CI: 1.5, 3.4) and 2.0 (95% CI: 1.3, 3.1) respectively 5.5 months after the second dose (**Figure 34**).

The 5.5-month to one-month ratio of IFNγ-SFC frequencies, as a marker of wane, was not statistically different for BNT/BNT and BNT/Mod. However, the more immunogenic heterologous ChAd-primed participants waned more quickly than ChAd/ChAd and the more immunogenic BNT/BNT waned more quickly than BNT/NVX (**Figure 35**).

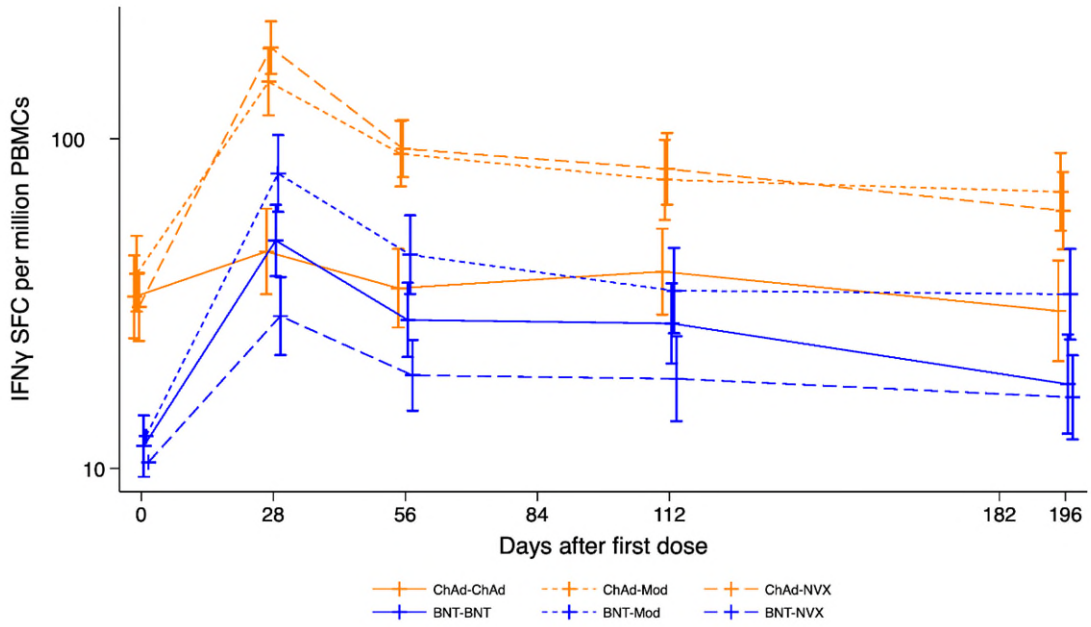

Figure 33 *– Com-COV2 Scatter plot showing the kinetics of the GMC ± 95% CI of IFNγ-SFC for all six Com-COV2 schedules for seronegative participants only. Solid lines represent homologous schedules, short-dashed line represent Mod boosted heterologous schedules and long-dashed lines represent NVX boosted heterologous schedules. Orange represents ChAd primed arms, blue represents BNT primed arms*

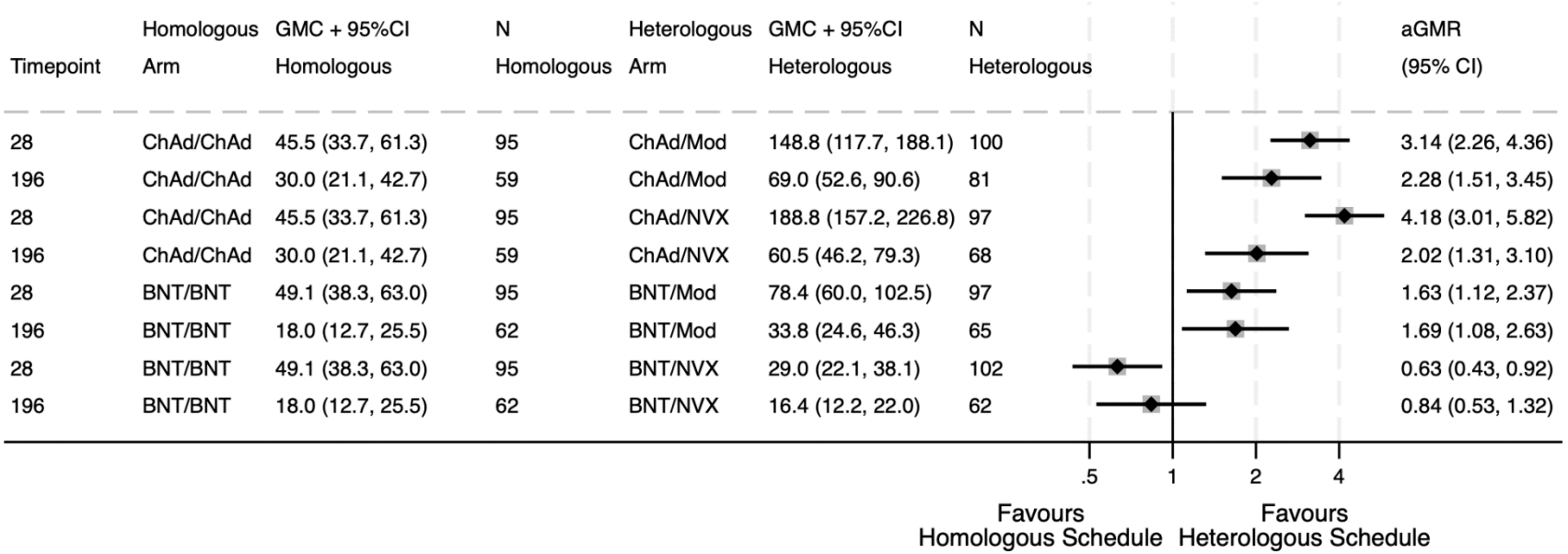

Figure 34 *– Com-COV2 Forest plot comparing peak (28 days post second dose) and 5.5-months post second dose IFNγ-SFC between homologous and heterologous schedules. Left sided columns represent the raw, unadjusted GMC ± 95% CI. The forest plot represents the right sided aGMR derived from a multivariate linear regression.*

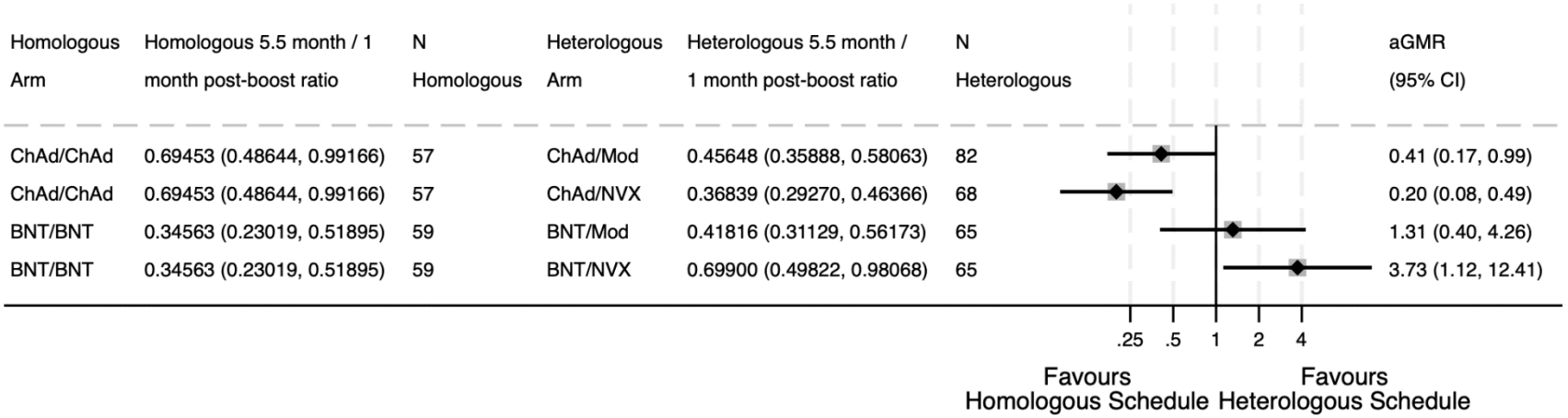

Figure 35 *– Com-COV2 Forest plot comparing rates of IFNγ-SFC wane between homologous and heterologous schedules. Left sided columns represent the raw, unadjusted GMC ± 95% CI of the ratio of the 5.5-month antibody level over the peak response. The forest plot represents the right sided aGMR derived from a multivariate linear regression*

3.4.2.2.3.1 Serostatus Subgroup Analysis

Seropositive participants had a higher T cell response than seronegative participants, but second doses of vaccine had little to no effect on the absolute level (**Figure 36**).

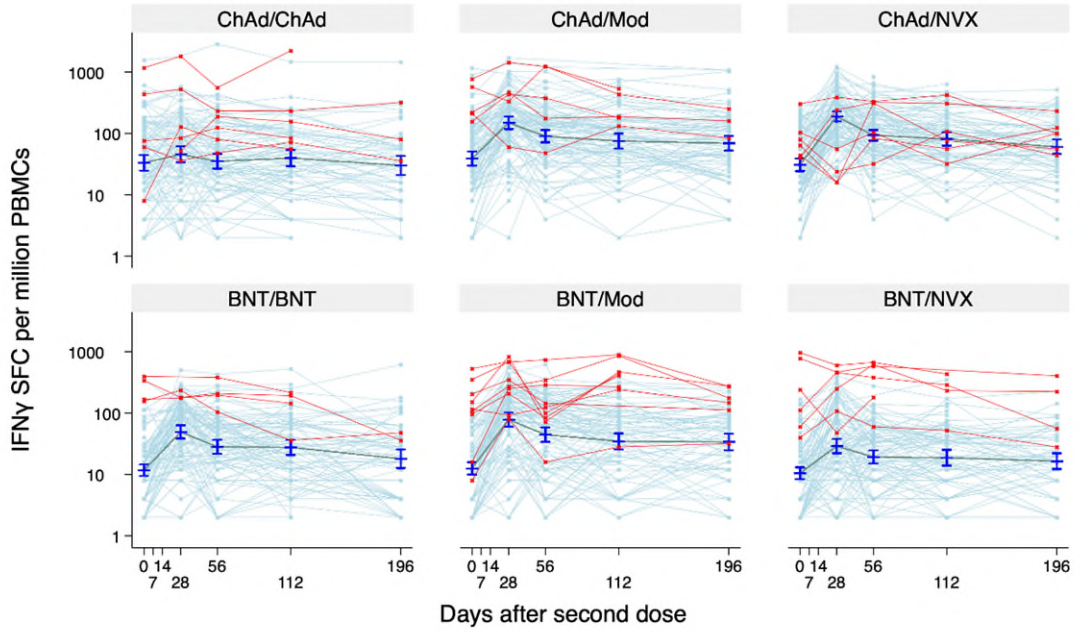

Figure 36 *– Com-COV2 Scatter plot showing the kinetics of IFNγ-SFC in seronegative (light blue) and seropositive individuals (red). The GMC ± 95% CI for seronegative participants are shown in dark blue/grey.*

3.4.3 Reactogenicity

3.4.3.1 Com-COV1

3.4.3.1.1 2nd dose: Homologous vs Heterologous

Overall, heterologous schedules more frequently caused systemic reactogenicity than homologous schedules regardless of interval (**Figure 37** & **Figure 38**). For those participants who did suffer systemic symptoms of any grade, the only comparison which suggested an increased likelihood for those symptoms to be moderate-severe (instead of mild) than the homologous schedule was BNT/ChAd-84 vs BNT/BNT-84 (**Figure 39**). In those who did suffer moderate-severe symptoms (Grade 2 or above), the symptoms lasted a little longer on average in BNT/ChAd-84 (vs BNT/BNT-84) and ChAd/BNT-28 (vs ChAd/ChAd-28) (**Figure 40**). **Supplementary Figure 1**, **Supplementary Figure 2** & **Supplementary Figure 3** show similar plots for local symptoms with no clear patterns emerging.

There were insufficient seropositive participants formally to assess the effects of serostatus on second dose reactogenicity.

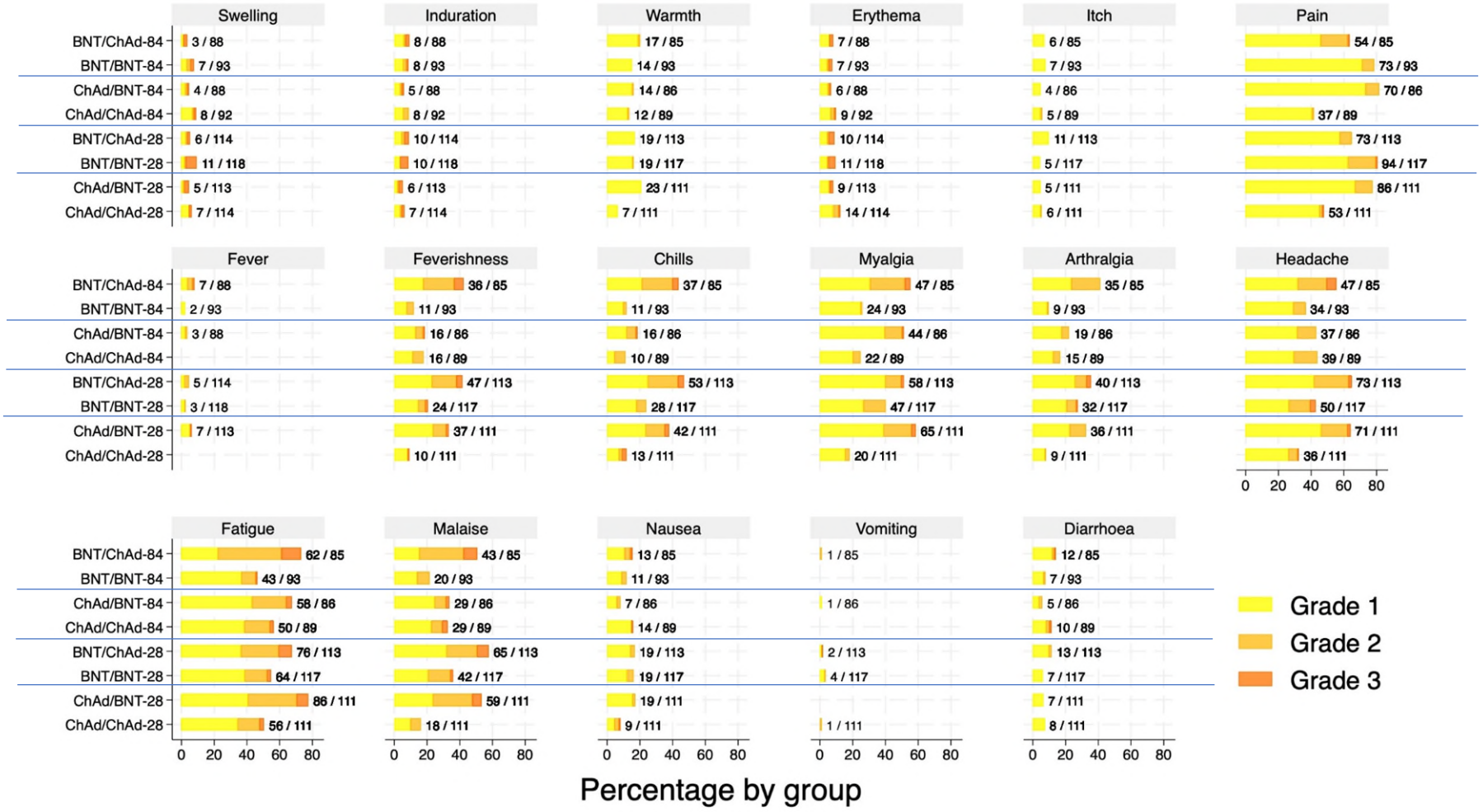

Figure 37 *– Com-COV1 Stacked bar chart showing total number of participants suffering each local & systemic symptom after the second dose for all schedules. The severity presented is each participant's highest severity across 7 days after vaccination. Seronegative and seropositive participants are grouped together*

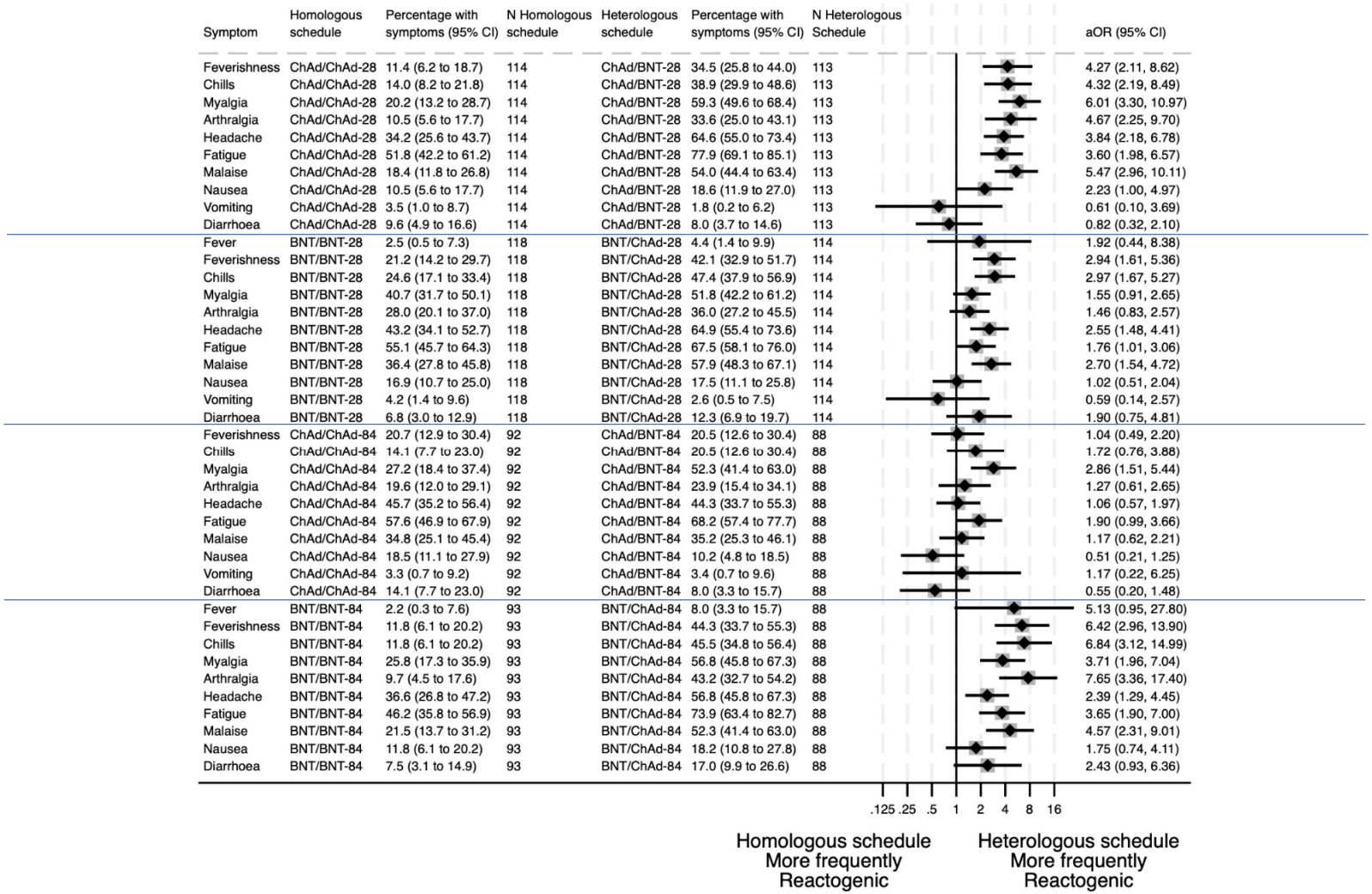

Figure 38 *– Com-COV1 Forest plot comparing proportions of participants (seronegative & seropositive combined) suffering systemic symptoms within 7 days of their second vaccination between homologous & heterologous schedules "Were you more likely to suffer a symptom of any grade after the second dose if the schedule was homologous or heterologous?"*

Symptom	Homologous schedule	Percentage with symptoms (95% CI)	N Homologous schedule	Heterologous schedule	Percentage with symptoms (95% CI)	N Heterologous Schedule	aOR (95% CI)
Feverishness	ChAd/ChAd-28	30.8 (9.1 to 61.4)	13	ChAd/BNT-28	33.3 (19.1 to 50.2)	39	1.12 (0.29, 4.35)
Chills	ChAd/ChAd-28	50.0 (24.7 to 75.3)	16	ChAd/BNT-28	40.9 (26.3 to 56.8)	44	0.69 (0.22, 2.19)
Myalgia	ChAd/ChAd-28	26.1 (10.2 to 48.4)	23	ChAd/BNT-28	35.8 (24.5 to 48.5)	67	1.58 (0.55, 4.55)
Arthralgia	ChAd/ChAd-28	33.3 (9.9 to 65.1)	12	ChAd/BNT-28	34.2 (19.6 to 51.4)	38	1.04 (0.26, 4.11)
Headache	ChAd/ChAd-28	25.6 (13.0 to 42.1)	39	ChAd/BNT-28	30.1 (19.9 to 42.0)	73	1.25 (0.52, 3.00)
Fatigue	ChAd/ChAd-28	35.6 (23.6 to 49.1)	59	ChAd/BNT-28	48.9 (38.1 to 59.8)	88	1.73 (0.88, 3.40)
Malaise	ChAd/ChAd-28	47.6 (25.7 to 70.2)	21	ChAd/BNT-28	57.4 (44.1 to 70.0)	61	1.48 (0.55, 4.01)
Nausea	ChAd/ChAd-28	58.3 (27.7 to 84.8)	12	ChAd/BNT-28	19.0 (5.4 to 41.9)	21	0.17 (0.03, 0.82)
Diarrhoea	ChAd/ChAd-28	27.3 (6.0 to 61.0)	11	ChAd/BNT-28	22.2 (2.8 to 60.0)	9	0.76 (0.10, 5.96)
Feverishness	BNT/BNT-28	32.0 (14.9 to 53.5)	25	BNT/ChAd-28	45.8 (31.4 to 60.8)	48	1.80 (0.65, 4.96)
Chills	BNT/BNT-28	27.6 (12.7 to 47.2)	29	BNT/ChAd-28	48.1 (34.3 to 62.2)	54	2.44 (0.92, 6.45)
Myalgia	BNT/BNT-28	35.4 (22.2 to 50.5)	48	BNT/ChAd-28	23.7 (13.6 to 36.6)	59	0.57 (0.24, 1.32)
Arthralgia	BNT/BNT-28	27.3 (13.3 to 45.5)	33	BNT/ChAd-28	29.3 (16.1 to 45.5)	41	1.10 (0.40, 3.06)
Headache	BNT/BNT-28	39.2 (25.8 to 53.9)	51	BNT/ChAd-28	36.5 (25.6 to 48.5)	74	0.89 (0.43, 1.86)
Fatigue	BNT/BNT-28	30.8 (19.9 to 43.4)	65	BNT/ChAd-28	46.8 (35.3 to 58.5)	77	1.98 (0.99, 3.94)
Malaise	BNT/BNT-28	44.2 (29.1 to 60.1)	43	BNT/ChAd-28	45.5 (33.1 to 58.2)	66	1.05 (0.49, 2.28)
Nausea	BNT/BNT-28	30.0 (11.9 to 54.3)	20	BNT/ChAd-28	20.0 (5.7 to 43.7)	20	0.58 (0.14, 2.50)
Diarrhoea	BNT/BNT-28	12.5 (0.3 to 52.7)	8	BNT/ChAd-28	21.4 (4.7 to 50.8)	14	1.91 (0.16, 22.20)
Feverishness	ChAd/ChAd-84	47.4 (24.4 to 71.1)	19	ChAd/BNT-84	38.9 (17.3 to 64.3)	18	0.71 (0.19, 2.61)
Chills	ChAd/ChAd-84	69.2 (38.6 to 90.9)	13	ChAd/BNT-84	44.4 (21.5 to 69.2)	18	0.36 (0.08, 1.59)
Myalgia	ChAd/ChAd-84	28.0 (12.1 to 49.4)	25	ChAd/BNT-84	26.1 (14.3 to 41.1)	46	0.91 (0.30, 2.71)
Arthralgia	ChAd/ChAd-84	38.9 (17.3 to 64.3)	18	ChAd/BNT-84	28.6 (11.3 to 52.2)	21	0.63 (0.16, 2.40)
Headache	ChAd/ChAd-84	38.1 (23.6 to 54.4)	42	ChAd/BNT-84	30.8 (17.0 to 47.6)	39	0.72 (0.29, 1.82)
Fatigue	ChAd/ChAd-84	35.8 (23.1 to 50.2)	53	ChAd/BNT-84	38.3 (26.1 to 51.8)	60	1.11 (0.52, 2.39)
Malaise	ChAd/ChAd-84	37.5 (21.1 to 56.3)	32	ChAd/BNT-84	32.3 (16.7 to 51.4)	31	0.79 (0.28, 2.24)
Nausea	ChAd/ChAd-84	23.5 (6.8 to 49.9)	17	ChAd/BNT-84	44.4 (13.7 to 78.8)	9	2.60 (0.46, 14.63)
Diarrhoea	ChAd/ChAd-84	46.2 (19.2 to 74.9)	13	ChAd/BNT-84	57.1 (18.4 to 90.1)	7	1.56 (0.24, 9.91)
Feverishness	BNT/BNT-84	36.4 (10.9 to 69.2)	11	BNT/ChAd-84	61.5 (44.6 to 76.6)	39	2.80 (0.70, 11.21)
Chills	BNT/BNT-84	18.2 (2.3 to 51.8)	11	BNT/ChAd-84	55.0 (38.5 to 70.7)	40	5.50 (1.05, 28.75)
Myalgia	BNT/BNT-84	4.2 (0.1 to 21.1)	24	BNT/ChAd-84	48.0 (33.7 to 62.6)	50	21.23 (2.66, 169.53)
Arthralgia	BNT/BNT-84	11.1 (0.3 to 48.2)	9	BNT/ChAd-84	47.4 (31.0 to 64.2)	38	7.20 (0.82, 63.32)
Headache	BNT/BNT-84	20.6 (8.7 to 37.9)	34	BNT/ChAd-84	46.0 (31.8 to 60.7)	50	3.29 (1.21, 8.93)
Fatigue	BNT/BNT-84	20.9 (10.0 to 36.0)	43	BNT/ChAd-84	70.8 (58.2 to 81.4)	65	9.15 (3.69, 22.69)
Malaise	BNT/BNT-84	35.0 (15.4 to 59.2)	20	BNT/ChAd-84	71.7 (56.5 to 84.0)	46	4.71 (1.54, 14.46)
Nausea	BNT/BNT-84	27.3 (6.0 to 61.0)	11	BNT/ChAd-84	43.8 (19.8 to 70.1)	16	2.07 (0.40, 10.84)
Diarrhoea	BNT/BNT-84	14.3 (0.4 to 57.9)	7	BNT/ChAd-84	33.3 (11.8 to 61.6)	15	3.00 (0.28, 32.21)

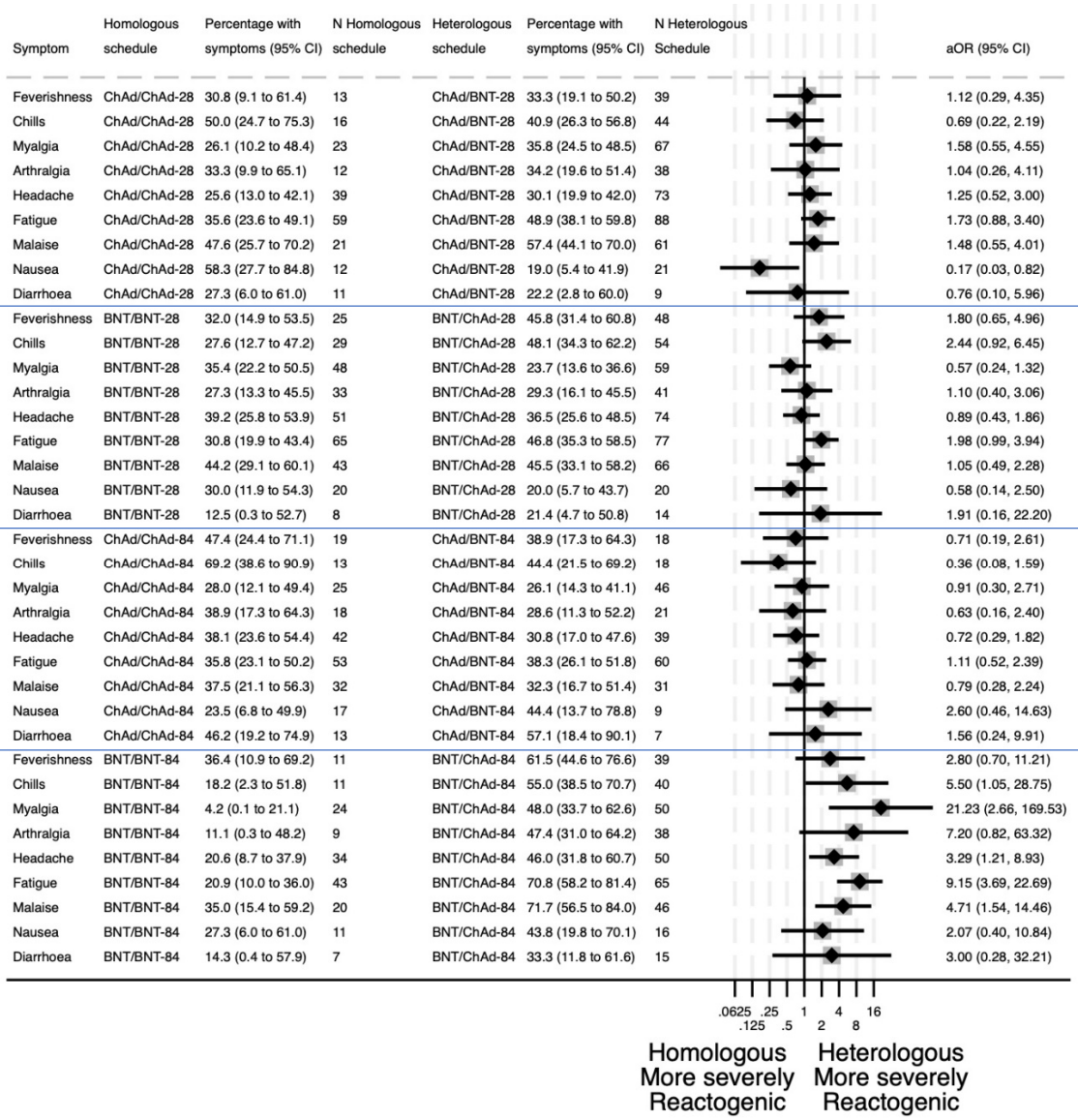

Figure 39 *– Com-COV1 Forest plot comparing proportions of participants (seronegative and seropositive combined) suffering moderate-severe (Grade 2 or above) systemic reactogenicity symptoms out of the total number of participants suffering any grade symptoms, in the first 7 days after a second COVID-19 vaccination between homologous and heterologous schedules. "If you did suffer a symptom of any grade after the second dose, was it more likely to be moderate/severe (Grade 2 or above) if the schedule was homologous or heterolgous?"*

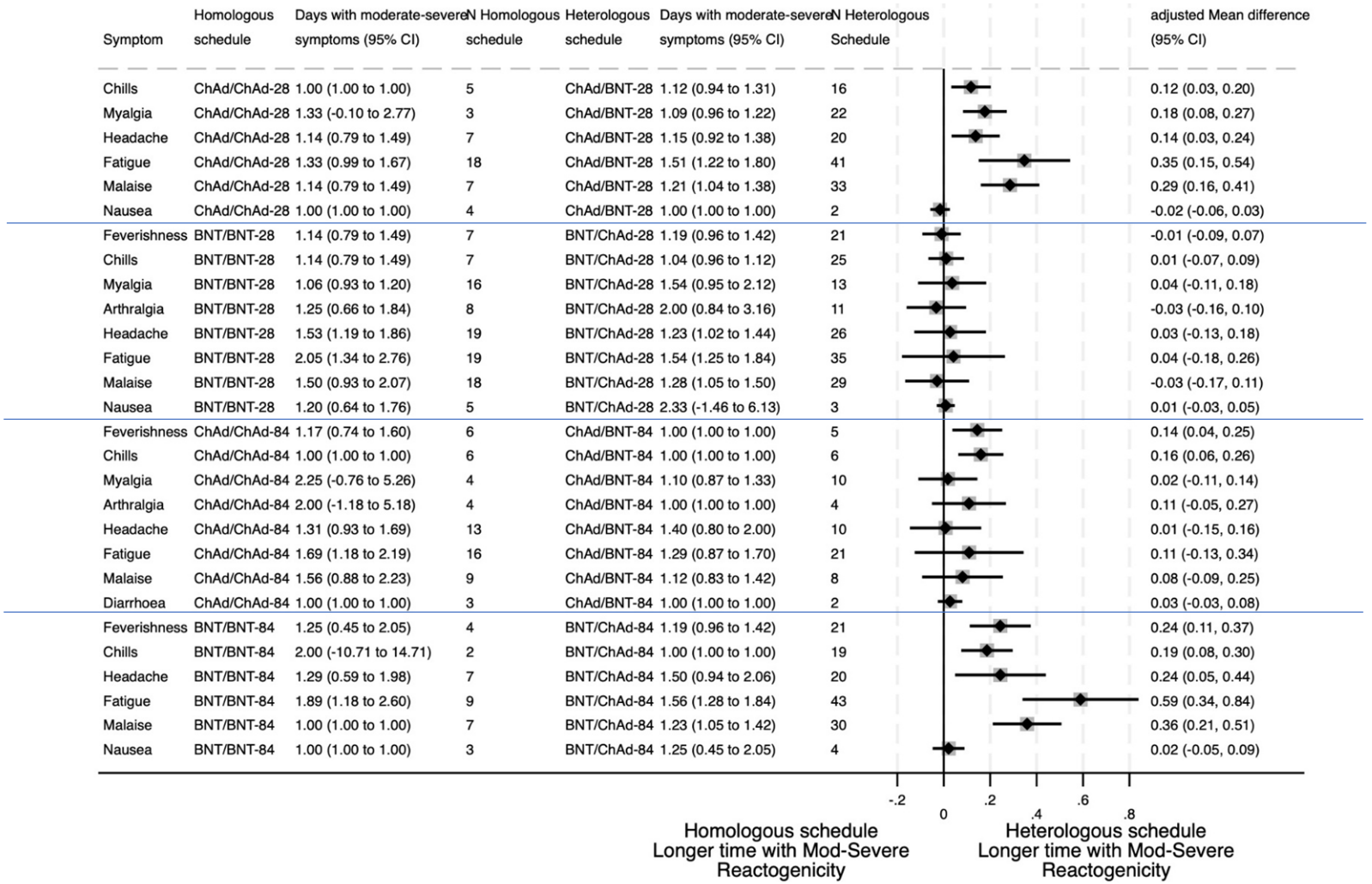

Figure 40 *– Com-COV1 Forest plot comparing durations of time suffering with grade 2 or higher systemic reactogenicity symptoms after the second COVID-19 vaccination between participants (seronegative and seropositive combined) receiving homologous or heterologous schedules . "If you did suffer with moderate/severe symptoms after the second dose, were the symptoms likely to last longer after a homologous or heterologous schedule?"*

3.4.3.1.2 1st dose: ChAd vs BNT

A BNT first dose is approximately three times less likely to cause systemic symptoms than a ChAd first dose. It is approximately half as likely for those systemic symptoms to be moderate-severe instead of mild. If moderate-severe symptoms were experienced, on average, they lasted a few hours less than for participants receiving ChAd as their first dose (**Figure 41**, **Figure 42**, **Figure 43** & **Figure 44**).

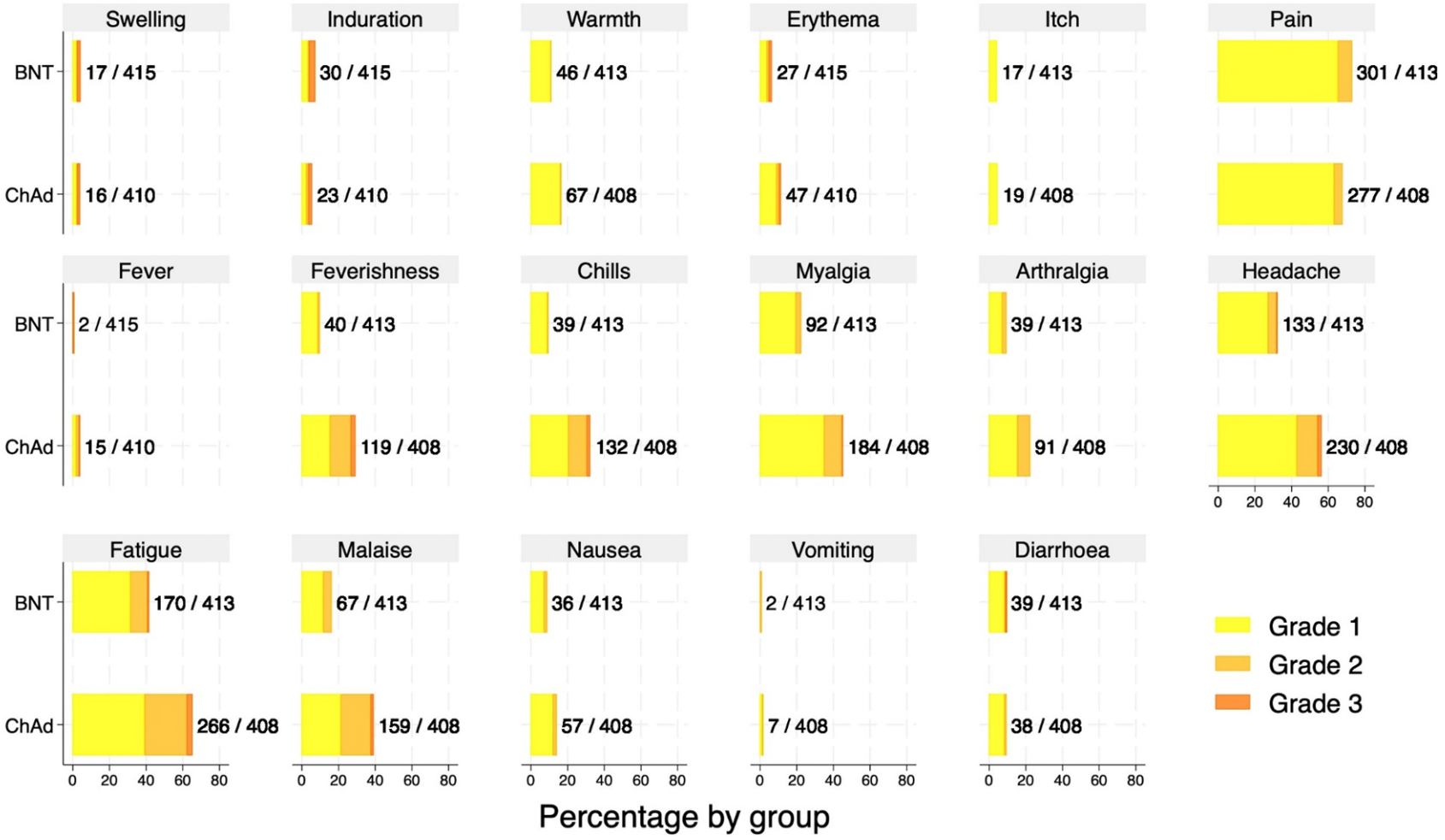

Figure 41 *– Com-COV1 Stacked bar chart showing the total number of participants suffering each local and systemic reactogenicity symptom after the first dose. The severity presented is each participant's highest severity across 7 days after vaccination. Data are divided by prime dose.*

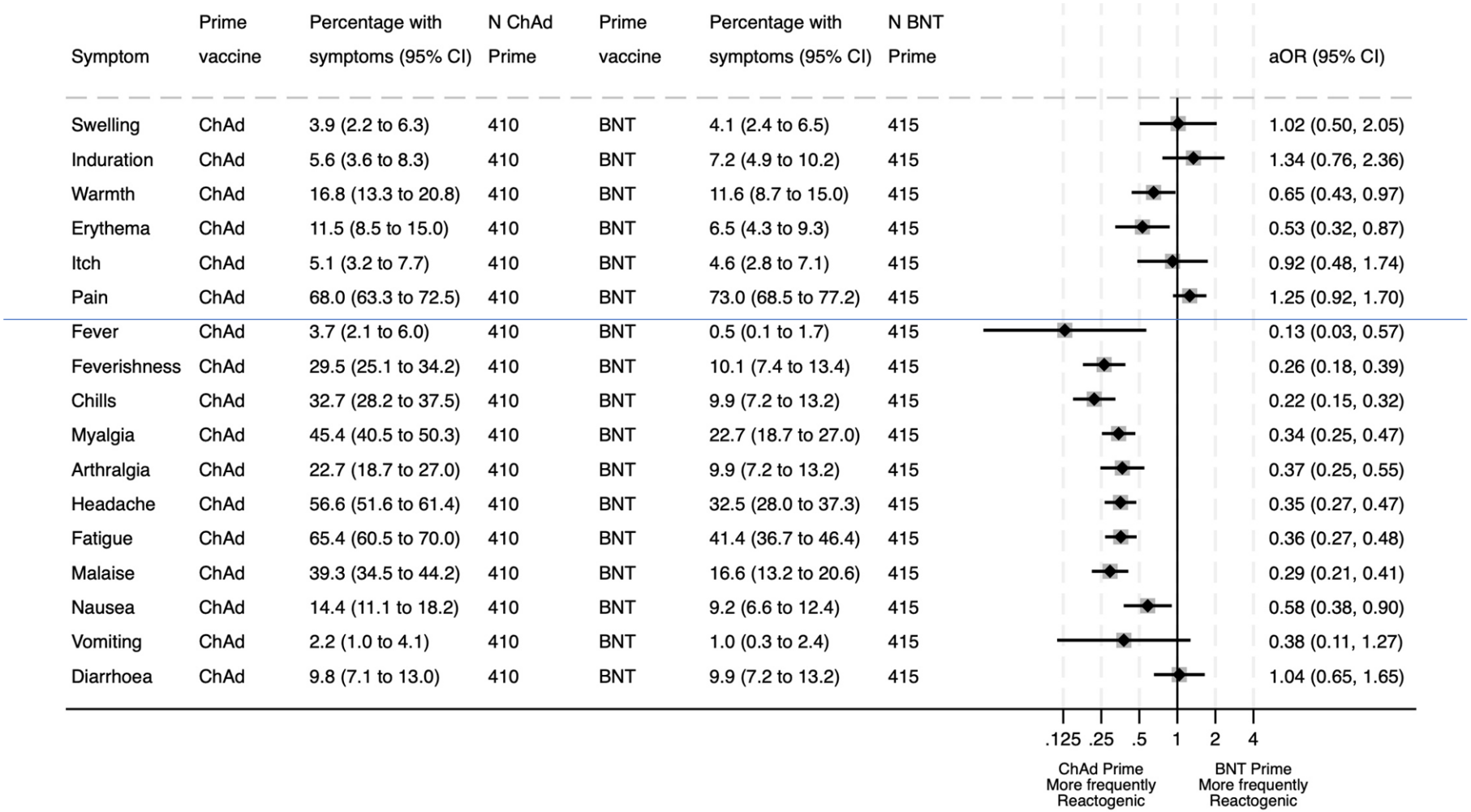

Figure 42 *– Com-COV1 Forest plot comparing the proportions of participants (seronegative and seropositive combined) suffering local and systemic reactogenicity symptoms in the first 7 days after the first COVID-19 vaccination between ChAd and BNT first doses "Were you more likely to suffer any grade symptom after a single dose of either ChAd or BNT?"*

Symptom	Prime vaccine	Percentage with symptoms (95% CI)	N ChAd Prime	Prime vaccine	Percentage with symptoms (95% CI)	N BNT Prime	aOR (95% CI)
Swelling	ChAd	50.0 (24.7 to 75.3)	16	BNT	52.9 (27.8 to 77.0)	17	1.08 (0.23, 5.03)
Induration	ChAd	56.5 (34.5 to 76.8)	23	BNT	53.3 (34.3 to 71.7)	30	0.99 (0.31, 3.12)
Warmth	ChAd	4.3 (0.9 to 12.2)	69	BNT	8.3 (2.3 to 20.0)	48	1.90 (0.40, 9.12)
Erythema	ChAd	23.4 (12.3 to 38.0)	47	BNT	44.4 (25.5 to 64.7)	27	2.55 (0.84, 7.70)
Itch	ChAd	9.5 (1.2 to 30.4)	21	BNT	10.5 (1.3 to 33.1)	19	1.17 (0.13, 10.65)
Pain	ChAd	7.9 (5.0 to 11.7)	279	BNT	11.2 (7.9 to 15.3)	303	1.39 (0.77, 2.49)
Fever	ChAd	53.3 (26.6 to 78.7)	15	BNT	50.0 (1.3 to 98.7)	2	0.51 (0.02, 11.86)
Feverishness	ChAd	48.8 (39.6 to 58.0)	121	BNT	16.7 (7.0 to 31.4)	42	0.20 (0.08, 0.51)
Chills	ChAd	38.1 (29.8 to 46.8)	134	BNT	12.2 (4.1 to 26.2)	41	0.22 (0.08, 0.61)
Myalgia	ChAd	23.7 (17.7 to 30.4)	186	BNT	14.9 (8.4 to 23.7)	94	0.57 (0.29, 1.11)
Arthralgia	ChAd	32.3 (22.9 to 42.7)	93	BNT	29.3 (16.1 to 45.5)	41	0.91 (0.40, 2.09)
Headache	ChAd	25.0 (19.6 to 31.1)	232	BNT	17.0 (11.1 to 24.5)	135	0.59 (0.34, 1.02)
Fatigue	ChAd	40.3 (34.4 to 46.4)	268	BNT	25.0 (18.7 to 32.2)	172	0.46 (0.30, 0.71)
Malaise	ChAd	46.6 (38.7 to 54.6)	161	BNT	30.4 (19.9 to 42.7)	69	0.42 (0.22, 0.80)
Nausea	ChAd	16.9 (8.4 to 29.0)	59	BNT	23.7 (11.4 to 40.2)	38	1.49 (0.52, 4.25)
Vomiting	ChAd	55.6 (21.2 to 86.3)	9	BNT	75.0 (19.4 to 99.4)	4	3.06 (0.11, 82.66)
Diarrhoea	ChAd	15.0 (5.7 to 29.8)	40	BNT	17.1 (7.2 to 32.1)	41	1.11 (0.32, 3.76)

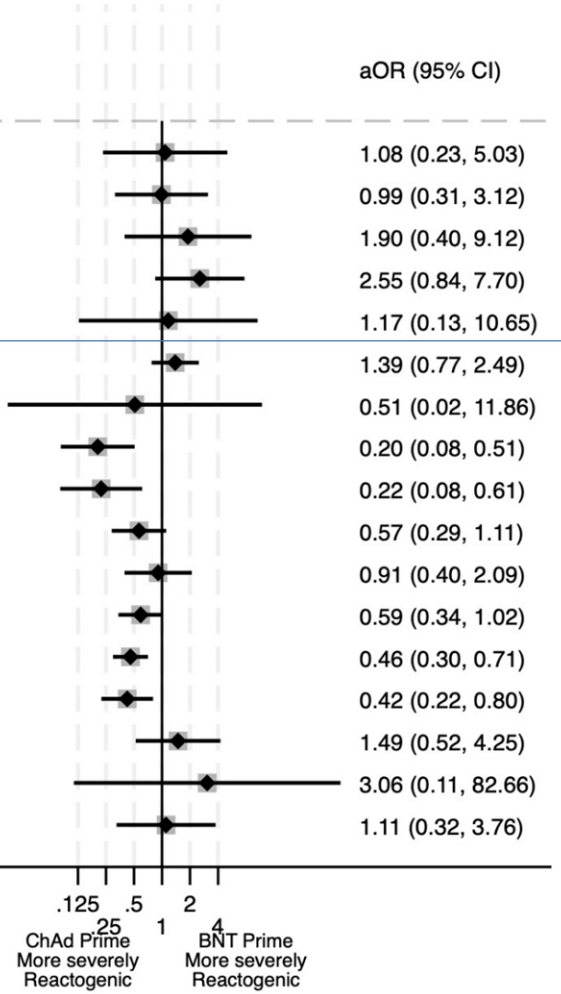

Figure 43 *– Com-COV1 Forest plot comparing proportions of participants (seronegative and seropositive combined) suffering moderate-severe (Grade 2 or above) local and systemic reactogenicity symptoms out of the total number of participants suffering any grade symptoms, in the first 7 days after a first COVID-19 vaccination of either ChAd or BNT. "If you did suffer a symptom of any grade, was it more likely to be moderate/severe after a single dose of either ChAd or BNT"*

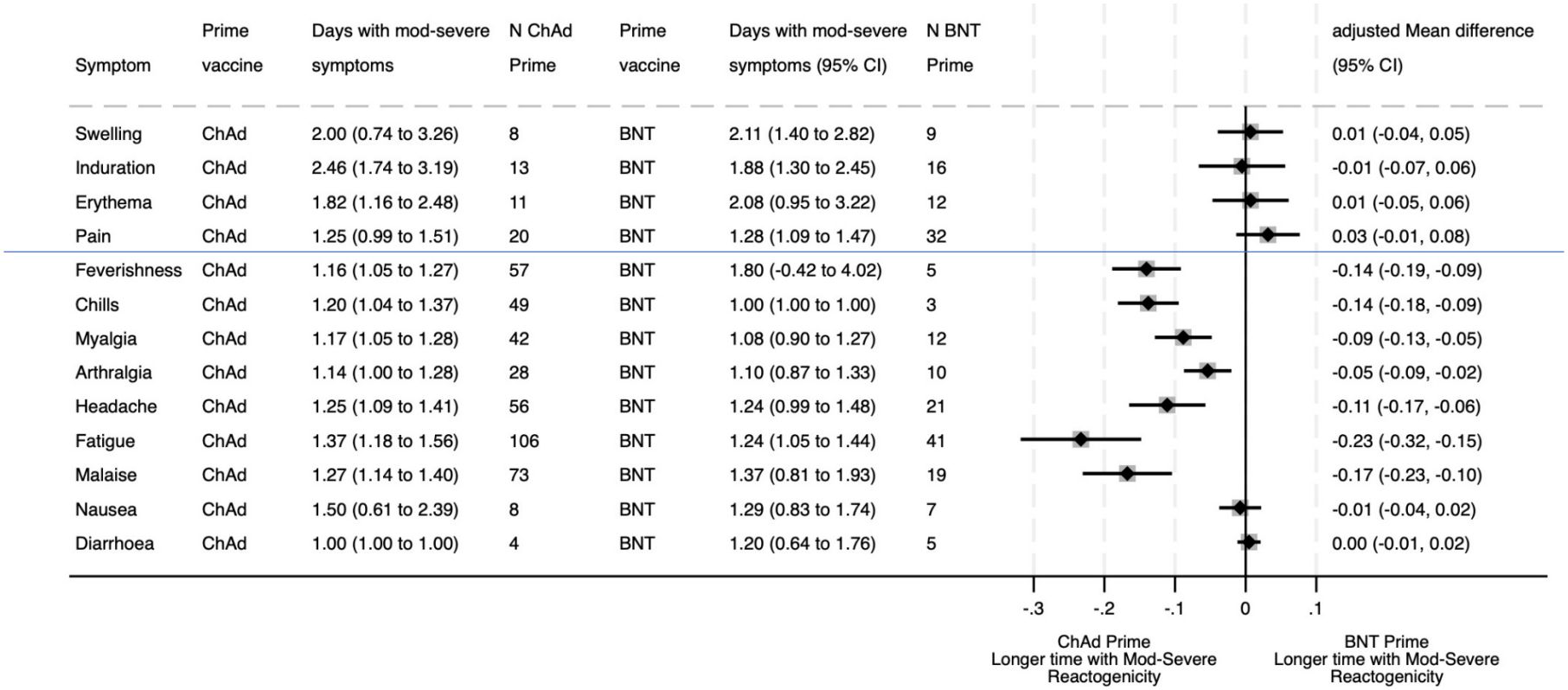

Figure 44 *– Com-COV1 Forest plot comparing durations of time spent with grade 2 or higher local and systemic reactogenicity symptoms after the first COVID-19 vaccination between participants (seronegative and seropositive combined) receiving ChAd or BNT . "If you did suffer with moderate/severe symptoms, were the symptoms likely to last longer after a single dose of either ChAd or BNT"*

3.4.3.1.3 1st dose: Serostatus

Despite the small number of seropositive participants in this trial, by combining the results of all participants who received the same first dose, a statistical comparison alongside descriptive display (**Figure 45**) becomes possible.

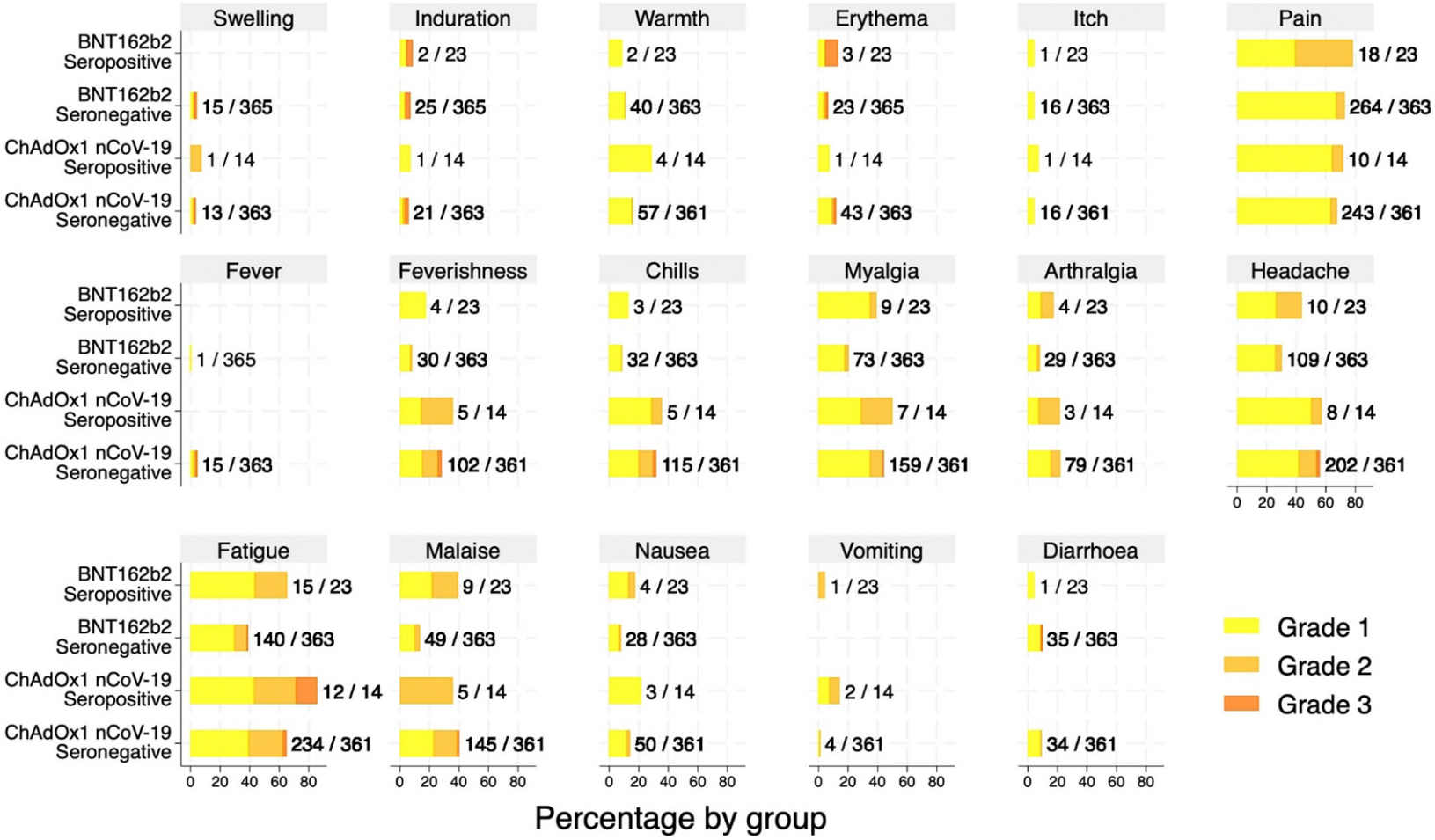

Figure 45 *– Com-COV1 Stacked bar chart showing the total number of participants suffering each local and systemic reactogenicity symptom after the first dose. The severity presented is each participant's highest severity across 7 days after vaccination. Data are divided by prime dose and pre-vaccination serostatus*

There was no difference in likelihood of suffering systemic reactogenicity symptoms between seropositive and seronegative participants receiving a ChAd first dose. However for those receiving a BNT first dose, seropositive participants were about twice as likely to suffer systemic symptoms as seronegative participants, although 7/10 of the systemic symptoms did not reach statistical significance. For participants receiving either vaccine as a first dose, who did suffer systemic symptoms, there was no difference in likelihood of symptoms being moderate-severe between seropositive and seronegative participants. However, moderate-severe systemic symptoms in seropositive participants who had received BNT, lasted a few hours more on average than in seronegative participants (**Figure 46**, **Figure 47** & **Figure 48**). There were no clear patterns for local reactogenicity symptoms.

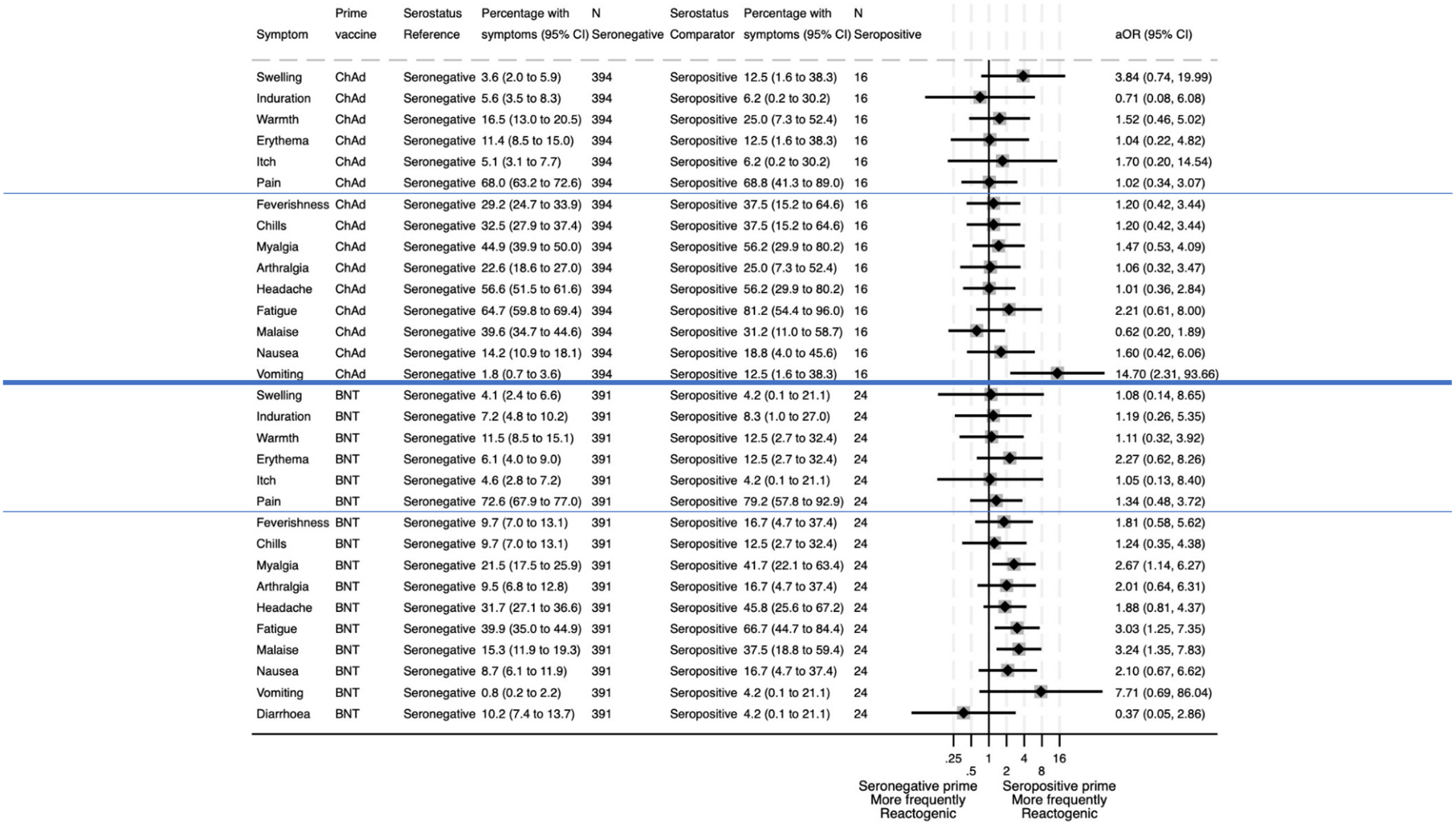

Figure 46 *– Com-COV1 Forest plot comparing the proportions of seronegative vs seropositive participants suffering local & systemic reactogenicity symptoms in the first 7 days after the first COVID-19 vaccination for both ChAd and BNT first doses "Were you more likely to suffer any grade symptom if you were seropositive or seronegative at the time of your single dose of either ChAd or BNT?"*

Symptom	Prime vaccine	Serostatus Reference	Percentage with symptoms (95% CI)	N Seronegative	Serostatus Comparator	Percentage with symptoms (95% CI)	N Seropositive	aOR (95% CI)
Swelling	ChAd	Seronegative	50.0 (23.0 to 77.0)	14	Seropositive	50.0 (1.3 to 98.7)	2	1.00 (0.05, 19.36)
Pain	ChAd	Seronegative	7.8 (4.9 to 11.7)	268	Seropositive	9.1 (0.2 to 41.3)	11	1.18 (0.14, 9.64)
Feverishness	ChAd	Seronegative	48.7 (39.3 to 58.2)	115	Seropositive	50.0 (11.8 to 88.2)	6	1.05 (0.20, 5.44)
Chills	ChAd	Seronegative	39.1 (30.6 to 48.1)	128	Seropositive	16.7 (0.4 to 64.1)	6	0.31 (0.04, 2.75)
Myalgia	ChAd	Seronegative	23.2 (17.2 to 30.1)	177	Seropositive	33.3 (7.5 to 70.1)	9	1.66 (0.40, 6.93)
Arthralgia	ChAd	Seronegative	31.5 (22.0 to 42.2)	89	Seropositive	50.0 (6.8 to 93.2)	4	2.18 (0.29, 16.27)
Headache	ChAd	Seronegative	25.6 (20.0 to 31.8)	223	Seropositive	11.1 (0.3 to 48.2)	9	0.36 (0.04, 2.97)
Fatigue	ChAd	Seronegative	40.0 (33.9 to 46.3)	255	Seropositive	46.2 (19.2 to 74.9)	13	1.29 (0.42, 3.94)
Induration	BNT	Seronegative	53.6 (33.9 to 72.5)	28	Seropositive	50.0 (1.3 to 98.7)	2	0.87 (0.05, 15.28)
Erythema	BNT	Seronegative	41.7 (22.1 to 63.4)	24	Seropositive	66.7 (9.4 to 99.2)	3	2.80 (0.22, 35.29)
Pain	BNT	Seronegative	8.5 (5.5 to 12.3)	284	Seropositive	52.6 (28.9 to 75.6)	19	12.04 (4.46, 32.49)
Myalgia	BNT	Seronegative	15.5 (8.5 to 25.0)	84	Seropositive	10.0 (0.3 to 44.5)	10	0.61 (0.07, 5.20)
Arthralgia	BNT	Seronegative	27.0 (13.8 to 44.1)	37	Seropositive	50.0 (6.8 to 93.2)	4	2.70 (0.33, 21.83)
Headache	BNT	Seronegative	15.3 (9.5 to 22.9)	124	Seropositive	36.4 (10.9 to 69.2)	11	3.16 (0.84, 11.85)
Fatigue	BNT	Seronegative	24.4 (17.9 to 31.9)	156	Seropositive	31.2 (11.0 to 58.7)	16	1.41 (0.46, 4.32)
Malaise	BNT	Seronegative	28.3 (17.5 to 41.4)	60	Seropositive	44.4 (13.7 to 78.8)	9	2.02 (0.48, 8.45)
Nausea	BNT	Seronegative	23.5 (10.7 to 41.2)	34	Seropositive	25.0 (0.6 to 80.6)	4	1.08 (0.10, 11.92)

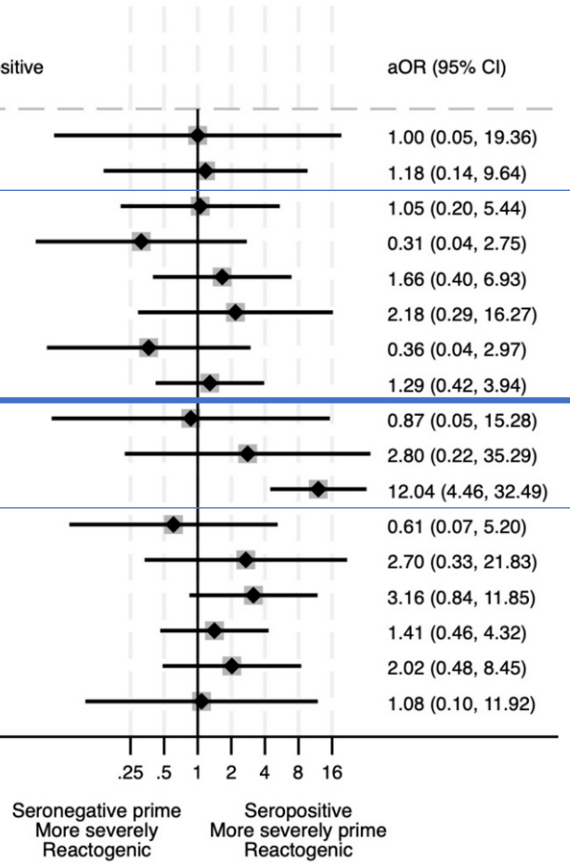

Figure 47 *– Com-COV1 Forest plot comparing the proportions of seronegative vs seropositive participants suffering moderate-severe (Grade 2 or above) local and systemic reactogenicity symptoms out of the total number of participants suffering any grade symptoms, in the first 7 days after a first COVID-19 vaccination of either ChAd or BNT. "If you did suffer a symptom of any grade, was it more likely to be moderate/severe if you were seropositive or seronegative for both ChAd and BNT first doses?"*

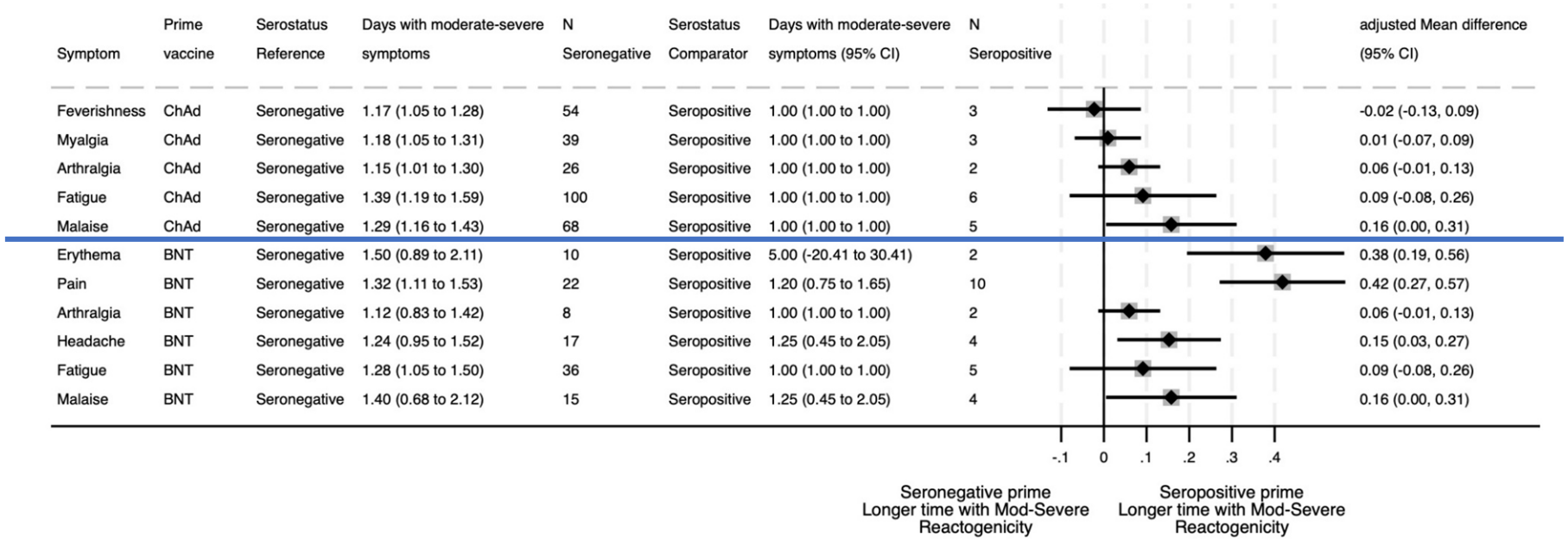

Figure 48 *– Com-COV1 Forest plot comparing durations of time spent with grade 2 or higher local and systemic reactogenicity symptoms after the first COVID-19 vaccination between seronegative and seropositive participants receiving ChAd or BNT first doses . "If you did suffer with moderate/severe symptoms, were the symptoms likely to last longer if you were seropositive or seronegative at baseline, after single doses of ChAd and BNT"*

3.4.3.1.4 Prediction of reactogenicity

Participants who were symptomatic at baseline were 1.6 to 29 times as likely to suffer the same systemic symptom again at boost regardless of schedule. In general the chance of suffering a symptom at the time of the second dose was, on average, about four times greater if it had been experienced after the first dose. A similar effect was seen for local symptoms, where participants were 1.8 to 62 times as likely to suffer the same symptoms again (**Figure 49** & **Figure 50**).

Symptom	Vaccine schedule	Prime Symptoms	Percentage with boost symptoms (95% CI)	N Prime Asymptomatic	Prime Symptoms	Percentage with boost symptoms (95% CI)	N Prime Symptomatic	aOR (95% CI)
Feverishness	ChAd/ChAd-28	Prime Asymptomatic	9.1 (3.7 to 17.8)	77	Prime Symptomatic	16.2 (6.2 to 32.0)	37	1.88 (0.58, 6.09)
Chills	ChAd/ChAd-28	Prime Asymptomatic	12.2 (5.7 to 21.8)	74	Prime Symptomatic	17.5 (7.3 to 32.8)	40	1.66 (0.56, 4.88)
Myalgia	ChAd/ChAd-28	Prime Asymptomatic	9.2 (3.5 to 19.0)	65	Prime Symptomatic	34.7 (21.7 to 49.6)	49	5.22 (1.87, 14.54)
Headache	ChAd/ChAd-28	Prime Asymptomatic	11.5 (4.4 to 23.4)	52	Prime Symptomatic	53.2 (40.1 to 66.0)	62	8.66 (3.19, 23.47)
Fatigue	ChAd/ChAd-28	Prime Asymptomatic	28.6 (15.7 to 44.6)	42	Prime Symptomatic	65.3 (53.1 to 76.1)	72	4.67 (2.04, 10.70)
Malaise	ChAd/ChAd-28	Prime Asymptomatic	12.5 (5.6 to 23.2)	64	Prime Symptomatic	26.0 (14.6 to 40.3)	50	2.45 (0.93, 6.50)
Nausea	ChAd/ChAd-28	Prime Asymptomatic	8.2 (3.6 to 15.6)	97	Prime Symptomatic	23.5 (6.8 to 49.9)	17	3.95 (1.02, 15.32)
Diarrhoea	ChAd/ChAd-28	Prime Asymptomatic	7.5 (3.3 to 14.3)	106	Prime Symptomatic	37.5 (8.5 to 75.5)	8	7.05 (1.42, 35.03)
Feverishness	ChAd/BNT-28	Prime Asymptomatic	27.4 (18.2 to 38.2)	84	Prime Symptomatic	55.2 (35.7 to 73.6)	29	3.40 (1.40, 8.26)
Chills	ChAd/BNT-28	Prime Asymptomatic	24.7 (15.8 to 35.5)	81	Prime Symptomatic	75.0 (56.6 to 88.5)	32	10.04 (3.77, 26.74)
Myalgia	ChAd/BNT-28	Prime Asymptomatic	47.6 (34.9 to 60.6)	63	Prime Symptomatic	74.0 (59.7 to 85.4)	50	3.08 (1.38, 6.90)
Headache	ChAd/BNT-28	Prime Asymptomatic	48.9 (33.7 to 64.2)	45	Prime Symptomatic	75.0 (63.0 to 84.7)	68	3.18 (1.42, 7.10)
Fatigue	ChAd/BNT-28	Prime Asymptomatic	65.9 (49.4 to 79.9)	41	Prime Symptomatic	84.7 (74.3 to 92.1)	72	2.87 (1.15, 7.15)
Malaise	ChAd/BNT-28	Prime Asymptomatic	43.1 (31.4 to 55.3)	72	Prime Symptomatic	73.2 (57.1 to 85.8)	41	3.58 (1.55, 8.28)
Nausea	ChAd/BNT-28	Prime Asymptomatic	12.4 (6.6 to 20.6)	97	Prime Symptomatic	56.2 (29.9 to 80.2)	16	8.68 (2.72, 27.65)
Feverishness	BNT/BNT-28	Prime Asymptomatic	17.9 (11.2 to 26.6)	106	Prime Symptomatic	50.0 (21.1 to 78.9)	12	4.19 (1.19, 14.73)
Chills	BNT/BNT-28	Prime Asymptomatic	21.8 (14.2 to 31.1)	101	Prime Symptomatic	41.2 (18.4 to 67.1)	17	2.40 (0.81, 7.10)
Myalgia	BNT/BNT-28	Prime Asymptomatic	29.8 (20.3 to 40.7)	84	Prime Symptomatic	67.6 (49.5 to 82.6)	34	5.63 (2.29, 13.81)
Headache	BNT/BNT-28	Prime Asymptomatic	26.6 (17.3 to 37.7)	79	Prime Symptomatic	76.9 (60.7 to 88.9)	39	9.15 (3.73, 22.47)
Fatigue	BNT/BNT-28	Prime Asymptomatic	37.5 (25.7 to 50.5)	64	Prime Symptomatic	75.9 (62.4 to 86.5)	54	5.23 (2.30, 11.87)
Malaise	BNT/BNT-28	Prime Asymptomatic	27.2 (18.4 to 37.4)	92	Prime Symptomatic	69.2 (48.2 to 85.7)	26	5.49 (2.09, 14.40)
Nausea	BNT/BNT-28	Prime Asymptomatic	14.4 (8.3 to 22.7)	104	Prime Symptomatic	35.7 (12.8 to 64.9)	14	3.35 (0.96, 11.64)
Diarrhoea	BNT/BNT-28	Prime Asymptomatic	3.9 (1.1 to 9.7)	102	Prime Symptomatic	25.0 (7.3 to 52.4)	16	7.58 (1.67, 34.36)
Feverishness	BNT/ChAd-28	Prime Asymptomatic	40.8 (31.2 to 50.9)	103	Prime Symptomatic	54.5 (23.4 to 83.3)	11	1.89 (0.54, 6.64)
Chills	BNT/ChAd-28	Prime Asymptomatic	46.7 (37.0 to 56.6)	107	Prime Symptomatic	57.1 (18.4 to 90.1)	7	1.50 (0.32, 7.04)
Myalgia	BNT/ChAd-28	Prime Asymptomatic	46.7 (36.3 to 57.4)	92	Prime Symptomatic	72.7 (49.8 to 89.3)	22	3.05 (1.09, 8.51)
Headache	BNT/ChAd-28	Prime Asymptomatic	57.8 (46.5 to 68.6)	83	Prime Symptomatic	83.9 (66.3 to 94.5)	31	4.11 (1.39, 12.09)
Fatigue	BNT/ChAd-28	Prime Asymptomatic	59.2 (46.8 to 70.7)	71	Prime Symptomatic	81.4 (66.6 to 91.6)	43	2.97 (1.20, 7.35)
Malaise	BNT/ChAd-28	Prime Asymptomatic	54.0 (43.7 to 64.0)	100	Prime Symptomatic	85.7 (57.2 to 98.2)	14	5.11 (1.09, 24.07)
Nausea	BNT/ChAd-28	Prime Asymptomatic	17.0 (10.4 to 25.5)	106	Prime Symptomatic	25.0 (3.2 to 65.1)	8	1.87 (0.34, 10.39)
Diarrhoea	BNT/ChAd-28	Prime Asymptomatic	7.9 (3.5 to 15.0)	101	Prime Symptomatic	46.2 (19.2 to 74.9)	13	10.90 (2.87, 41.39)
Feverishness	ChAd/ChAd-84	Prime Asymptomatic	13.8 (6.1 to 25.4)	58	Prime Symptomatic	32.4 (17.4 to 50.5)	34	2.93 (1.04, 8.26)
Chills	ChAd/ChAd-84	Prime Asymptomatic	8.3 (2.8 to 18.4)	60	Prime Symptomatic	25.0 (11.5 to 43.4)	32	3.60 (1.07, 12.15)
Myalgia	ChAd/ChAd-84	Prime Asymptomatic	15.9 (6.6 to 30.1)	44	Prime Symptomatic	37.5 (24.0 to 52.6)	48	3.28 (1.21, 8.91)
Headache	ChAd/ChAd-84	Prime Asymptomatic	18.9 (8.0 to 35.2)	37	Prime Symptomatic	63.6 (49.6 to 76.2)	55	7.25 (2.69, 19.54)
Fatigue	ChAd/ChAd-84	Prime Asymptomatic	43.8 (26.4 to 62.3)	32	Prime Symptomatic	65.0 (51.6 to 76.9)	60	2.51 (1.04, 6.06)
Malaise	ChAd/ChAd-84	Prime Asymptomatic	24.5 (13.8 to 38.3)	53	Prime Symptomatic	48.7 (32.4 to 65.2)	39	2.85 (1.17, 6.93)
Nausea	ChAd/ChAd-84	Prime Asymptomatic	13.5 (6.7 to 23.5)	74	Prime Symptomatic	38.9 (17.3 to 64.3)	18	4.01 (1.26, 12.78)
Diarrhoea	ChAd/ChAd-84	Prime Asymptomatic	11.4 (5.3 to 20.5)	79	Prime Symptomatic	30.8 (9.1 to 61.4)	13	3.41 (0.87, 13.37)
Feverishness	ChAd/BNT-84	Prime Asymptomatic	13.4 (6.3 to 24.0)	67	Prime Symptomatic	42.9 (21.8 to 66.0)	21	5.30 (1.69, 16.65)
Chills	ChAd/BNT-84	Prime Asymptomatic	12.1 (5.0 to 23.3)	58	Prime Symptomatic	36.7 (19.9 to 56.1)	30	4.19 (1.40, 12.49)
Myalgia	ChAd/BNT-84	Prime Asymptomatic	39.2 (25.8 to 53.9)	51	Prime Symptomatic	70.3 (53.0 to 84.1)	37	3.67 (1.49, 9.03)
Headache	ChAd/BNT-84	Prime Asymptomatic	23.3 (11.8 to 38.6)	43	Prime Symptomatic	64.4 (48.8 to 78.1)	45	6.25 (2.42, 16.10)
Fatigue	ChAd/BNT-84	Prime Asymptomatic	42.3 (23.4 to 63.1)	26	Prime Symptomatic	79.0 (66.8 to 88.3)	62	6.97 (2.42, 20.04)
Malaise	ChAd/BNT-84	Prime Asymptomatic	25.9 (15.3 to 39.0)	58	Prime Symptomatic	53.3 (34.3 to 71.7)	30	3.28 (1.29, 8.36)
Nausea	ChAd/BNT-84	Prime Asymptomatic	8.8 (3.6 to 17.2)	80	Prime Symptomatic	25.0 (3.2 to 65.1)	8	3.83 (0.62, 23.52)
Diarrhoea	ChAd/BNT-84	Prime Asymptomatic	6.3 (2.1 to 14.2)	79	Prime Symptomatic	22.2 (2.8 to 60.0)	9	3.83 (0.62, 23.52)
Feverishness	BNT/BNT-84	Prime Asymptomatic	8.5 (3.5 to 16.8)	82	Prime Symptomatic	36.4 (10.9 to 69.2)	11	6.95 (1.58, 30.66)
Chills	BNT/BNT-84	Prime Asymptomatic	9.0 (4.0 to 16.9)	89	Prime Symptomatic	75.0 (19.4 to 99.4)	4	28.94 (2.43, 345.11)
Myalgia	BNT/BNT-84	Prime Asymptomatic	20.3 (12.0 to 30.8)	79	Prime Symptomatic	57.1 (28.9 to 82.3)	14	4.64 (1.36, 15.83)
Headache	BNT/BNT-84	Prime Asymptomatic	21.7 (12.1 to 34.2)	60	Prime Symptomatic	63.6 (45.1 to 79.6)	33	6.15 (2.40, 15.79)
Fatigue	BNT/BNT-84	Prime Asymptomatic	36.7 (24.6 to 50.1)	60	Prime Symptomatic	63.6 (45.1 to 79.6)	33	2.96 (1.22, 7.19)
Malaise	BNT/BNT-84	Prime Asymptomatic	20.0 (11.9 to 30.4)	80	Prime Symptomatic	30.8 (9.1 to 61.4)	13	1.69 (0.43, 6.59)
Nausea	BNT/BNT-84	Prime Asymptomatic	9.3 (4.1 to 17.5)	86	Prime Symptomatic	42.9 (9.9 to 81.6)	7	6.63 (1.21, 36.37)
Feverishness	BNT/ChAd-84	Prime Asymptomatic	44.3 (33.1 to 55.9)	79	Prime Symptomatic	42.9 (9.9 to 81.6)	7	0.96 (0.20, 4.58)
Chills	BNT/ChAd-84	Prime Asymptomatic	42.5 (31.0 to 54.6)	73	Prime Symptomatic	61.5 (31.6 to 86.1)	13	2.25 (0.66, 7.64)
Myalgia	BNT/ChAd-84	Prime Asymptomatic	52.4 (39.4 to 65.1)	63	Prime Symptomatic	69.6 (47.1 to 86.8)	23	2.06 (0.74, 5.71)
Headache	BNT/ChAd-84	Prime Asymptomatic	47.3 (33.7 to 61.2)	55	Prime Symptomatic	74.2 (55.4 to 88.1)	31	3.24 (1.23, 8.55)
Fatigue	BNT/ChAd-84	Prime Asymptomatic	63.0 (47.5 to 76.8)	46	Prime Symptomatic	87.5 (73.2 to 95.8)	40	4.52 (1.44, 14.26)
Malaise	BNT/ChAd-84	Prime Asymptomatic	46.5 (34.5 to 58.7)	71	Prime Symptomatic	80.0 (51.9 to 95.7)	15	4.67 (1.21, 18.07)
Nausea	BNT/ChAd-84	Prime Asymptomatic	15.2 (8.1 to 25.0)	79	Prime Symptomatic	42.9 (9.9 to 81.6)	7	4.11 (0.81, 20.83)
Diarrhoea	BNT/ChAd-84	Prime Asymptomatic	16.0 (8.8 to 25.9)	81	Prime Symptomatic	40.0 (5.3 to 85.3)	5	3.34 (0.50, 22.28)

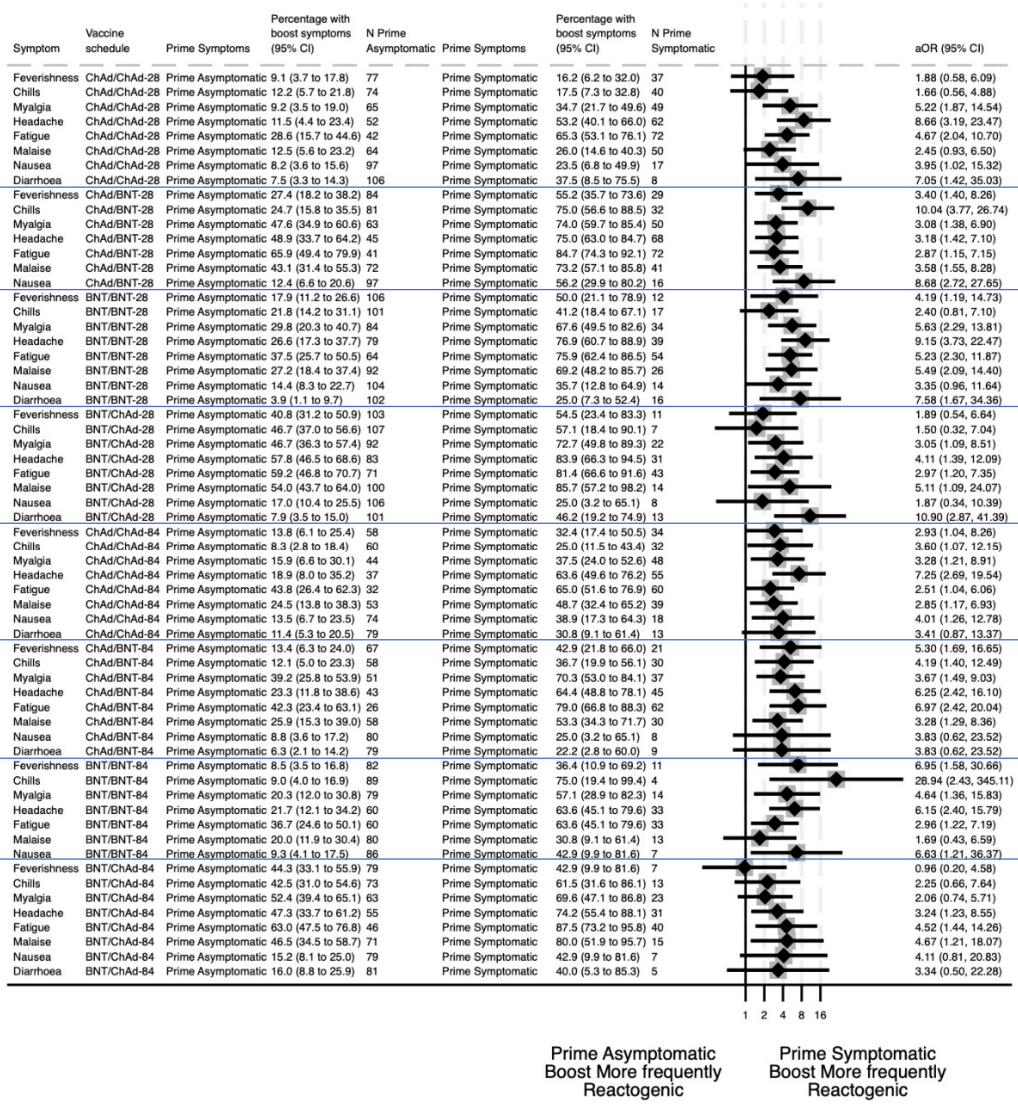

Figure 49 *– Com-COV1 Forest plot comparing the presence/absence of systemic reactogenicity symptoms in the first 7 days after second COVID-19 vaccination between those who were or were not symptomatic after the first dose . "If you were symptomatic at the first dose, were you more likely to suffer any grade symptom at the second dose?"*

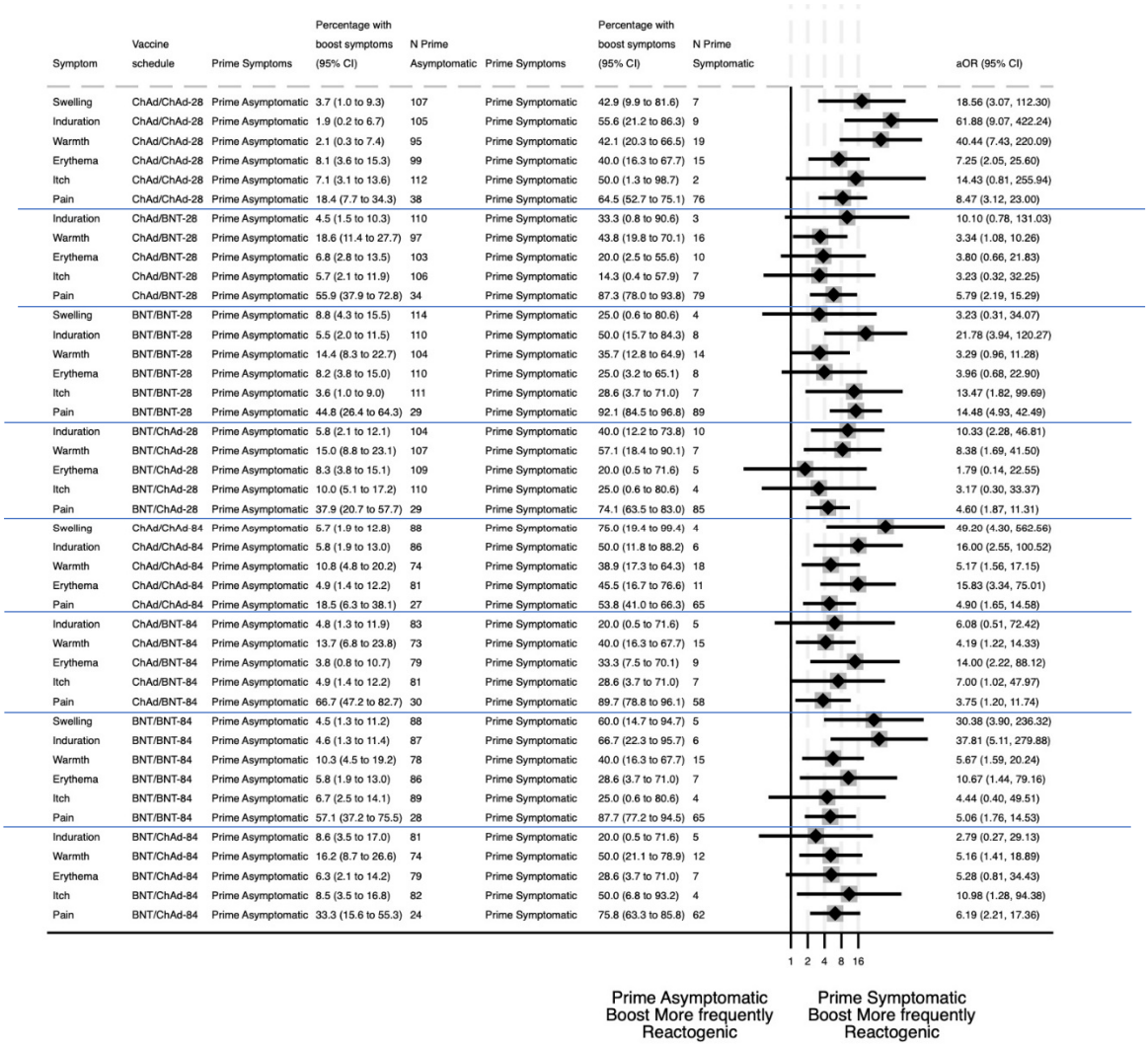

Figure 50 *– Com-COV1 Forest plot comparing the presence/absence of local reactogenicity symptoms in the first 7 days after the second COVID-19 vaccination if you were or were not symptomatic after the first dose . "If you were symptomatic at the first dose, were you more likely to suffer any grade symptom at the second dose?"*

3.4.3.1.5 First vs second dose reactogenicity

The first dose of homologous ChAd is generally more frequently reactogenic (first dose in ChAd/ChAd-28, ChAd/ChAd-84), but the first ever exposure to ChAd (second dose in BNT/ChAd-28 and BNT/ChAd-84) is also more frequently and more severely reactogenic than the first dose with longer lasting reactogenicity. BNT, when given as a second dose at a short interval (BNT/BNT-28, ChAd/BNT-28), results in the second dose being more frequently and severely reactogenic (**Figure 51**, **Figure 52** & **Figure 53**). No obvious trends were observed for local symptoms (**Supplementary Figure 4**, **Supplementary Figure 5** & **Supplementary Figure 6**).

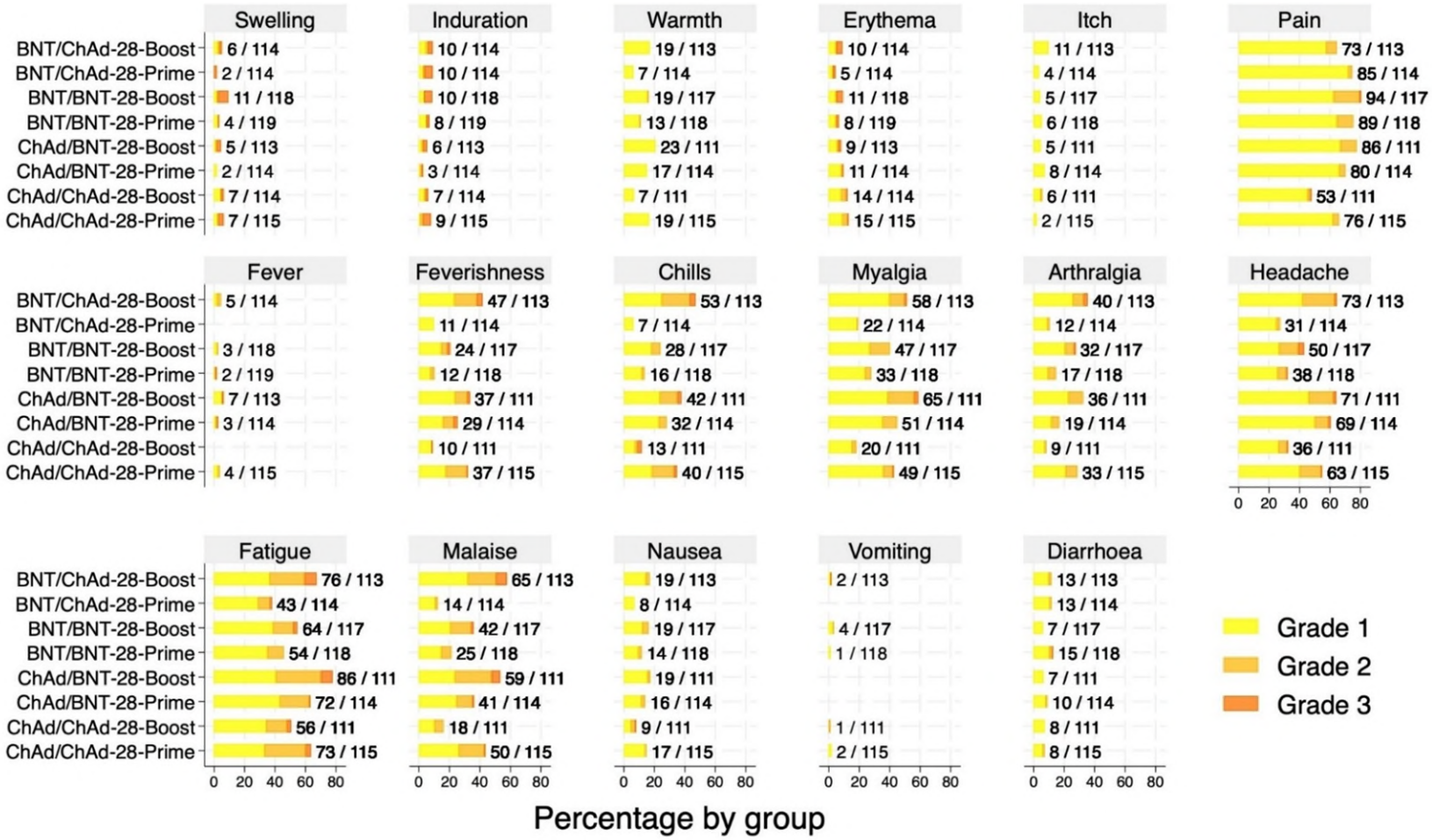

Figure 51 *– Com-COV1 Stacked bar chart showing the total number of participants suffering each local and systemic reactogenicity symptom after first and second doses. 4-week interval only . The severity presented is each participant's highest severity across 7 days after vaccination. Data are divided by schedule and dose.*

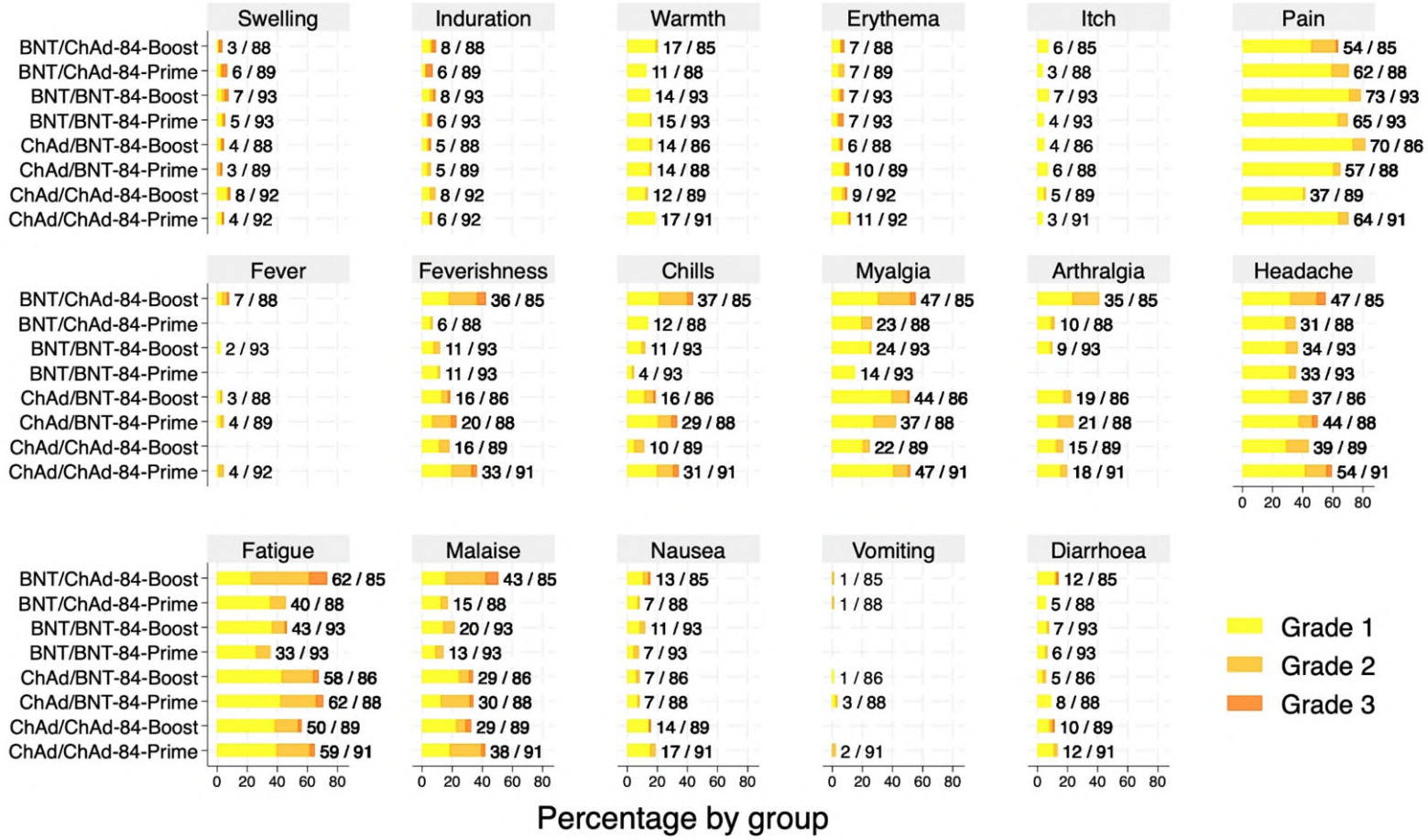

Figure 52 *– Com-COV1 Stacked bar chart showing the total number of participants suffering each local and systemic reactogenicity symptom after first and second doses. 12-week interval only . The severity presented is each participant's highest severity across 7 days after vaccination. Data are divided by schedule and dose.*

Symptom	Vaccine schedule	Vaccine	Percentage with symptoms (95% CI)	N Boost	Vaccine	Percentage with symptoms (95% CI)	N Prime	aOR (95% CI)
Feverishness	ChAd/ChAd-28	Prime	32.2 (23.8 to 41.5)	115	Boost	11.4 (6.2 to 18.7)	114	0.27 (0.13, 0.54)
Chills	ChAd/ChAd-28	Prime	34.8 (26.1 to 44.2)	115	Boost	14.0 (8.2 to 21.8)	114	0.30 (0.15, 0.57)
Myalgia	ChAd/ChAd-28	Prime	42.6 (33.4 to 52.2)	115	Boost	20.2 (13.2 to 28.7)	114	0.33 (0.18, 0.60)
Arthralgia	ChAd/ChAd-28	Prime	28.7 (20.6 to 37.9)	115	Boost	10.5 (5.6 to 17.7)	114	0.28 (0.13, 0.59)
Headache	ChAd/ChAd-28	Prime	54.8 (45.2 to 64.1)	115	Boost	34.2 (25.6 to 43.7)	114	0.39 (0.22, 0.69)
Fatigue	ChAd/ChAd-28	Prime	63.5 (54.0 to 72.3)	115	Boost	51.8 (42.2 to 61.2)	114	0.60 (0.35, 1.03)
Malaise	ChAd/ChAd-28	Prime	43.5 (34.3 to 53.0)	115	Boost	18.4 (11.8 to 26.8)	114	0.27 (0.15, 0.51)
Nausea	ChAd/ChAd-28	Prime	14.8 (8.9 to 22.6)	115	Boost	10.5 (5.6 to 17.7)	114	0.67 (0.30, 1.49)
Diarrhoea	ChAd/ChAd-28	Prime	7.0 (3.1 to 13.2)	115	Boost	9.6 (4.9 to 16.6)	114	1.48 (0.56, 3.93)
Fever	ChAd/BNT-28	Prime	2.6 (0.5 to 7.5)	114	Boost	6.2 (2.5 to 12.3)	113	2.45 (0.62, 9.73)
Feverishness	ChAd/BNT-28	Prime	25.4 (17.7 to 34.4)	114	Boost	34.5 (25.8 to 44.0)	113	1.55 (0.87, 2.76)
Chills	ChAd/BNT-28	Prime	28.1 (20.1 to 37.3)	114	Boost	38.9 (29.9 to 48.6)	113	1.67 (0.95, 2.95)
Myalgia	ChAd/BNT-28	Prime	44.7 (35.4 to 54.3)	114	Boost	59.3 (49.6 to 68.4)	113	1.81 (1.06, 3.07)
Arthralgia	ChAd/BNT-28	Prime	16.7 (10.3 to 24.8)	114	Boost	33.6 (25.0 to 43.1)	113	2.62 (1.38, 4.96)
Headache	ChAd/BNT-28	Prime	60.5 (50.9 to 69.6)	114	Boost	64.6 (55.0 to 73.4)	113	1.20 (0.69, 2.07)
Fatigue	ChAd/BNT-28	Prime	63.2 (53.6 to 72.0)	114	Boost	77.9 (69.1 to 85.1)	113	2.09 (1.16, 3.78)
Malaise	ChAd/BNT-28	Prime	36.0 (27.2 to 45.5)	114	Boost	54.0 (44.4 to 63.4)	113	2.12 (1.24, 3.62)
Nausea	ChAd/BNT-28	Prime	14.0 (8.2 to 21.8)	114	Boost	18.6 (11.9 to 27.0)	113	1.44 (0.69, 3.01)
Diarrhoea	ChAd/BNT-28	Prime	8.8 (4.3 to 15.5)	114	Boost	8.0 (3.7 to 14.6)	113	0.90 (0.35, 2.31)
Fever	BNT/BNT-28	Prime	1.7 (0.2 to 5.9)	119	Boost	2.5 (0.5 to 7.3)	118	1.55 (0.25, 9.55)
Feverishness	BNT/BNT-28	Prime	10.9 (5.9 to 18.0)	119	Boost	21.2 (14.2 to 29.7)	118	2.27 (1.08, 4.76)
Chills	BNT/BNT-28	Prime	14.3 (8.5 to 21.9)	119	Boost	24.6 (17.1 to 33.4)	118	1.99 (1.02, 3.92)
Myalgia	BNT/BNT-28	Prime	28.6 (20.7 to 37.6)	119	Boost	40.7 (31.7 to 50.1)	118	1.75 (1.01, 3.04)
Arthralgia	BNT/BNT-28	Prime	15.1 (9.2 to 22.8)	119	Boost	28.0 (20.1 to 37.0)	118	2.25 (1.17, 4.34)
Headache	BNT/BNT-28	Prime	32.8 (24.4 to 42.0)	119	Boost	43.2 (34.1 to 52.7)	118	1.62 (0.93, 2.79)
Fatigue	BNT/BNT-28	Prime	46.2 (37.0 to 55.6)	119	Boost	55.1 (45.7 to 64.3)	118	1.46 (0.86, 2.47)
Malaise	BNT/BNT-28	Prime	21.8 (14.8 to 30.4)	119	Boost	36.4 (27.8 to 45.8)	118	2.17 (1.19, 3.93)
Nausea	BNT/BNT-28	Prime	12.6 (7.2 to 19.9)	119	Boost	16.9 (10.7 to 25.0)	118	1.44 (0.69, 3.00)
Diarrhoea	BNT/BNT-28	Prime	13.4 (7.9 to 20.9)	119	Boost	6.8 (3.0 to 12.9)	118	0.46 (0.19, 1.14)
Feverishness	BNT/ChAd-28	Prime	9.6 (4.9 to 16.6)	114	Boost	42.1 (32.9 to 51.7)	114	7.16 (3.43, 14.98)
Chills	BNT/ChAd-28	Prime	6.1 (2.5 to 12.2)	114	Boost	47.4 (37.9 to 56.9)	114	14.65 (6.18, 34.72)
Myalgia	BNT/ChAd-28	Prime	19.3 (12.5 to 27.7)	114	Boost	51.8 (42.2 to 61.2)	114	4.53 (2.50, 8.23)
Arthralgia	BNT/ChAd-28	Prime	10.5 (5.6 to 17.7)	114	Boost	36.0 (27.2 to 45.5)	114	4.85 (2.37, 9.90)
Headache	BNT/ChAd-28	Prime	27.2 (19.3 to 36.3)	114	Boost	64.9 (55.4 to 73.6)	114	5.09 (2.87, 9.01)
Fatigue	BNT/ChAd-28	Prime	37.7 (28.8 to 47.3)	114	Boost	67.5 (58.1 to 76.0)	114	3.73 (2.11, 6.58)
Malaise	BNT/ChAd-28	Prime	12.3 (6.9 to 19.7)	114	Boost	57.9 (48.3 to 67.1)	114	11.62 (5.70, 23.71)
Nausea	BNT/ChAd-28	Prime	7.0 (3.1 to 13.4)	114	Boost	17.5 (11.1 to 25.8)	114	2.93 (1.21, 7.08)
Diarrhoea	BNT/ChAd-28	Prime	11.4 (6.2 to 18.7)	114	Boost	12.3 (6.9 to 19.7)	114	1.09 (0.48, 2.46)
Feverishness	ChAd/ChAd-84	Prime	37.0 (27.1 to 47.7)	92	Boost	20.7 (12.9 to 30.4)	92	0.41 (0.21, 0.82)
Chills	ChAd/ChAd-84	Prime	34.8 (25.1 to 45.4)	92	Boost	14.1 (7.7 to 23.0)	92	0.27 (0.12, 0.58)
Myalgia	ChAd/ChAd-84	Prime	52.2 (41.5 to 62.7)	92	Boost	27.2 (18.4 to 37.4)	92	0.33 (0.18, 0.62)
Arthralgia	ChAd/ChAd-84	Prime	20.7 (12.9 to 30.4)	92	Boost	19.6 (12.0 to 29.1)	92	0.93 (0.45, 1.93)
Headache	ChAd/ChAd-84	Prime	59.8 (49.0 to 69.9)	92	Boost	45.7 (35.2 to 56.4)	92	0.55 (0.31, 1.01)
Fatigue	ChAd/ChAd-84	Prime	65.2 (54.6 to 74.9)	92	Boost	57.6 (46.9 to 67.9)	92	0.71 (0.39, 1.31)
Malaise	ChAd/ChAd-84	Prime	42.4 (32.1 to 53.1)	92	Boost	34.8 (25.1 to 45.4)	92	0.71 (0.39, 1.31)
Nausea	ChAd/ChAd-84	Prime	19.6 (12.0 to 29.1)	92	Boost	18.5 (11.1 to 27.9)	92	0.93 (0.44, 1.95)
Diarrhoea	ChAd/ChAd-84	Prime	14.1 (7.7 to 23.0)	92	Boost	14.1 (7.7 to 23.0)	92	1.00 (0.44, 2.30)
Fever	ChAd/BNT-84	Prime	4.5 (1.2 to 11.1)	89	Boost	3.4 (0.7 to 9.6)	88	0.75 (0.16, 3.47)
Feverishness	ChAd/BNT-84	Prime	23.6 (15.2 to 33.8)	89	Boost	20.5 (12.6 to 30.4)	88	0.83 (0.41, 1.71)
Chills	ChAd/BNT-84	Prime	33.7 (24.0 to 44.5)	89	Boost	20.5 (12.6 to 30.4)	88	0.50 (0.25, 0.99)
Myalgia	ChAd/BNT-84	Prime	42.7 (32.3 to 53.6)	89	Boost	52.3 (41.4 to 63.0)	88	1.47 (0.81, 2.66)
Arthralgia	ChAd/BNT-84	Prime	24.7 (16.2 to 35.0)	89	Boost	23.9 (15.4 to 34.1)	88	0.95 (0.48, 1.90)
Headache	ChAd/BNT-84	Prime	50.6 (39.8 to 61.3)	89	Boost	44.3 (33.7 to 55.3)	88	0.76 (0.41, 1.40)
Fatigue	ChAd/BNT-84	Prime	70.8 (60.2 to 79.9)	89	Boost	68.2 (57.4 to 77.7)	88	0.88 (0.46, 1.68)
Malaise	ChAd/BNT-84	Prime	34.8 (25.0 to 45.7)	89	Boost	35.2 (25.3 to 46.1)	88	1.02 (0.54, 1.90)
Nausea	ChAd/BNT-84	Prime	9.0 (4.0 to 16.9)	89	Boost	10.2 (4.8 to 18.5)	88	1.15 (0.41, 3.23)
Diarrhoea	ChAd/BNT-84	Prime	10.1 (4.7 to 18.3)	89	Boost	8.0 (3.3 to 15.7)	88	0.74 (0.25, 2.19)
Feverishness	BNT/BNT-84	Prime	11.8 (6.1 to 20.2)	93	Boost	11.8 (6.1 to 20.2)	93	1.00 (0.41, 2.44)
Chills	BNT/BNT-84	Prime	4.3 (1.2 to 10.6)	93	Boost	11.8 (6.1 to 20.2)	93	3.15 (0.94, 10.59)
Myalgia	BNT/BNT-84	Prime	15.1 (8.5 to 24.0)	93	Boost	25.8 (17.3 to 35.9)	93	2.08 (0.97, 4.48)
Headache	BNT/BNT-84	Prime	35.5 (25.8 to 46.1)	93	Boost	36.6 (26.8 to 47.2)	93	1.05 (0.57, 1.93)
Fatigue	BNT/BNT-84	Prime	35.5 (25.8 to 46.1)	93	Boost	46.2 (35.8 to 56.9)	93	1.57 (0.87, 2.84)
Malaise	BNT/BNT-84	Prime	14.0 (7.7 to 22.7)	93	Boost	21.5 (13.7 to 31.2)	93	1.71 (0.79, 3.72)
Nausea	BNT/BNT-84	Prime	7.5 (3.1 to 14.9)	93	Boost	11.8 (6.1 to 20.2)	93	1.67 (0.61, 4.58)
Diarrhoea	BNT/BNT-84	Prime	6.5 (2.4 to 13.5)	93	Boost	7.5 (3.1 to 14.9)	93	1.18 (0.38, 3.69)
Feverishness	BNT/ChAd-84	Prime	7.9 (3.2 to 15.5)	89	Boost	44.3 (33.7 to 55.3)	88	9.74 (4.00, 23.71)
Chills	BNT/ChAd-84	Prime	14.6 (8.0 to 23.7)	89	Boost	45.5 (34.8 to 56.4)	88	5.30 (2.51, 11.21)
Myalgia	BNT/ChAd-84	Prime	27.0 (18.1 to 37.4)	89	Boost	56.8 (45.8 to 67.3)	88	3.68 (1.94, 6.97)
Arthralgia	BNT/ChAd-84	Prime	12.4 (6.3 to 21.0)	89	Boost	43.2 (32.7 to 54.2)	88	5.47 (2.55, 11.73)
Headache	BNT/ChAd-84	Prime	36.0 (26.1 to 46.8)	89	Boost	56.8 (45.8 to 67.3)	88	2.35 (1.28, 4.33)
Fatigue	BNT/ChAd-84	Prime	46.1 (35.4 to 57.0)	89	Boost	73.9 (63.4 to 82.7)	88	3.36 (1.77, 6.37)
Malaise	BNT/ChAd-84	Prime	18.0 (10.6 to 27.5)	89	Boost	52.3 (41.4 to 63.0)	88	5.12 (2.56, 10.22)
Nausea	BNT/ChAd-84	Prime	9.0 (4.0 to 16.9)	89	Boost	18.2 (10.8 to 27.8)	88	2.25 (0.91, 5.60)
Diarrhoea	BNT/ChAd-84	Prime	6.7 (2.5 to 14.1)	89	Boost	17.0 (9.9 to 26.6)	88	2.91 (1.06, 7.95)

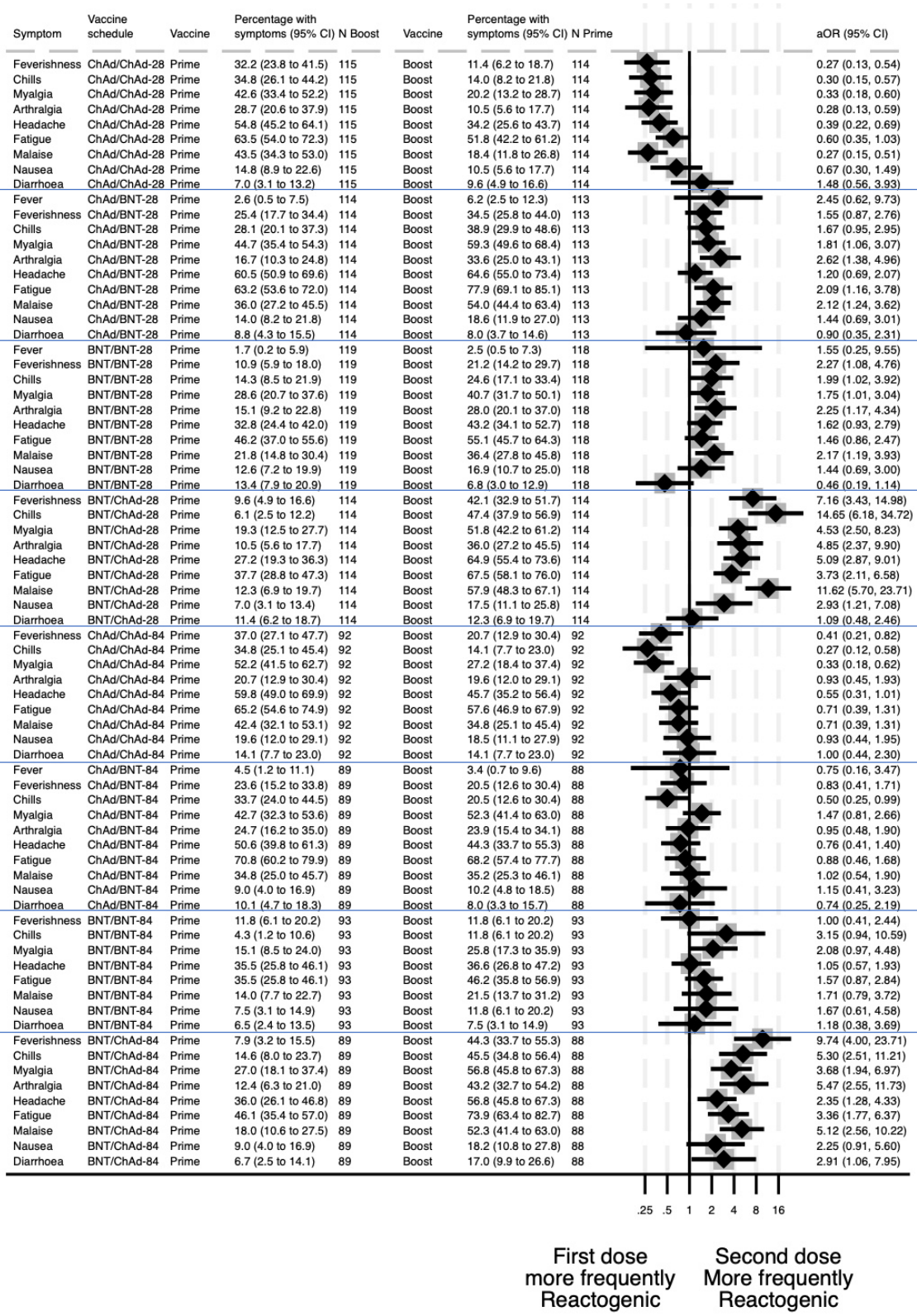

Figure 53 *– Com-COV1 Forest plot comparing presence/absence of systemic reactogenicity symptoms in the first 7 days after the first vs second COVID-19 vaccination . "Were you more likely to be symptomatic for your first or second dose?"*

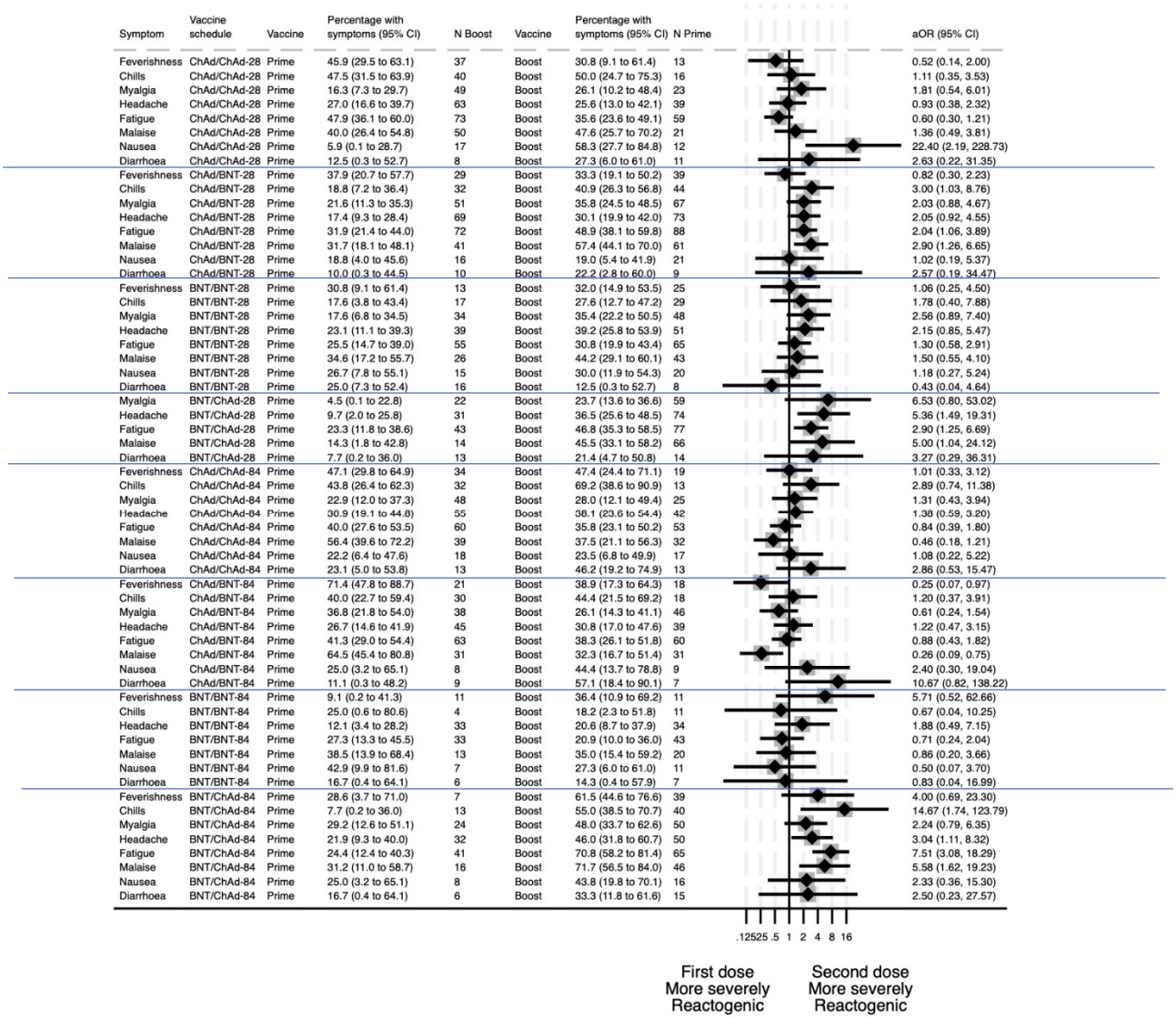

Figure 54 *– Com-COV1 Forest plot comparing the proportions of seronegative vs seropositive participants suffering moderate-severe (Grade 2 or above) systemic reactogenicity symptoms out of the total number of participants suffering any grade symptoms, in the first 7 days after a COVID-19 vaccination for first vs second doses. "If you did suffer a symptom of any grade after a vaccine dose, was it more likely to be moderate/severe if you it was you first or second dose?"*

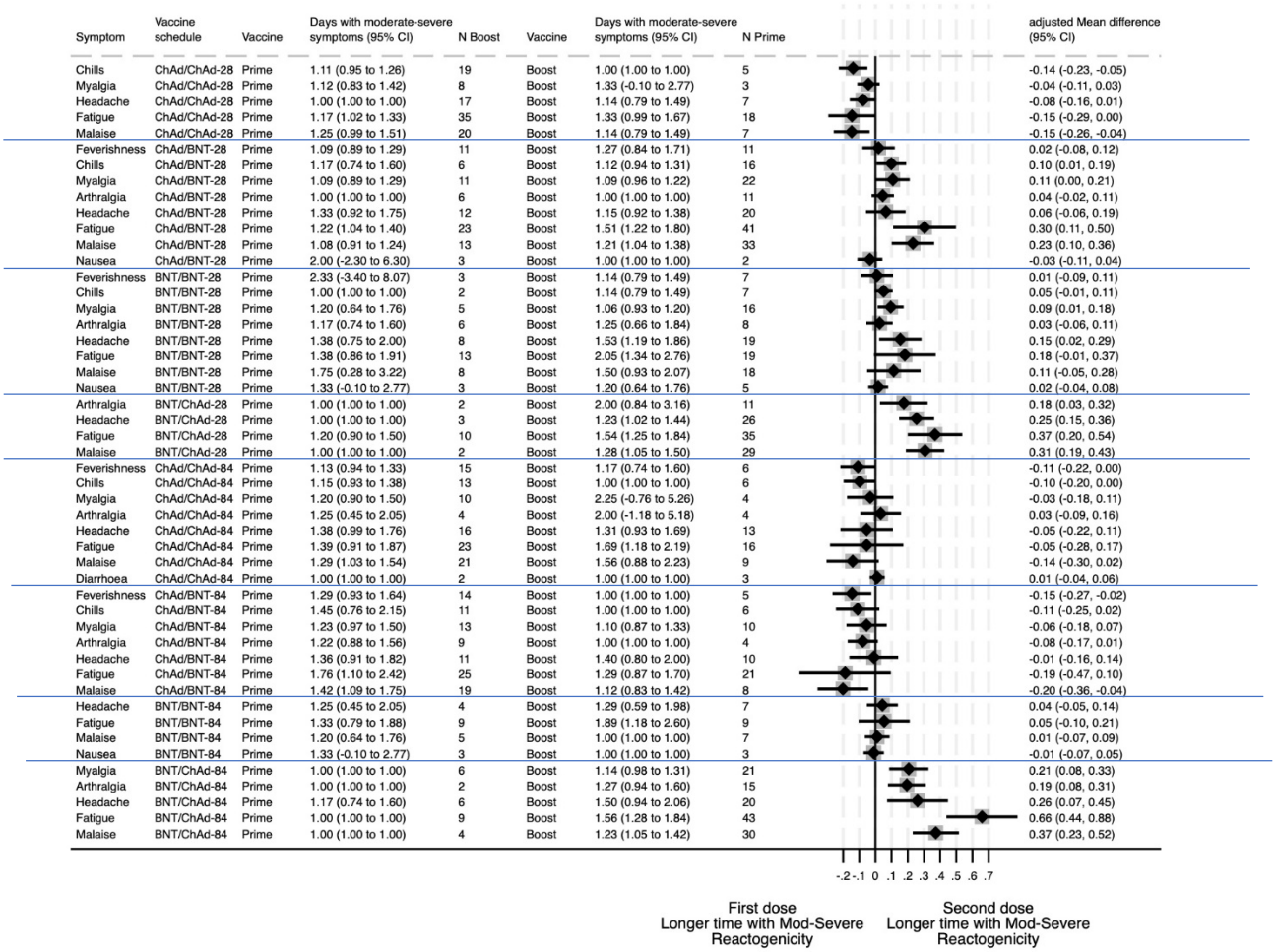

Figure 55 *– Com-COV1 Forest plot comparing durations of time spent with grade 2 or higher systemic reactogenicity symptoms after a COVID-19 vaccination between first and second dose . "If you did suffer with moderate/severe symptoms (Grade 2 or above), were the symptoms likely to last longer after a single dose of either ChAd or BNT"*

3.4.3.2 Com-COV2

3.4.3.2.1 2nd dose: Homologous vs Heterologous

Since participants recruited to Com-COV2 had already received their first dose of vaccine outside of the study, there are no available data on the reactogenicity on the first dose and therefore also no comparison of first vs second dose reactogenicity. There were larger numbers of seropositive participants at the start of the study than in Com-COV1, and so these are graphically demonstrated in comparison to the seronegative population (**Figure 56** & **Figure 57**).

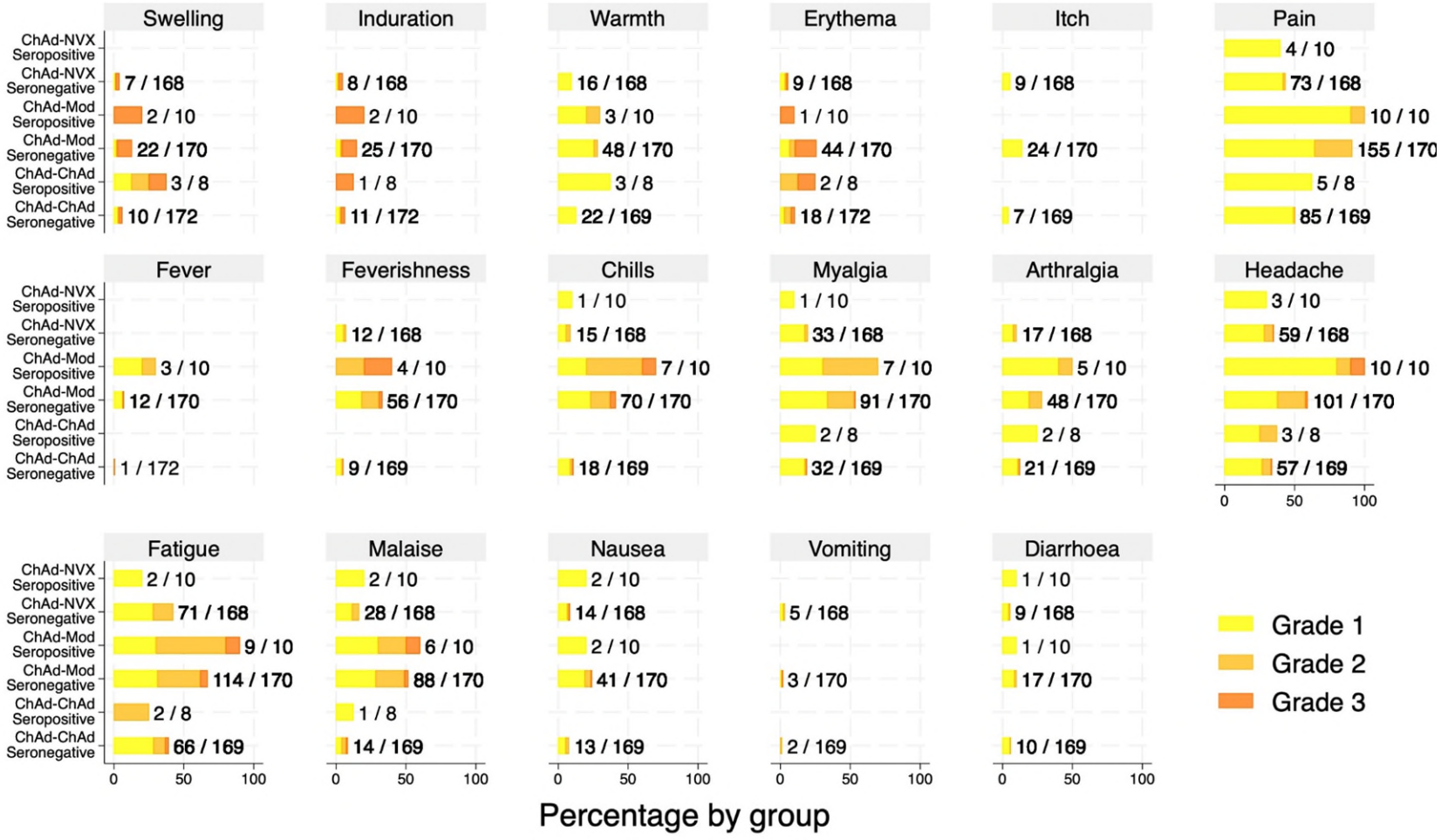

Figure 56 *– Com-COV2 Stacked bar chart showing the total number of participants suffering each local and systemic reactogenicity symptom after the second dose of ChAd-primed participants . The severity presented is each participant's highest severity across 7 days after vaccination. Data are divided by schedule and pre-second vaccination serostatus*

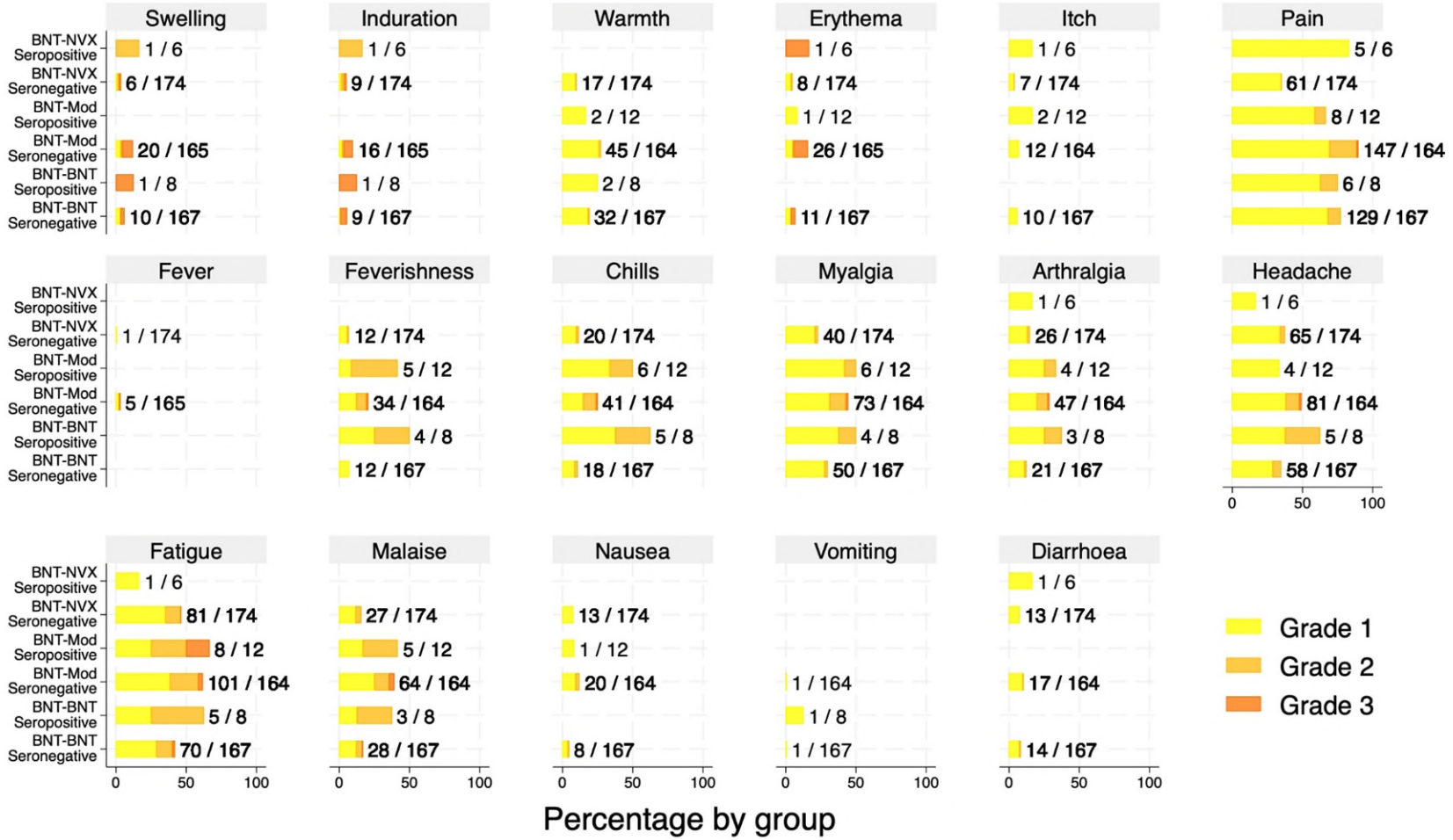

Figure 57 *– Com-COV2 Stacked bar chart showing the total number of participants suffering each local and systemic reactogenicity symptom after the second dose of BNT-primed participants . The severity presented is each participant's highest severity across 7 days after vaccination. Data are divided by schedule and pre-second vaccination serostatus*

Moderna boosted heterologous schedules were more frequently reactogenic than homologous schedules, but Novavax-boosted heterologous schedules were similar to homologous schedules in terms of frequency of systemic symptoms. There was a trend that the Modern-boosted heterologous schedules might also give more severe symptoms, but this was non-significant. Where participants did have more severe symptoms, however, the duration of these symptoms was slightly longer for Moderna-boosted schedules (**Figure 58**, **Figure 59** & **Figure 60**). A similar trend was observed for the local reactogenicity symptoms (**Supplementary Figure 7**, **Supplementary Figure 8** & **Supplementary Figure 9**)

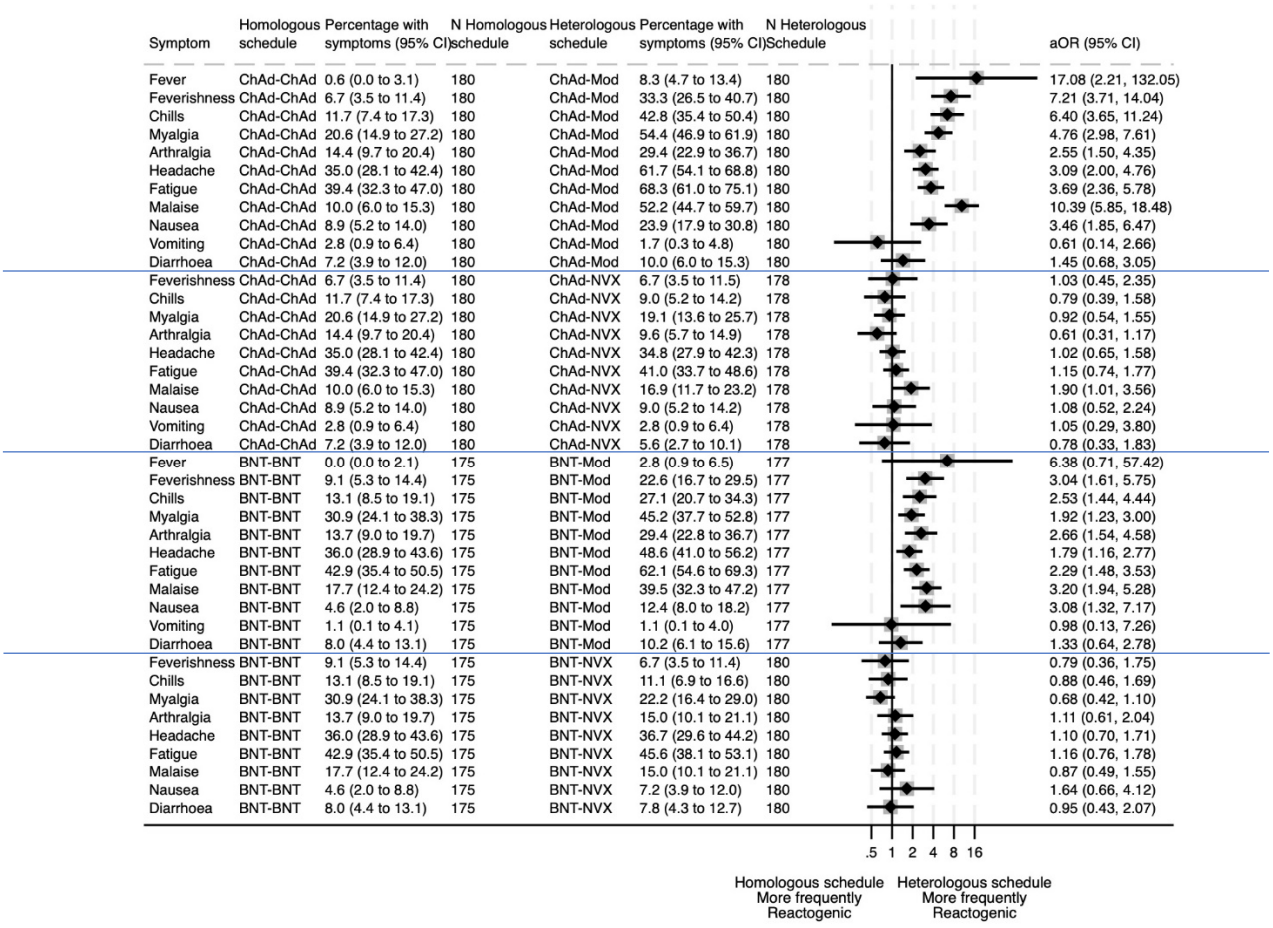

Figure 58 *– Com-COV2 Forest plot comparing the proportions of participants (seronegative and seropositive combined) suffering systemic reactogenicity symptoms in the first 7 days after the second COVID-19 vaccination between homologous and heterologous schedules "Were you more likely to suffer a symptom of any grade after the second dose if the schedule was homologous or heterologous?"*

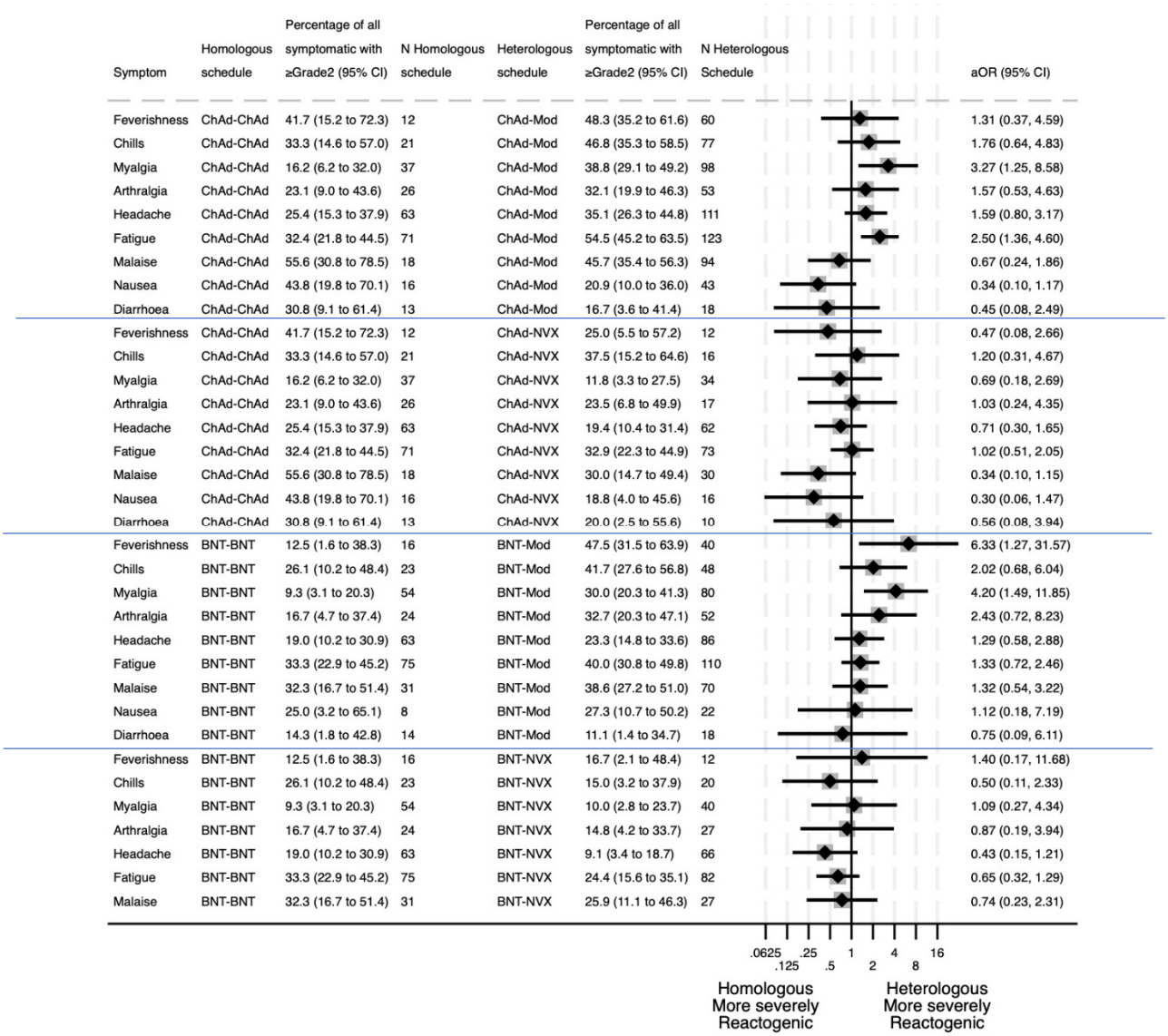

Figure 59 *– Com-COV2 Forest plot comparing proportions of participants (seronegative and seropositive combined) suffering moderate-severe (Grade 2 or above) systemic reactogenicity symptoms out of the total number of participants suffering any grade symptoms, in the first 7 days after a second COVID-19 vaccination between homologous and heterologous schedules. "If you did suffer a symptom of any grade after the second dose, was it more likely to be moderate/severe if the schedule was homologous or heterolgous?"*

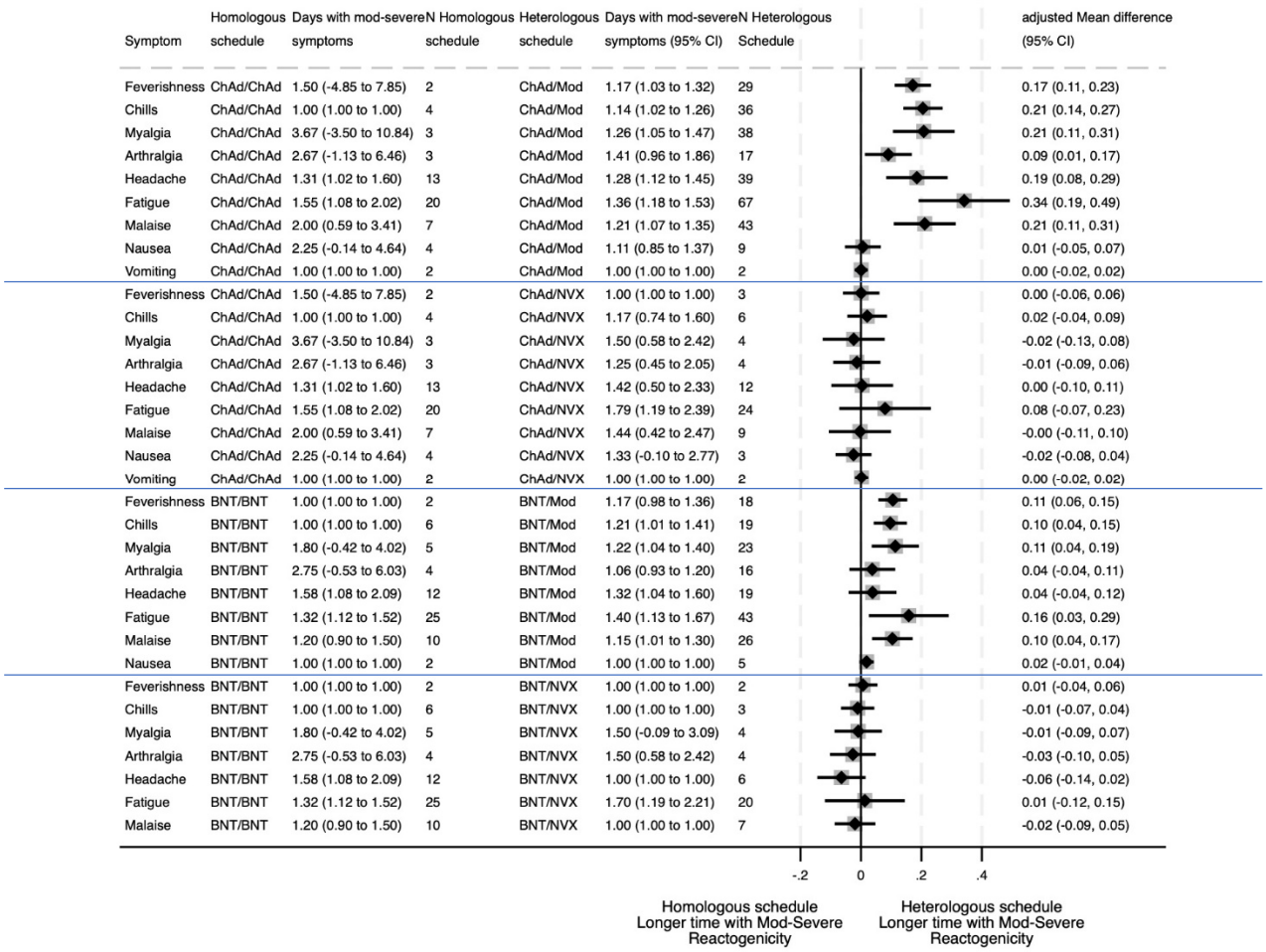

Figure 60 *– Com-COV2 Forest plot comparing durations of time spent with grade 2 or higher systemic reactogenicity symptoms after the second COVID-19 vaccination between participants (seronegative and seropositive combined) receiving homologous or heterologous schedules . "If you did suffer with moderate/severe symptoms (Grade 2 or above) after the second dose, were the symptoms likely to last longer after a homologous or heterologous schedule?"*

3.4.3.2.2 2nd dose: Serostatus

There were sufficient seropositive participants to allow a statistical comparison in Com-COV2. Seropositive participants receiving homologous BNT/BNT showed an increase in frequency of systemic reactogenicity at the point of the second dose in comparison to seronegative participants, although many symptoms did not reach a level of statistical significance. Homologous ChAd/ChAd showed no difference in frequency of reactogenicity between seropositive and seronegative participants. For all other schedules, there were not convincing signals to say whether seropositive and seronegative participants had a difference in their frequency of reactogenicity. Analysis

of local reactogenicity suggested that seropositive BNT/NVX recipients might have more frequent symptoms than seronegative participants (**Supplementary Figure 10**).

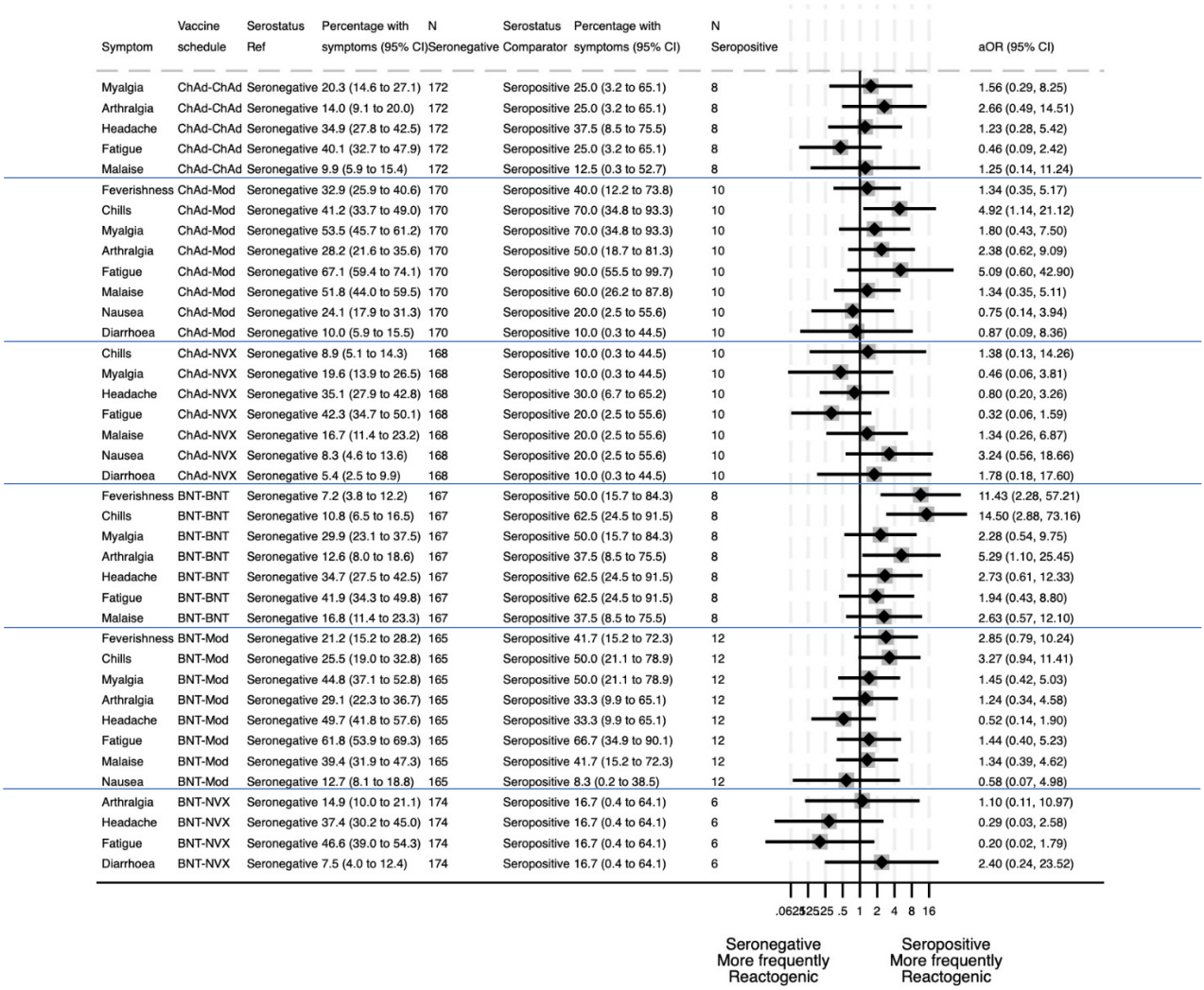

Figure 61 *– Com-COV2 Forest plot comparing the proportions of seronegative vs seropositive participants suffering systemic reactogenicity symptoms in the first 7 days after the second COVID-19 vaccination "Were you more likely to suffer a symptom of any grade after the second dose if you were seropositive or seronegative at the point of second dose administration?"*

3.4.4 Safety

3.4.4.1 Com-COV1

Over the course of the trial there were 1004 adverse events in 462 participants (**Table 26**), proportionally split across arms. Descriptions of all non-serious adverse events (AEs) of grade 3 or above are presented in **Table 27**. There were five AEs of special interest, excluding SARS-CoV-2/COVID-19 events (**Table 28**) and eleven serious AEs across all arms (**Table 29**). One of these SAEs was deemed possibly related to immunisation (IgA nephropathy/minimal change disease overlap, possibly precipitated by COVID infection soon after the first dose of BNT). The participant is under further follow up with regards to an ongoing fall in renal function. 40 participants tested positive for SARS-CoV-2 (all but four cases occurred at least 2 weeks post second dose). Combining over both intervals these were distributed evenly by group: ChAd/ChAd (eleven), ChAd/BNT (nine), BNT/BNT (eleven), BNT/ChAd (seven) (**Table 30**). No participants were hospitalised.

Table 26 *– Com-COV1 Summary of Adverse Events*

	4-week interval arms				12-week interval arms				
	ChAd/ChAd (N=115)	**ChAd/BNT (N=114)**	**BNT/BNT (N=119)**	**BNT/ChAd (N=115)**	**ChAd/ChAd (N=92)**	**ChAd/BNT (N=90)**	**BNT/BNT (N=93)**	**BNT/ChAd (N=92)**	**Total (N=830)**
Number of adverse events*	127	133	158	154	99	113	98	122	1004
Number of unique participants with at least one adverse event	64 (55.6%)	68 (59.6%)	61 (51.2%)	67 (58.2%)	54 (58.7%)	55 (61.1%)	40 (43.0%)	53 (57.6%)	462 (48.1%)
Timing of AE									
Between first and second doses	53 (43.1%)	54 (42.5%)	59 (38.3%)	61 (42.4%)	52 (57.1%)	63 (57.8%)	50 (53.2%)	61 (51.7%)	453 (47.2%)
Post 1st dose†	3 (2.4%)							4 (3.4%)	7 (0.7%)
Post 2nd dose	71 (57.7%)	79 (62.2%)	99 (64.3%)	93 (64.6%)	47 (51.6%)	50 (45.9%)	48 (51.1%)	57 (48.3%)	544 (56.7%)
Severity									
Grade 1	57 (46.3%)	80 (63.0%)	80 (51.9%)	69 (47.9%)	57 (62.6%)	63 (57.8%)	60 (63.8%)	68 (57.6%)	534 (55.6%)
Grade 2	56 (45.5%)	41 (32.3%)	71 (46.1%)	74 (51.4%)	33 (36.3%)	42 (38.5%)	30 (31.9%)	42 (35.6%)	389 (40.5%)
Grade 3	13 (10.6%)	10 (7.9%)	6 (3.9%)	10 (6.9%)	9 (9.9%)	8 (7.3%)	7 (7.4%)	11 (9.3%)	74 (7.7%)
Grade 4	1 (0.8%)	2 (1.6%)	1 (0.6%)	1 (0.7%)			1 (1.1%)	1 (0.8%)	7 (0.7%)
Causality									
No relationship	62 (50.4%)	49 (38.6%)	58 (37.7%)	52 (36.1%)	45 (49.5%)	48 (44.0%)	50 (53.2%)	63 (53.4%)	427 (44.5%)
Unlikely	42 (34.1%)	52 (40.9%)	53 (34.4%)	64 (44.4%)	30 (33.0%)	31 (28.4%)	29 (30.9%)	32 (27.1%)	333 (34.7%)
Possible	13 (10.6%)	16 (12.6%)	38 (24.7%)	23 (16.0%)	5 (5.5%)	17 (15.6%)	11 (11.7%)	16 (13.6%)	139 (14.5%)
Probable	5 (4.1%)	9 (7.1%)	8 (5.2%)	11 (7.6%)	14 (15.4%)	14 (12.8%)	7 (7.4%)	9 (7.6%)	77 (8.0%)
Definite	5 (4.1%)	7 (5.5%)	1 (0.6%)	4 (2.8%)	5 (5.5%)	3 (2.8%)	1 (1.1%)	2 (1.7%)	28 (2.9%)

**Denominator for percentage calculations. †Did not receive second dose.*

Table 27 *– Com-COV1 Summary of Non-serious Adverse Events of grade ≥3*

Study Arm	Causality	Days since first dose	Days since second dose	MedDRA Preferred Term	MedDRA System Order Class
ChAd/ChAd 4-week	Unlikely	33	5	Migraine	Vascular disorders
ChAd/ChAd 4-week	No relationship	67	39	Chest pain	General disorders and administration site conditions
ChAd/ChAd 4-week	No relationship	1	-	Back pain	Musculoskeletal and connective tissue disorders
ChAd/ChAd 4-week	No relationship	0	-	Cold type haemolytic anaemia	Immune system disorders
ChAd/ChAd 4-week	Possible	53	23	Pain in extremity	Musculoskeletal and connective tissue disorders
ChAd/ChAd 4-week	Unlikely	48	19	Headache	Nervous system disorders
ChAd/ChAd 4-week	No relationship	206	176	Post viral fatigue syndrome	Nervous system disorders
ChAd/ChAd 4-week	No relationship	32	3	Limb injury	Injury, poisoning and procedural complications
ChAd/ChAd 4-week	No relationship	182	153	Infected dermal cyst	Infections and infestations
ChAd/ChAd 4-week	No relationship	3	-	Environmental exposure~	Injury, poisoning and procedural complications
ChAd/ChAd 4-week	Possible	0	-	Fatigue	General disorders & administration site conditions
ChAd/ChAd 4-week	No relationship	55	27	Back pain	Musculoskeletal and connective tissue disorders
ChAd/ChAd 4-week	No relationship	27	-	Glaucoma	Eye disorders
ChAd/ChAd 12-week	No relationship	99	15	Bunion operation	Surgical and medical procedures
ChAd/ChAd 12-week	No relationship	257	173	Coronavirus infections	Infections and infestations
ChAd/ChAd 12-week	No relationship	31	-	Tonsillitis	Infections and infestations
ChAd/ChAd 12-week	No relationship	81	-	Tooth abscess	Infections and infestations
ChAd/ChAd 12-week	No relationship	62	-	Thyroid mass	Endocrine disorders
ChAd/ChAd 12-week	No relationship	103	19	Vertigo	Ear and labyrinth disorders
ChAd/ChAd 12-week	Unlikely	244	160	Abdominal pain	Gastrointestinal disorders
ChAd/ChAd 12-week	No relationship	93	9	Migraine	Vascular disorders
ChAd/BNT 4-week	Definite	0	-	Chills§	General disorders and administration site conditions
ChAd/BNT 4-week	Unlikely	92	64	Deep vein thrombosis	Vascular disorders

ChAd/BNT 4-week	Probable	1	-	Meniere's disease	Ear and labyrinth disorders
ChAd/BNT 4-week	No relationship	43	15	Back Pain	Musculoskeletal and connective tissue disorders
ChAd/BNT 4-week	No relationship	100	72	Basal cell carcinoma	Neoplasms benign, malignant and unspecified (incl cysts and polyps)
ChAd/BNT 4-week	Unlikely	15	-	Fatigue	General disorders and administration site conditions
ChAd/BNT 4-week	No relationship	56	28	Abdominal pain	Gastrointestinal disorders
ChAd/BNT 4-week	No relationship	38	10	Headache	Nervous system disorders
ChAd/BNT 4-week	No relationship	43	14	Foot fracture	Musculoskeletal and connective tissue disorders
ChAd/BNT 4-week	Unlikely	48	20	Fatigue	General disorders and administration site conditions
ChAd/BNT 12-week	No relationship	108	25	Cluster headache	Nervous system disorders
ChAd/BNT 12-week	No relationship	165	82	Radioactive iodine therapy	Surgical and medical procedures
ChAd/BNT 12-week	Unlikely	58		Periarthritis	Musculoskeletal and connective tissue disorders
ChAd/BNT 12-week	No relationship	8		Urinary tract infection	Infections and infestations
ChAd/BNT 12-week	No relationship	109	25	Respiratory tract infection	Infections and infestations
ChAd/BNT 12-week	Unlikely	136	52	Renal mass	Renal and urinary disorders
ChAd/BNT 12-week	Unlikely	101	17	Lethargy	General disorders and administration site conditions
ChAd/BNT 12-week	Possible	0		Tremor	Nervous system disorders
BNT/BNT 4-week	No relationship	166	138	Hypertension	Vascular disorders
BNT/BNT 4-week	No relationship	26		Pneumonia	Infections and infestations
BNT/BNT 4-week	Unlikely	3		Coronavirus infections	Infections and infestations
BNT/BNT 4-week	No relationship	48	20	Rotator cuff syndrome	Injury, poisoning and procedural complications
BNT/BNT 4-week	No relationship	10		Bursitis	Musculoskeletal and connective tissue disorders
BNT/BNT 4-week	No relationship	211	181	Road traffic accident	Injury, poisoning and procedural complications
BNT/BNT 12-week	Unlikely	62		Depressed mood	Psychiatric disorders
BNT/BNT 12-week	Unlikely	89	5	Diarrhoea	Gastrointestinal disorders
BNT/BNT 12-week	Unlikely	102	16	Sinusitis	Respiratory, thoracic and mediastinal disorders
BNT/BNT 12-week	Unlikely	104	20	Vertigo	Ear and labyrinth disorders

BNT/BNT 12-week	No relationship	2		Rotator cuff repair	Surgical and medical procedures
BNT/BNT 12-week	No relationship	109	23	Tooth extraction	Surgical and medical procedures
BNT/ChAd 4-week	Probable	29	1	Decreased appetite	Metabolism and nutrition disorders
BNT/ChAd 4-week	Probable	31	1	Migraine	Vascular disorders
BNT/ChAd 4-week	No relationship	44	16	Pyrexia	General disorders and administration site conditions
BNT/ChAd 4-week	Unlikely	47	19	Fatigue	General disorders and administration site conditions
BNT/ChAd 4-week	No relationship	28	0	Depressed mood	Psychiatric disorders
BNT/ChAd 4-week	No relationship	92	64	Hypersensitivity	Immune system disorders
BNT/ChAd 4-week	Probable	28	0	Arthralgia	Musculoskeletal and connective tissue disorders
BNT/ChAd 4-week	Unlikely	45	17	Headache	Nervous system disorders
BNT/ChAd 4-week	Unlikely	45	17	Viral infection*	Infections and infestations
BNT/ChAd 4-week	Possible	33	5	Back Pain	Musculoskeletal and connective tissue disorders
BNT/ChAd 12-week	No relationship	87	3	Melanocytic naevus	Skin and subcutaneous tissue disorders
BNT/ChAd 12-week	Possible	84	1	Tachycardia	Cardiac disorders
BNT/ChAd 12-week	No relationship	80		Skin injury	Injury, poisoning and procedural complications
BNT/ChAd 12-week	No relationship	91	0	Trigeminal palsy	Nervous system disorders
BNT/ChAd 12-week	No relationship	56		Transurethral prostatectomy	Surgical and medical procedures
BNT/ChAd 12-week	Probable	85	0	Ear pain	Ear and labyrinth disorders
BNT/ChAd 12-week	Unlikely	104	19	Upper respiratory tract infection	Infections and infestations
BNT/ChAd 12-week	Probable	84	0	Sinus headache	Respiratory, thoracic and mediastinal disorders
BNT/ChAd 12-week	No relationship	40		Ligament sprain	Injury, poisoning and procedural complications
BNT/ChAd 12-week	Unlikely	14		Anaphylactoid reaction	Immune system disorders
BNT/ChAd 12-week	No relationship	99		Knee arthroplasty	Surgical and medical procedures

~ Participant developed respiratory irritation after performing DIY. § Episode of rigors with fever, entered in unsolicited diary. Tested for COVID-19 and negative. No AEs were of grade 4 severity.*

Table 28 *– Com-COV1 Adverse Events of Special Interest**

Study arm	Severity	Causality	Serious AE	Days since 1st dose	Days since 2nd dose	MedDRA Preferred Term	MedDRA System Order Class
ChAd/BNT 4-week	Grade 3	Unlikely	No	92	64	Deep vein thrombosis	Vascular disorders
ChAd/BNT 4-week	Grade 4	Unlikely	Hospitalisation	84	56	Cardiac failure	Cardiac disorders
BNT/ChAd 4-week	Grade 3	No relationship	No	92	64	Hypersensitivity	Immune system disorders
BNT/ChAd 12-week	Grade 3	No relationship	No	91	0	Trigeminal palsy	Nervous system disorders
BNT/ChAd 12-week	Grade 3	Unlikely	No	14	-	Anaphylactoid reaction	Immune system disorders

** excluding SARS-CoV-2 infection/COVID-19*

Table 29 *– Com-COV1 Serious Adverse Events*

Study arm	Severity	Causality	Serious adverse event	Days since 1st dose	Days since 2nd dose	MedDRA Preferred Term	MedDRA System Order Class
ChAd/ChAd 4-week	Grade 4	Unlikely	Hospitalisation	7	-	Arthritis bacterial	Infections & infestations
ChAd/ChAd 12-week	Grade 2	Unlikely	Hospitalisation	106	22	Orchitis	Reproductive system & breast disorders
ChAd/ChAd 12-week	Grade 3	No relationship	Hospitalisation	85	^	Tubo-ovarian abscess	Infections & infestations
ChAd/BNT 4-week	Grade 4	No relationship	Important medical event	144	116	Cellulitis	Infections & infestations
ChAd/BNT 4-week	Grade 4	Unlikely	Hospitalisation	84	56	Cardiac failure	Cardiac disorders
BNT/BNT 4-week	Grade 4	No relationship	Hospitalisation	265	236	Ankle fracture	Musculoskeletal & connective tissue disorders
BNT/BNT 12-week	Grade 4	No relationship	Hospitalisation	88	0	Acute kidney injury	Renal & urinary disorders
BNT/BNT 12-week	Grade 3	No relationship	Important medical event	197	113	Joint dislocation	Musculoskeletal & connective tissue disorders
BNT/ChAd 4-week	Grade 4	No relationship	Hospitalisation	109	81	Clavicle fracture	Musculoskeletal & connective tissue disorders
BNT/ChAd 4-week	Grade 2	No relationship	Hospitalisation	132	104	Hand fracture	Musculoskeletal & connective tissue disorders
BNT/ChAd 12-week	Grade 3	Possible	Important medical event	113	29	IgA nephropathy	Immune system disorders

^Second dose at D94

Table 30 *– Com-COV1 SARS-CoV2 positive cases*

Study arm	Severity	Causality	Days since 1st dose	Days since 2nd dose
ChAd/ChAd 4-week	Grade 1	No relationship	149	121
ChAd/ChAd 4-week	Grade 2	No relationship	145	117
ChAd/ChAd 4-week	Grade 1	No relationship	219	191
ChAd/ChAd 4-week	Grade 2	No relationship	124	96
ChAd/ChAd 4-week	Grade 1	No relationship	193	165
ChAd/ChAd 4-week	Grade 1	No relationship	215	187
ChAd/ChAd 12-week	Grade 3	No relationship	257	173
ChAd/ChAd 12-week	Grade 2	Unlikely	194	110
ChAd/ChAd 12-week	Grade 2	No relationship	228	144
ChAd/ChAd 12-week	Grade 1	No relationship	191	105
ChAd/ChAd 12-week	Grade 1	Unlikely	225	140
ChAd/BNT 4-week	Grade 1	No relationship	188	160
ChAd/BNT 4-week	Grade 1	No relationship	210	182
ChAd/BNT 4-week	Grade 1	No relationship	149	121
ChAd/BNT 4-week	Grade 2	No relationship	53^	-
ChAd/BNT 4-week	Grade 2	Unlikely	139	111
ChAd/BNT 12-week	Grade 1	No relationship	265	181
ChAd/BNT 12-week	Grade 2	Unlikely	256	172
ChAd/BNT 12-week	Grade 2	No relationship	210	126
ChAd/BNT 12-week	Grade 2	No relationship	247	161
ChAd/BNT 12-week	Grade 2	No relationship	265	179
BNT/ChAd 4-week	Grade 1	No relationship	177	149
BNT/ChAd 4-week	Grade 1	Unlikely	169	141
BNT/ChAd 4-week	Grade 2	Unlikely	196	168
BNT/ChAd 4-week	Grade 1	No relationship	156	128
BNT/ChAd 4-week	Grade 2	No relationship	235	207
BNT/ChAd 12-week	Grade 1	No relationship	253	169
BNT/ChAd 12-week	Grade 1	No relationship	196	112
BNT/ChAd 12-week	Grade 2	No relationship	6	-
BNT/BNT 4-week	Grade 3	Unlikely	3	-
BNT/BNT 4-week	Grade 2	No relationship	228	200
BNT/BNT 4-week	Grade 1	No relationship	148	120
BNT/BNT 4-week	Grade 1	No relationship	179	151
BNT/BNT 4-week	Grade 1	No relationship	245	216
BNT/BNT 4-week	Grade 2	Unlikely	4	-
BNT/BNT 4-week	Grade 1	No relationship	142	114
BNT/BNT 4-week	Grade 2	Unlikely	160	132
BNT/BNT 12-week	Grade 1	No relationship	147	62
BNT/BNT 12-week	Grade 2	No relationship	177	92
BNT/BNT 12-week	Grade 1	No relationship	252	166

^Participant had not received second dose prior to infection, dose delayed due to travel

3.4.4.2 Com-COV2

Over the course of the trial, there were 756 adverse events in 436 participants, proportionally split across arms (**Table 31**). Descriptions of all non-serious AEs of grade 3 or above are presented in **Table 32**. There remained five AEs of special interest, excluding SARS-CoV-2/COVID-19 events, one deemed possibly related to study vaccination (**Table 33**). There were 21 serious AEs across all arms (**Table 34**), none of which were deemed related to immunisation. 34 participants tested positive for SARS-CoV-2 with infections spread equally per arm), of whom a single participant was hospitalised but did not require invasive ventilation (**Table 35**).

Table 31 *– Com-COV2 Summary of Adverse Events*

	ChAd/ChAd (N=180)	ChAd/Mod (N=181)	ChAd/NVX (N=179)	BNT/ChAd (N=175)	BNT/Mod (N=177)	BNT/NVX (N=180)	Total (N=1072)
Number of adverse events	140	148	116	122	122	108	756
Number of unique participants	83 (45.6%)	81 (44.8%)	65 (36.3%)	70 (40.0%)	71 (40.1%)	66 (36.7%)	436 (40.7%)
AE within 28 days post boost	96 (68.6%)	119 (80.4%)	90 (77.6%)	91 (74.6%)	93 (76.2%)	81 (75.0%)	570 (75.4%)
AE within 3 months post boost	129 (92.1%)	143 (96.6%)	112 (96.6%)	115 (94.3%)	117 (95.9%)	102 (94.4%)	718 (95.0%)
Adverse of special interest	12 (8.6%)	7 (4.7%)	6 (5.2%)	5 (4.1%)	5 (4.1%)	7 (6.5%)	42 (5.6%)
Serious adverse event	4 (2.9%)	5 (3.4%)	1 (0.9%)	3 (2.5%)	5 (4.1%)	3 (2.8%)	21 (2.8%)
Severity							
Grade 1	79 (56.4%)	90 (60.8%)	62 (53.4%)	62 (50.8%)	66 (54.1%)	53 (49.1%)	412 (54.5%)
Grade 2	46 (32.9%)	47 (31.8%)	45 (38.8%)	49 (40.2%)	43 (35.2%)	44 (40.7%)	274 (36.2%)
Grade 3	13 (9.3%)	10 (6.8%)	8 (6.9%)	10 (8.2%)	9 (7.4%)	10 (9.3%)	60 (7.9%)
Grade 4	2 (1.4%)	1 (0.7%)	1 (0.9%)	1 (0.8%)	4 (3.3%)	1 (0.9%)	10 (1.3%)
Causality							
No relationship	52 (37.1%)	43 (29.1%)	48 (41.4%)	52 (42.6%)	51 (41.8%)	44 (40.7%)	290 (38.4%)
Unlikely	52 (37.1%)	60 (40.5%)	46 (39.7%)	51 (41.8%)	43 (35.2%)	40 (37.0%)	292 (38.6%)
Possible	26 (18.6%)	25 (16.9%)	16 (13.8%)	9 (7.4%)	18 (14.8%)	12 (11.1%)	106 (14.0%)
Probable	8 (5.7%)	18 (12.2%)	4 (3.4%)	6 (4.9%)	6 (4.9%)	6 (5.6%)	48 (6.3%)
Definite	2 (1.4%)	2 (1.4%)	2 (1.7%)	4 (3.3%)	4 (3.3%)	6 (5.6%)	20 (2.6%)
Type of SAE							
An important medical event	1 (0.7%)	3 (2.0%)	0 (0.0%)	1 (0.8%)	1 (0.8%)	1 (0.9%)	7 (0.9%)
Hospitalisation	2 (1.4%)	2 (1.4%)	1 (0.9%)	2 (1.6%)	3 (2.5%)	2 (1.9%)	12 (1.6%)
Life threatening	1 (0.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.8%)	0 (0.0%)	2 (0.3%)
Outcome							
Ongoing	0 (0.0%)	2 (1.4%)	0 (0.0%)	1 (0.8%)	0 (0.0%)	1 (0.9%)	4 (0.5%)
Recovered	2 (1.4%)	3 (2.0%)	1 (0.9%)	1 (0.8%)	4 (3.3%)	0 (0.0%)	11 (1.5%)
Recovered with sequelae	2 (1.4%)	0 (0.0%)	0 (0.0%)	1 (0.8%)	1 (0.8%)	2 (1.9%)	6 (0.8%)

Table 32 *– Com-COV2 Non-serious Adverse Events of grade ≥3*

Prime vaccine	Study arm	Days since 2nd dose	MedDRA Preferred Term	MedDRA System Order Class	Duration (days)	Severity	Causality assessment
ChAd	ChAd	9	Wrist fracture	Musculoskeletal & connective tissue disorders	39	Grade 3	No relationship
ChAd	ChAd	0	Hyperkalaemia^	Metabolism & nutrition disorders	2	Grade 3	No relationship
ChAd	ChAd	9	Dental caries	Gastrointestinal disorders	49	Grade 3	No relationship
ChAd	ChAd	65	Polytrauma	Injury, poisoning & procedural complications	107	Grade 3	No relationship
ChAd	ChAd	2	Vertigo	Ear & labyrinth disorders	1	Grade 3	Probable
ChAd	ChAd	39	Lower respiratory tract infection bacterial	Infections & infestations	13	Grade 3	Unlikely
ChAd	ChAd	3	Fatigue	General disorders & administration site conditions	9	Grade 3	Probable
ChAd	ChAd	0	Headache	Nervous system disorders	2	Grade 3	Definite
ChAd	ChAd	1	Musculoskeletal chest pain	Musculoskeletal & connective tissue disorders	35	Grade 3	Possible
ChAd	ChAd	7	Sleep apnoea syndrome	Respiratory, thoracic & mediastinal disorders	29	Grade 3	Unlikely
ChAd	ChAd	126	COVID-19	Infections & infestations	62	Grade 3	No relationship
ChAd	Mod	9	Headache	Nervous system disorders	1	Grade 3	Possible
ChAd	Mod	11	Prostate cancer	Neoplasms benign, malignant & unspecified (incl cysts and polyps)	178	Grade 3	Unlikely
ChAd	Mod	0	Fever	General disorders & administration site conditions	2	Grade 3	Definite
ChAd	Mod	23	Limb injury	Injury, poisoning & procedural complications	.	Grade 3	No relationship
ChAd	Mod	1	Fever	General disorders & administration site conditions	2	Grade 3	Probable
ChAd	Mod	58	UTI	Infections & infestations	4	Grade 3	No relationship
ChAd	NVX	48	Pacemaker insertion (cardiac)	Surgical & medical procedures	1	Grade 3	Unlikely
ChAd	NVX	27	Fatigue	General disorders & administration site conditions	2	Grade 3	Unlikely
ChAd	NVX	65	Dizziness	Nervous system disorders	4	Grade 4	No relationship
ChAd	NVX	1	Menopausal symptoms	Reproductive system & breast disorders	31	Grade 3	Possible
ChAd	NVX	91	Head injury	Injury, poisoning & procedural complications	.	Grade 3	Unlikely
ChAd	NVX	26	Chest pain*	General disorders & administration site conditions	.	Grade 3	Unlikely
ChAd	NVX	52	Urinary tract infection	Infections & infestations	4	Grade 3	No relationship

Prime vaccine	Study arm	Days since 2nd dose	MedDRA Preferred Term	MedDRA System Order Class	Duration (days)	Severity	Causality assessment
ChAd	NVX	43	Cystitis	Renal & urinary disorders	8	Grade 3	No relationship
BNT	BNT	5	Tooth repair	Surgical & medical procedures	1	Grade 3	No relationship
BNT	BNT	11	Back pain	Musculoskeletal & connective tissue disorders	17	Grade 3	Unlikely
BNT	BNT	64	Eyelid operation	Surgical & medical procedures	1	Grade 3	No relationship
BNT	BNT	0	Hypomagnesaemia	Metabolism & nutrition disorders	15	Grade 3	No relationship
BNT	BNT	42	Back injury	Injury, poisoning & procedural complications	2	Grade 3	No relationship
BNT	BNT	21	Tooth extraction	Surgical & medical procedures	4	Grade 3	No relationship
BNT	BNT	74	Orthostatic hypotension	Vascular disorders	40	Grade 3	Unlikely
BNT	BNT	27	Labyrinthitis	Ear & labyrinth disorders	8	Grade 3	Unlikely
BNT	BNT	16	Muscle spasms	Musculoskeletal & connective tissue disorders	3	Grade 3	Unlikely
BNT	Mod	10	Tooth extraction	Surgical & medical procedures	16	Grade 3	No relationship
BNT	Mod	9	Tooth extraction	Surgical & medical procedures	6	Grade 3	No relationship
BNT	Mod	24	Fatigue	General disorders & administration site conditions	7	Grade 3	Possible
BNT	Mod	19	Chest pain*	General disorders & administration site conditions	2	Grade 4	Unlikely
BNT	Mod	24	Joint injury	Injury, poisoning & procedural complications	4	Grade 3	No relationship
BNT	Mod	5	Joint dislocation	Musculoskeletal & connective tissue disorders	5	Grade 4	No relationship
BNT	Mod	11	Back pain	Musculoskeletal & connective tissue disorders	3	Grade 3	Unlikely
BNT	Mod	1	Fever	General disorders & administration site conditions	2	Grade 3	Definite
BNT	NVX	12	Oropharyngeal pain	Respiratory, thoracic & mediastinal disorders	3	Grade 3	Unlikely
BNT	NVX	12	Fatigue	General disorders & administration site conditions	1	Grade 3	Unlikely
BNT	NVX	12	Abdominal discomfort	Gastrointestinal disorders	3	Grade 3	Unlikely
BNT	NVX	12	Myalgia	Musculoskeletal & connective tissue disorders	3	Grade 3	Unlikely
BNT	NVX	16	Fatigue	General disorders & administration site conditions	4	Grade 3	Unlikely
BNT	NVX	16	Abdominal discomfort	Gastrointestinal disorders	4	Grade 3	Unlikely
BNT	NVX	77	Basal cell carcinoma	Neoplasms benign, malignant & unspecified (incl cysts and polyps)	33	Grade 3	Unlikely
BNT	NVX	37	Tendon sheath incision	Surgical & medical procedures	1	Grade 3	No relationship

Table 33 *– Com-COV2 Adverse Events of Special Interest excluding SAEs*

Prime vaccine	Study arm	Days to onset from boost	MedDRA Preferred Term	MedDRA System Order Class	Duration (days)	Severity	Causality assessment
ChAd	Mod	75	Seizure	Nervous system disorders	1	Grade 2	Unlikely
ChAd	NVX	28	Acute kidney injury	Renal and urinary disorders	12	Grade 1	Unlikely
BNT	Mod	29	Eosinophilia	Blood and lymphatic system disorders	105	Grade 2	Possible
BNT	NVX	105	Contusion	Vascular disorders	Ongoing	Grade 1	Unlikely
BNT	NVX	83	Retinal vein thrombosis	Eye disorders	Ongoing	Grade 2	Unlikely

Table 34 *– Com-COV2 Serious Adverse Events*

Prime vaccine	Study arm	Days to onset from boost	MedDRA Preferred Term	MedDRA System Organ Class	Duration (days)	Severity	Causality assessment	SAE Type
ChAd	Mod	28	Neutropaenia	Blood and lymphatic system disorders	10	Grade 4	No relationship	Important medical event
ChAd	ChAd	142	Diverticulitis	Gastrointestinal disorders	8	Grade 3	No relationship	Hospitalisation
BNT	Mod	84	Renal colic	Renal and urinary tract disorders	2	Grade 3	Unlikely	Hospitalisation
BNT	Mod	183	Dog bite	Injury, poisoning & procedural complications	3	Grade 3	No relationship	Hospitalisation
BNT	Mod	184	Penicillin allergy	Immune system disorders	1	Grade 3	No relationship	Important medical event
BNT	NVX	94	Intervertebral disc protrusion Intervertebral disc operation	Musculoskeletal & connective tissue disorders Surgical and medical procedures	.	Grade 3	Unlikely	Hospitalisation
ChAd	Mod	39	Coronary arterial stent insertion	Surgical and medical procedures	143	Grade 3	Unlikely	Important medical event
ChAd	Mod	67	Jaw fracture	Injury, poisoning & procedural complications	133	Grade 3	Unlikely	Hospitalisation
ChAd	NVX	56	Sebaceous cyst excision	Surgical and medical procedures	3	Grade 3	No relationship	Hospitalisation
ChAd	ChAd	109	Humerus fracture	Injury, poisoning & procedural complications	22	Grade 3	No relationship	Hospitalisation
ChAd	Mod	21	Headache	Nervous system disorders	.	Grade 3	Unlikely	Hospitalisation
BNT	BNT	179	Hysterectomy	Surgical and medical procedures	42	Grade 2	No relationship	Hospitalisation
BNT	NVX	18	Transient ischaemic attack**	Nervous system disorders	1	Grade 3	Unlikely	Important medical event
BNT	Mod	45	Aortic dissection	Vascular disorders	33	Grade 4	No relationship	Life threatening
BNT	BNT	133	Hysteroscopy	Investigations	.	Grade 3	Unlikely	Hospitalisation
ChAd	ChAd	52	Acute myocardial infarction	Cardiac disorders	10	Grade 4	Unlikely	Life threatening
ChAd	ChAd	111	Breast cancer	Neoplasms benign, malignant and unspecified (incl cysts and polyps)	77	Grade 4	No relationship	Important medical event
BNT	Mod	0	Cholecystitis	Hepatobiliary disorders	4	Grade 4	Unlikely	Hospitalisation

Prime vaccine	Study arm	Days to onset from boost	MedDRA Preferred Term	MedDRA System Organ Class	Duration (days)	Severity	Causality assessment	SAE Type
BNT	BNT	94	Retinal detachment	Eye disorders	.	Grade 4	No relationship	Important medical event
BNT	NVX	134	COVID-19**	Infections and infestations	14	Grade 4	No relationship	Hospitalisation
ChAd	Mod	179	Oesophageal varices	Gastrointestinal disorders	.	Grade 3	No relationship	Important medical event

Table 35 *– Com-COV2 SARS-CoV2 positive cases*

Prime vaccine	Study arm	Days to onset from boost	Severity
ChAd	ChAd	174	Grade 2
ChAd	ChAd	130	Grade 1
ChAd	ChAd	68	Grade 1
ChAd	ChAd	183	Grade 2
ChAd	ChAd	96	Grade 1
ChAd	ChAd	67	Grade 2
ChAd	ChAd	84	Grade 1
ChAd	ChAd	151	Grade 1
ChAd	ChAd	104	Grade 1
ChAd	ChAd	118	Grade 1
ChAd	ChAd	126	Grade 3
ChAd	Mod	77	Grade 1
ChAd	Mod	134	Grade 2
ChAd	Mod	108	Grade 1
ChAd	Mod	180	Grade 2
ChAd	Mod	173	Grade 1
ChAd	Mod	70	Grade 2
ChAd	NVX	168	Grade 1
ChAd	NVX	148	Grade 1
ChAd	NVX	91	Grade 2
ChAd	NVX	109	Grade 2
ChAd	NVX	117	Grade 2
BNT	BNT	137	Grade 1
BNT	BNT	82	Grade 1
BNT	BNT	177	Grade 2
BNT	BNT	217	Grade 2
BNT	BNT	155	Grade 2
BNT	Mod	76	Grade 1
BNT	Mod	165	Grade 2
BNT	Mod	144	Grade 1
BNT	Mod	142	Grade 1
BNT	NVX	197	Grade 2
BNT	NVX	194	Grade 1
BNT	NVX	166	Grade 2

3.5 Discussion & Conclusions

These two trials, Com-COV1 and Com-COV2 were designed rapidly to answer the immunisation policy-influencing questions of 1) are heterologous schedules safe, 2) are they tolerable and 3) are they sufficiently immunogenic (as measured by the serum anti-SARS-CoV2 spike IgG response) so that they are likely to be effective against preventing symptomatic SARS-CoV2 infection as well as severe disease.

3.5.1 Reactogenicity

3.5.1.1 Policy question & Hypothesis answer

The answers to the first two of these questions are more straightforward. In terms of safety, none of the schedules had any kind of safety signal and so were all deemed ‘safe’ (100,101,133,134). However, it should be noted that given the relatively small scale of these trials they are unable to pick up on rare serious adverse events such as vaccine induced thrombocytopaenic thrombosis or myocarditis. In fact, these rare, but serious adverse events associated with adenoviral-vectored vaccines and mRNA vaccines respectively, were not identified in the large efficacy trials that led to emergency authorisation (87,135), but only picked up in post-emergency authorisation monitoring.

In terms of tolerability, with the exception of the Novavax-boosted schedules in Com-COV2, heterologous schedules are more frequently reactogenic than their counterpart homologous schedules, particularly BNT/ChAd-84 in Com-COV1 and the Moderna-boosted schedules in Com-COV2. These same three more frequently reactogenic schedules were also found to be more severely reactogenic, with symptoms lasting slightly longer than in comparison to their homologous counterparts.

When passing judgement on the tolerability of these schedules, comparative measures alone are insufficient to determine their suitability and tolerability for a population. Across both trials no grade 4 symptoms were reported and the number of grade 3 symptoms

was small and the duration short. In summary, all evaluated schedules are tolerable and their reactogenicity profiles should not be a barrier to their use.

3.5.1.2 Further observations & analyses

The patterns of reactogenicity seen in these analyses, can be related to what is already known about the cellular and cytokine responses to these vaccines. Together, these allow theoretical postulation as to how reactogenicity may be mediated.

Interferon gamma levels have been associated with reactogenicity, but a definitive causative link has not yet been established (136). It is possible that initial reactogenicity represents the type I interferon response from the human body's innate nucleic acid sensing (DNA in the case of ChAd and mRNA in the case of BNT). The reduced reactogenicity of first dose BNT in comparison to first dose Chad, may, in part, be explained by the modification of the mRNA with pseudouridine bases, as this reduces activation of this innate response (137).

Non-classical IFNγ-producing cells such as MAIT cells (mucosally associated invariant T cells), Vδ2 $\gamma\delta$ T cells and iNKT cells are themselves stimulated by an initial type I interferon response and amplify the interferon in a feed-forward mechanism (138–140). It is therefore possible that the majority of early interferon is not produced by antigen-specific stimulation, but rather as part of this positive feedback system that is responsive to an initial antigen-specific production of interferon gamma.

In the homologous ChAd/ChAd regimen, greater reactogenicity is seen following the first dose than following the second dose. Conversely, in homologous BNT/BNT, greater reactogenicity is seen following the second dose than following the first dose. This pattern mirrors the activation of MAIT cells in response to these schedules, as defined by CD69+ expression (138,139).

The difference in second dose reactogenicity seen between BNT/BNT-28 and BNT/BNT-84 mirrors the activated T-cell level at the time of second dose, suggesting that second dose reactogenicity may be mediated in part by antigen-specific T cells. The fact that seropositive participants receiving a BNT first dose have greater reactogenicity than seronegative participants would be in keeping with this, as seropositive participants will already have spike-specific activated T-cells present following natural infection. This difference in reactogenicity is not seen between seropositive and seronegative participants receiving a ChAd first dose and may be masked by a large initial MAIT cell response.

Higher anti-vector neutralising antibody responses after one dose of ChAd have been shown to be associated with reduced anti-SARS-CoV2 spike responses in the original vaccine trials (141). It may be postulated that anti-vector immunity might reduce the effective dose of ChAd, prior to further MAIT-cell activation, and thus also reduce second dose reactogenicity in comparison to a first dose of ChAd. Increasing the ChAd/ChAd interval increases the second dose reactogenicity. This would be in keeping with a waning of anti-vector immunity over time. However, this idea is not supported by data from the paediatric immunogenicity trial, which showed no difference in pre-second dose levels of anti-vector neutralising antibody titre between schedules with different priming intervals (142). Anti-vector T cell responses have not been extensively investigated and may provide further insight into this in the future.

The greater second dose reactogenicity of Mod/BNT over BNT/BNT might be due to differences in doses of mRNA (100μg vs 30μg), however it is not possible to exclude differences in the proprietary make up of the lipid nanoparticle formulations as the cause of this difference in reactogenicity.

Finally, the presence of reactogenicity at prime increased the likelihood of second-dose reactogenicity by a factor of four. The magnitude of this increase in likelihood is similar

to other factors such as vaccine schedule, suggesting that the individual and their own specific immune response, is at least as important in defining the reactogenic response as external factors such as which vaccine schedule was received.

3.5.2 Immunogenicity

3.5.2.1 Policy question & Hypothesis answer

Evaluation of the third question regarding the immunological assessment of these heterologous schedules is more complicated. For the comparison to have any worth requires there to be a correlate of protection associated with protection either from severe disease or from symptomatic infection. Although no correlate has been formally accepted, serum binding anti-SARS-CoV2 spike IgG, and serum neutralising antibody responses in particular, have been used as acceptable correlates as they appear to mitigate risk of symptomatic infection (95). It is, as yet, unclear which aspect or aspects of the immune response might mitigate the risk of severe disease.

ChAd/BNT-28 met the criterion for non-inferiority against ChAd/ChAd-28, which was one of the two co-primary immunological endpoints of Com-COV1, however BNT/ChAd-28 did not, being less immunogenic than BNT/BNT-28. ChAd/BNT-84 similarly achieved non-inferiority against ChAd/ChAd-84, but BNT/ChAd-84 against BNT/BNT-84 did not. All longer interval schedules were at least as immunogenic as shorter interval schedules.

Again, however, comparative measures alone are insufficient to judge each of these heterologous schedules as to their potential worth and usefulness. When compared to the least immunogenic schedule, ChAd/ChAd-28, all other schedules produced a greater serum binding anti-SARS-CoV2 spike IgG titre. Although not the primary endpoint of this trial, from a pragmatic point of view, all schedules appear immunologically superior (as measured by serum binding anti-SARS-CoV2 spike IgG) to ChAd/ChAd-28, a schedule

which has been shown to have high protection against severe disease (87), and moderate-high levels of protection against symptomatic infection.

3.5.2.2 Further observations & analyses

The value of these trials lies not just in their primary outcome, but also evaluating the different vaccine platforms and their combinations, as this may give insight into how best to use these platforms in the future.

3.5.2.2.1 Antibody & T cell decay

Initially the antibody titres of more immunogenic schedules decayed faster, but as titres decayed, the differences in both the absolute antibody titres and the speed at which the antibody titre waned, reduced. On a graph with a logarithmically transformed y axis, this resulted in a concave shape of the antibody kinetics lines. This could have three possible interpretations: 1) Antibody decay is a function that is faster than logarithmic decay, which would be unusual for a biological system; 2) the decay pattern is more complicated than a simple logarithmic one – for example there may be different populations of antibody that wane at different rates, or there may be concurrent antibody production by a cell population, that may itself be waning (such as short- and long-lived plasma cells); 3) Finally, if decay is indeed logarithmic, then the baseline to which it is decaying (the asymptote) may be non-zero.

The Com-COV2 T cell responses show significantly different waning dynamics to antibody kinetics. There is a short period of rapid decay from 28 days post second dose to 56 days post second dose, followed by a period of either plateau or very shallow decay. In Com-COV1, the frequency of sampling post second dose was insufficient to demonstrate the same picture. The immunological implication of this is unclear, but this non-linear decay means that the ratios used to estimate wane need to be interpreted with caution as they will over-estimate the rate of wane, and there may be a stable

plateau. Future studies may benefit from more frequent early PBMC sampling better to describe the kinetics.

These observations of antibody and T cell wane, when taken together might suggest that two doses of vaccine may provide persistently raised levels of both T cells and antibody, although what this might mean in terms of clinical protection is unknown.

3.5.2.2.2 Qualitative response

There already exists evidence that different vaccine platforms are qualitatively different, with virally vectored vaccines being dominated by IgG1 and IgG3, whereas mRNA vaccines produce significant populations of IgG2 and IgG4 in addition to IgG1 and IgG3 (143).

Measurement of total serum binding anti-SARS-CoV2 spike IgG gives an incomplete picture of the humoral response as this assay does not elucidate the functional activity of these antibodies. Neutralising activity is one of the important antibody functions and was measured in this body of work.

In both Com-COV1 and Com-COV2, the binding anti-SARS-CoV2 spike IgG response to a first dose of ChAd was lower than the response to BNT, but the neutralising response was comparable. After the second dose differences in the neutralising response emerge. Homologous ChAd/ChAd-28 and ChAd/ChAd-84 schedules produce a significantly lower neutralisation response when compared to the six other schedules.

The proportion of neutralising activity per unit serum binding IgG appeared greater for ChAd primed participants, although since the absolute neutralising activity was similar for BNT-primed participants, this difference in proportional neutralising activity appears driven by increased production of non-neutralising antibody in BNT-primed participants. After a second dose of any vaccine, the differences in proportional neutralising activity

reduce, suggesting that BNT/ChAd and BNT/BNT produce a larger proportion of neutralising antibodies than ChAd/ChAd after the second dose.

This measured difference in proportional neutralising response was largely removed by a second dose of any vaccine dose across both trials. Interestingly, schedules with a NVX second dose proportionally give the highest neutralising responses. Previously published live virus microneutralisation titres, whose data is not presented in this thesis, showed an even more dramatic difference, with BNT/BNT having twice the binding anti-SARS-CoV2 spike IgG responses as BNT-NVX, but an indistinguishable level of neutralisation (133).

3.5.2.2.3 Serostatus

The majority of the analyses presented focus on seronegative participants. Given the low number of seropositive participants, statistical analysis of this group may be of limited value, however, despite small numbers there are some trends seen.

Infection followed by one dose of vaccination gives a higher response than two doses of vaccination without infection for all measured immune markers, but these are not significantly augmented by having a second dose of vaccine. This is supportive of current WHO vaccine advice, which takes into account the rate of seropositivity in the world, and currently advises only one dose of vaccine in the unvaccinated, provided they are not in an at risk group (144).

3.5.2.3 General Caveats

In Com-COV2, first doses had been administered in the community and not randomised in the trial. The underlying populations receiving the two different vaccines in the community were different and therefore comparisons between ChAd-primed and BNT-primed schedules must be made with caution. The same caution must be applied to

comparisons between Com-COV1 and Com-COV2 schedules, given the differences in population between these two trials.

After review of the second dose reactogenicity for the 4-week interval Com-COV1 schedules, a protocol amendment was submitted for a sub-study to randomise participants in the 12-week schedule at the time of their second dose to either “advice for prophylactic paracetamol” (where participants would self administer four doses of paracetamol over 24 hours) or “advice for reactive paracetamol” (where participants would self administer paracetamol only if they felt symptomatic). The results are not displayed here, but have been previously published (134). In short, there was no clear effect on reactogenicity or immunogenicity. This sub-study might conceivably have increased the overall amount of paracetamol usage in the 12-week schedules at the point of the second dose. It may therefore reduce the power to detect any differences in second dose reactogenicity seen between homologous and heterologous schedules amongst the 12-week arms. This sub-study might also affect the analysis of the effect of interval in Chapter 4 and is discussed there.

3.5.2.4 Further work

Longitudinal follow up of the participants in these trials is not sufficient definitively to clarify what may be driving the shape of antibody waning kinetics as discussed in Section 3.5.2.2.1. Further mathematical modelling using non-linear mixed effect models is planned to look for evidence of a non-zero asymptote, and if this level might be the same for all schedules.

Memory B cell ELISpot assays are planned, as these may inform the underlying differences in shape of antibody decay despite not being the primary producers of antibodies (short- and long-lived plasma cells are difficult to detect in the peripheral blood beyond eight days post immunisation).

3.5.3 Final comment

The heterologous COVID-19 vaccine schedules studied in Com-COV1 and Com-COV2, which incorporated ChAd, BNT, Mod and NVX are safe, tolerable and immunogenic. They are viable options to act as suitable priming schedules, depending on vaccine availability and immunisation programme logistics.

Chapter 4 COVID-19 Vaccine Priming Interval

4.1 Background

4.1.1 Pre-COVID

The time interval between the first and second vaccine doses (priming interval), is important in determining vaccine response and, in some cases, clinical protection (145). In order to understand how interval affects the development of the immune response, it is key to understand the timeline of immunological events in the body after antigen exposure.

The first antigenic exposure in humans results in specialised antigen presenting cells (APCs), such as dendritic cells, presenting antigen to circulating mature naïve T cells. This process happens most efficiently, and possibly exclusively, in the peripheral lymphoid tissue such as in the cortical regions of lymph nodes. Here, APCs are kept in close proximity to T cells, increasing the chances that a T cell with affinity for a particular peptide:MHC ligand complex will meet an APC displaying that peptide.

At the point that this recognition occurs, T cell activation occurs only if there are sufficient co-stimulatory signals: 1) the B7/CD28 interaction which stimulates survival and expansion of the T cell and 2) cytokines including IL-6, IL-12 and TGF-β which stimulate subset differentiation of effector T cells. When all three signals are received, activation is achieved resulting in clonal expansion and differentiation into effector T cells. This process typically takes 4-5 days during which time the naïve T cell upregulates all the proteins it requires to perform its functions as an activated T cell.

These activated T cells interact with naïve B cells through linked recognition, which typically occurs in secondary lymphoid tissue such as the spleen or lymph nodes, creating a primary focus. The formation of these primary foci takes about 5 days, in

keeping with T-cell activation timeframes. Both B and T cell proliferation then continues for several days, which constitutes the first phase of the primary humoral response. Some B cells differentiate into antibody-producing plasmablasts, of which some differentiate into more competent antibody secreting plasma cells after a few more days still.

An alternative route by which some activated B cells become plasma cells after prime vaccination is via migration to primary lymphoid follicles, where, with their partner T cell, they proliferate and form a germinal centre. Whereas the primary focus response produces a small, but fast response against the antigenic stimulus, it is the germinal centre response that provides a slightly delayed, but more effective response. It is also during this germinal centre phase that VDJ B cell receptor gene rearrangement, somatic hypermutation and class switching occurs – the processes by which B cells affinity mature and change production from IgM and IgD classes to, most frequently, IgG, which changes the range of available effector functions. The timeline of evolution of this germinal centre response, as defined by visualisable structural changes in the lymph node, is thought to continue for approximately 3-4 weeks following antigen exposure. However, the process of immunological maturation might last longer than this still.

The secondary anamnestic response on secondary antigenic exposure is principally differentiated by the fact that mature T and B cells exist, which may then be activated directly by suitable antigenic presentation of the pathogen, without requiring co-stimulation and a more prolonged maturation process.

The time taken for maturation of immune response following first dose directly impacts on the effect of priming interval until the second antigenic dose.

Various studies have demonstrated improved humoral response with increasing priming interval. This includes retrospective analyses of responses to an anthrax vaccine (anthrax vaccine adsorbed [AVA] – a cell free filtrate predominantly made up of

‘protective antigen’) have shown an improved humoral response for the first two doses as the interval was increased from two, to three, to four weeks (146). The spectrum of priming interval here is quite short and still relatively early in the maturation of the humoral response.

Another study using an H1N1 adjuvanted protein sub-unit vaccine has shown similar antibody production levels across both younger and older adult age groups in two dose schedules at both a 3-week and 6-month interval (147).

Most relevantly, the heterologous Ebola vaccine schedule referenced in Section 3.1.1, which used an adenovirus vectored first dose followed by a modified vaccina Ankara virus second dose, also investigated the effect of interval. Groups had been randomised to receive their second doses at 28, 56 and 84 day intervals (117). There was a marked 2.2 fold increase in humoral response of the 56 day interval group in comparison to the 28 day interval group. The additional benefit of increasing the dose from 56 days to 84 days was marginal (1.1 fold) and not statistically significant.

T cell responses were measured by intracellular cytokine staining rather than ELISpot, with no clear differences between schedules.

This study demonstrated a clear quantitative immunological benefit in serum binding IgG with prolonged priming interval, however, there was no clear additional benefit by increasing interval beyond 56 days. It is tempting to infer that this larger response is clinically desirable, although correlation with clinical outcome is lacking. This is potentially supported by an improved neutralising response in the 56-day interval arm when compared to the 4-week interval arm. Other data on the qualitative effector functions of the antibody response are lacking.

A hypothesis for the increased antibody response with prolonged interval is that a slightly delayed second dose may allow a more complete maturation of the priming response,

including germinal centre maturation and this may allow a stronger secondary anamnestic response.

4.1.2 COVID-19

The first evidence that interval might play a role in COVID vaccination schedules came from the immunological analyses (COV001, COV002) and efficacy analyses (COV001, COV002, COV003, COV005) of the ChAdOx1 nCoV-19 efficacy trials (87,148). Due to the speed at which these trials were delivered and unavoidable delays in vaccine manufacture, not all participants received their doses within the originally intended timeframe and so there was a variation in priming interval. The binding anti-SARS-CoV2 spike IgG response in those with a 10-weeks interval was on average twice as large as those with a 20-weeks interval. There was a further 2-fold increase when the interval was increased to 44-weeks. The efficacy analysis compared 'short interval' (<6 weeks) with 'long interval' (>6 weeks) and found that the efficacy against symptomatic infection increased from 60% to 90% by prolonging interval. Subsequent analyses, however, have not shown any differences in levels of protection against severe SARS-CoV2 infection.

The PITCH consortium conducted a cohort study on healthcare workers who received mostly homologous BNT/BNT (149). Due to the rapidly changing immunisation program, a portion of their cohort received their doses at the manufacturer recommended interval of 2-5 weeks, whilst the majority received their second dose at a longer interval of 6-14 weeks. Binding anti-SARS-CoV2 spike IgG titres were 1.5 times higher in the longer interval group than in the shorter interval group for seronegative participants. The neutralising response was 2-4 times higher depending on the SARS-CoV2 variant. B cell induction was also 7 times higher in longer interval participants. However, IFNγ-SFC T cell responses were slightly lower, at about 62% of the short interval value, however this reduction failed to reach statistical significance.

Although not randomised, this paper extensively immunophenotyped the differences between short and long interval BNT/BNT schedules and gave some reassuring and validating results that prolonging priming interval was likely a reasonable strategy.

4.2 Introduction

There were great logistical difficulties with respect to delivering vaccines in the manufacturer-recommended timeframe. This was primarily due to the initial shortfall in supply due to a massive global demand. There was, additionally, uncertainty about how robust the vaccine supply would be to disruption. Governments, including the UK government, made a decision to prioritise vaccinating larger numbers of the population with one dose rather than smaller numbers of the population with two doses. This decision was supported, in part, by evidence that short term protection after one dose against symptomatic infection was still high, at a rate of 70-90% depending on the vaccine (150). Subsequent analyses also showed there was significant protection against death after one dose of vaccine of 44-55%, depending on the vaccine (151). This policy aimed to maximise the lives saved with a limited vaccine stock, but necessarily increased the interval between first and second doses of vaccine.

As reviewed above, there was some evidence to support prolonging interval, but robust evidence exploring interval with mRNA lipid nanoparticle vaccines, as well as heterologous priming schedules which included different vaccine technologies was lacking.

Com-COV1 included randomisation of intervals in order to address this question to give confidence to policy makers and the public. The results of each schedule at both short and long intervals are presented in Chapter 3 . In this chapter I will present the results of the analyses comparing interval randomisation in Com-COV1 as well as the exploratory subgroup analysis of non-randomised interval in Com-COV2.

4.3 Hypotheses

1) 12-week interval schedules are non-inferior to their respective 4-week interval schedules in terms of magnitude of peak binding anti-SARS-CoV2 spike antibody response
2) 12-week schedules are non-inferior to their relative 4-week schedule in terms of magnitude of peak IFNγ ELISpot result
3) 12-week schedules are similarly reactogenic in comparison to their relative 4-week schedules

4.4 Results

4.4.1 Demographics & Analysis Population

4.4.1.1 Com-COV1

Recruitment numbers are presented in Section 3.4.1. However, the analysis population was different as outlined in the modified CONSORT diagram (**Figure 62**). The only pertinent difference is that the immunology cohort was excluded from this analysis as there were no 12-week schedule arms in this cohort. Participants who had serological or virological evidence of infection were included as seronegative or seropositive participants based on their status at baseline, up until the point of diagnosis. Out of window visits were included in the analysis.

Reactogenicity analyses included all participants correctly vaccinated. Safety analyses included all participants who received any immunisation.

Detailed baseline demographic information is displayed in **Table 36**.

The mean age of participants was 58, 44% (407/729) were female and 56% (322/729) were male. 23% (165/729) were of a self-defined non-white ethnicity, whilst the remaining 77% (564/729) were of a white ethnicity. 31% (2229/729) had at least one respiratory or cardiovascular comorbidity or diabetes, whilst 69% (500/729) were non-comorbid. Groups were well-balanced between all arms.

Table 36 *– Demographics of Com-COV1 participants (Interval analysis)*

	ChAd/ChAd-28 N=90	ChAd-BNT-28 N=90	BNT-BNT-28 N=93	BNT-ChAd-28 N=89	ChAd-ChAd-84 N=92	ChAd-BNT-84 N=90	BNT-BNT-84 N=93	BNT-ChAd-84 N=92	Total N=729
Age (years)									
Mean (SD)	58.2 (4.8)	58.0 (4.8)	58.3 (4.9)	57.3 (4.6)	57.5 (5.0)	58.6 (4.3)	58.1 (4.6)	58.1 (4.7)	58.0 (4.7)
Sex									
Female	38 (42%)	40 (44%)	49 (53%)	40 (45%)	42 (46%)	36 (40%)	44 (47%)	33 (36%)	322 (44%)
Male	52 (58%)	50 (56%)	44 (47%)	49 (55%)	50 (54%)	54 (60%)	49 (53%)	59 (64%)	407 (56%)
Ethnicity									
White	70 (78%)	65 (72%)	76 (82%)	65 (73%)	71 (77%)	72 (80%)	72 (77%)	73 (79%)	564 (77%)
Non-White	20 (22%)	25 (28%)	17 (18%)	24 (27%)	21 (23%)	18 (20%)	21 (23%)	19 (21%)	165 (23%)
Black	1 (1%)	1 (1%)	0 (0%)	2 (2%)	3 (3%)	1 (1%)	2 (2%)	1 (1%)	11 (2%)
Asian	13 (14%)	16 (18%)	7 (8%)	9 (10%)	8 (9%)	11 (12%)	9 (10%)	11 (12%)	84 (12%)
Mixed	6 (7%)	6 (7%)	8 (9%)	10 (11%)	7 (8%)	5 (6%)	7 (8%)	4 (4%)	53 (7%)
Other	0 (0%)	2 (2%)	2 (2%)	3 (3%)	3 (3%)	1 (1%)	3 (3%)	3 (3%)	17 (2%)
Comorbidity									
Non-comorbid	56 (62%)	65 (72%)	66 (71%)	60 (67%)	65 (71%)	60 (67%)	65 (70%)	63 (68%)	500 (69%)
Comorbid	34 (38%)	25 (28%)	27 (29%)	29 (33%)	27 (29%)	30 (33%)	28 (30%)	29 (32%)	229 (31%)
Cardiovascular	19 (21%)	16 (18%)	18 (19%)	20 (22%)	18 (20%)	21 (23%)	16 (17%)	20 (22%)	148 (20%)
Respiratory	16 (18%)	11 (12%)	11 (12%)	11 (12%)	7 (8%)	10 (11%)	14 (15%)	10 (11%)	90 (12%)
Diabetes	7 (8%)	8 (9%)	0 (0%)	2 (2%)	2 (2%)	1 (1%)	1 (1%)	4 (4%)	25 (3%)
BMI									
Mean (SD)	27.4 (4.9)	27.1 (4.8)	26.9 (4.8)	27.5 (4.0)	28.1 (4.3)	26.8 (4.5)	26.9 (4.6)	27.2 (5.1)	27.2 (4.6)

Percentages may sum to more than 100% due to rounding or due to having more than one co-morbidity. Combined Immunology & General cohorts

4.4.1.1.1 Sub-study

The introduction of an ‘advice for paracetamol’ sub-study as a late amendment (as described in Section 2.8.4.1) for the second dose of the 12-week interval schedules only, introduces a source of potential confounding to the interval analysis. Sensitivity analyses are therefore presented including a binary variable representing randomisation to “advice for prophylactic paracetamol for 24 hours” or “advice for reactive paracetamol for 24 hours”. Those who were not randomised were regarded as being part of the ‘reactive paracetamol’ group. Those in 4-week interval schedules were also regarded as being part of the ‘reactive paracetamol’ group.

4.4.1.2 Com-COV2

There is no change in the analysis population described in Section 3.4.1.2.

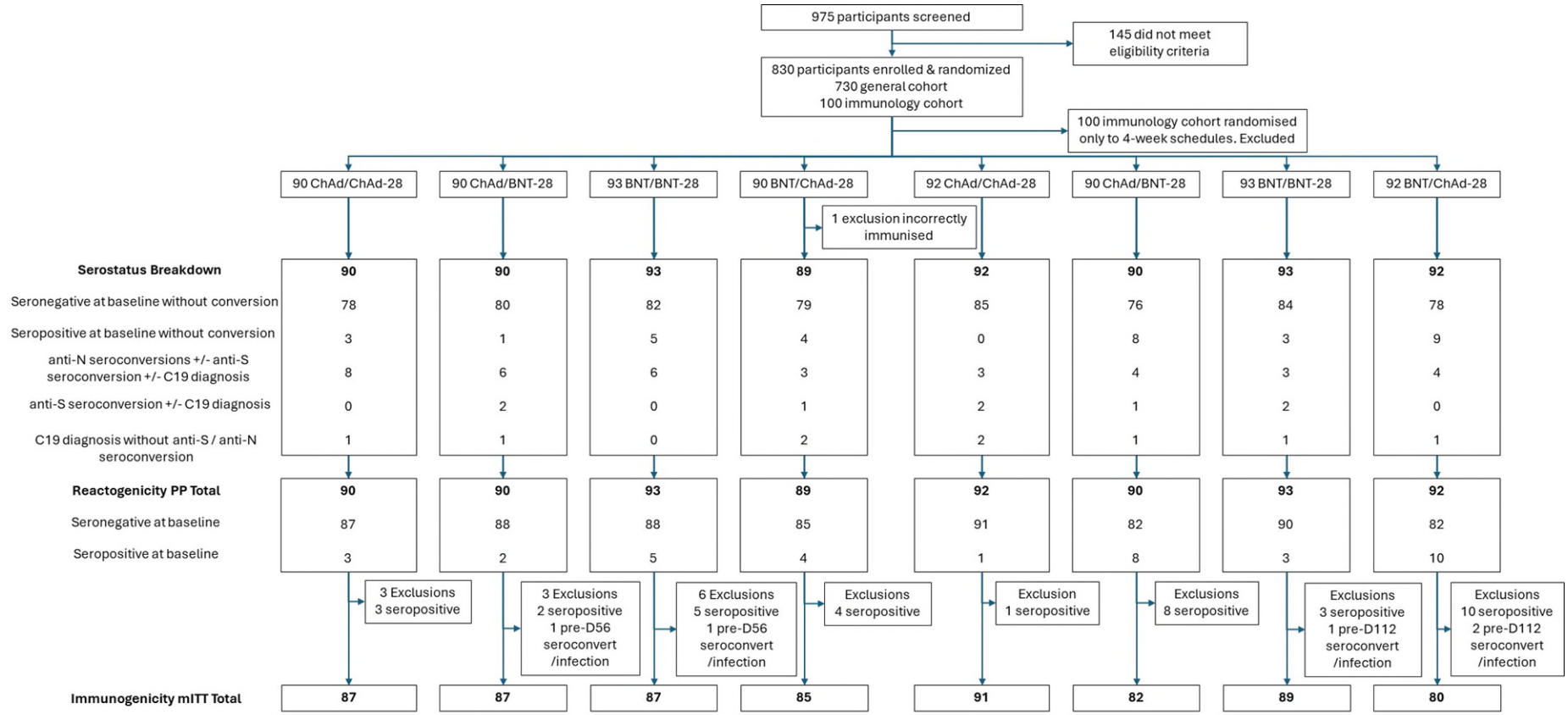

Figure 62 *– Com-COV1 CONSORT for Interval analysis. Seronegative: <1.0 anti-N IgG at baseline and for study duration. Seropositive: ≥1.0 anti-N IgG at baseline but with no evidence of further anti-N increases or anti-S increases at non-vaccination timepoints nor any virological evidence of infection by PCR or LFA. "Anti-N seroconversions +/- anti-S seroconversion +/- C19 diagnosis": ≥2-fold increase in anti-N IgG at any timepoint with the second titre ≥1.0 regardless of anti-S IgG, virological diagnosis or baseline serostatus. "Anti-S seroconversion +/- C19 diagnosis": No evidence of anti-N seroconversion, with ≥2-fold increase in anti-S IgG at a timepoint with no expected increase in titre due to vaccination, regardless of virological diagnosis or baseline serostatus. "C19 diagnosis without anti-S / anti-N seroconversion": No evidence of anti-S or anti-N seroconversion, but virological evidence of infection, regardless of baseline serostatus.*

4.4.2 Immunogenicity

4.4.2.1 Com-COV1

4.4.2.1.1 Anti-SARS-CoV2 spike IgG

Increasing the priming interval increased the peak antibody response by 1.9 (95% CI: 1.4, 2.4) times for ChAd/ChAd, 1.3 (95% CI: 1.0, 1.5) times for BNT/BNT and 1.5 (95% CI: 1.2, 1.8) times for BNT/ChAd, but not for ChAd/BNT. The antibody titre advantage is maintained only in homologous long interval schedules at 5 months post second dose: ChAd/ChAd 1.9 (95% CI: 1.4, 2.4) to 1.9 (95% CI: 1.1, 3.4), BNT/BNT 1.3 (95% CI: 1.0, 1.6) to 1.6 (95% CI: 1.1, 2.4) (**Figure 63**).

The 5 month:1 month ratio was again used as a measure of wane. None of the short interval:long interval aGMRs were statistically significantly different (indicating that each heterologous schedule waned at similar rate to its relative homologous schedule), including for BNT/BNT.

4.4.2.1.1.1 Paracetamol sub-study sensitivity analysis

Results from this sensitivity analysis were qualitatively and quantitatively very similar to the main analysis, although long interval peak BNT/BNT titres were no longer statistically significantly greater than short interval titres (**Supplementary Figure 11** & **Supplementary Figure 12**).

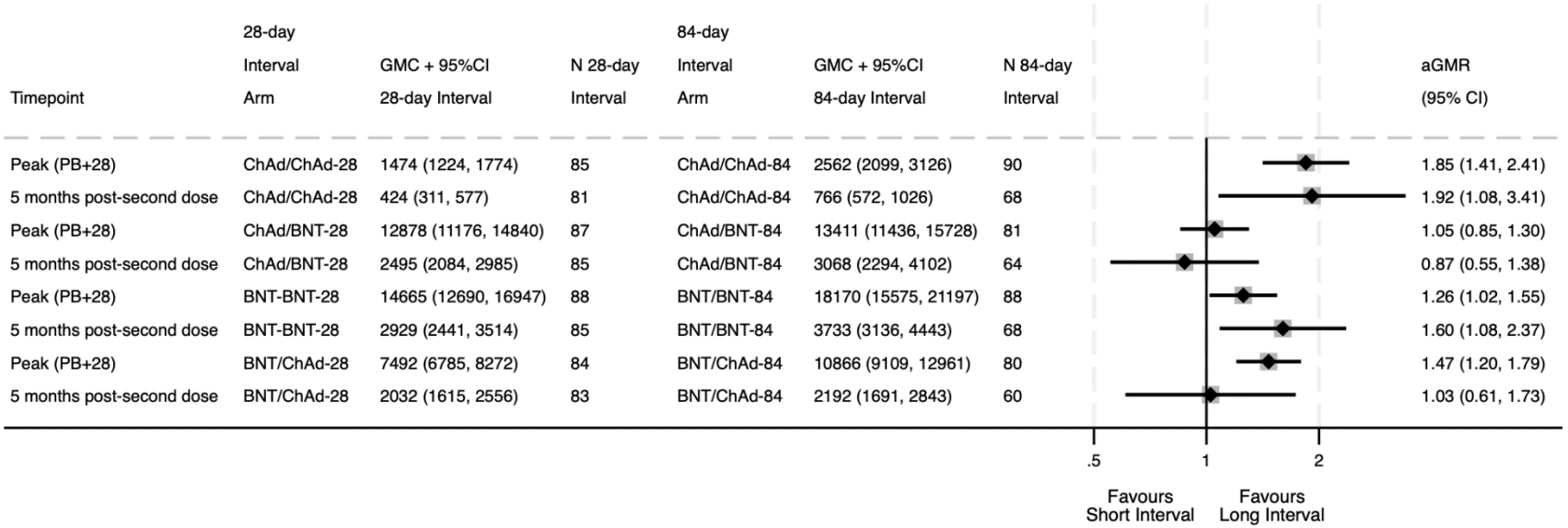

Figure 63 *– Com-COV1 Forest plot comparing peak (28 days post second dose) and 5-months post second dose anti-SARS-CoV2 spike IgG between 4-week and 12-week interval schedules . Peak antibody response for the 4 week schedules was at 56 days, whilst for 12-week this was at 112 days. The 5-month timepoint was at day 182 for 4-week schedules, and at day 255 for the 12-week schedules. Left sided columns represent the raw, unadjusted GMC ± 95% CI. The forest plot represents the right sided aGMR derived from a multivariate linear regression.*

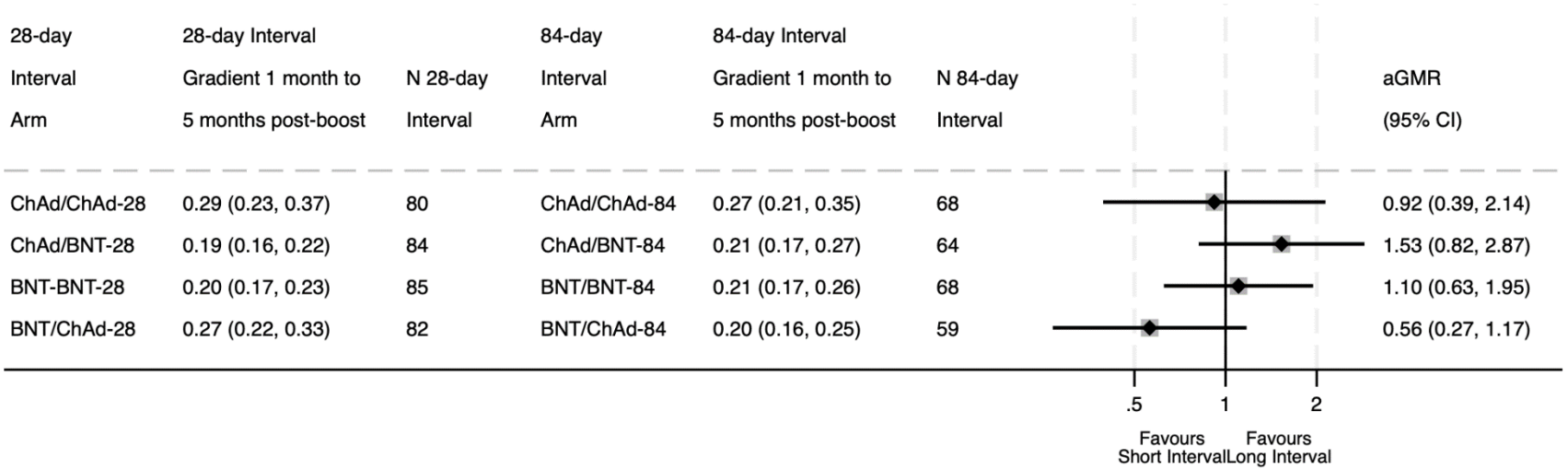

Figure 64 *– Com-COV1 Forest plot comparing rates of anti-SARS-CoV2 spike IgG wane between 4-week and 12-week interval schedules . Left sided columns represent the raw, unadjusted GMC ± 95% CI of the ratio of the 5-month antibody level over the peak antibody level. The forest plot represents the right sided aGMR derived from a multivariate linear regression.*

4.4.2.1.2 Pseudotype-virus neutralisation titre

Pseudotype-virus neutralisation titre was consistently greater in the longer interval schedules by a factor of 2.6 (95% CI: 2.0, 3.5) for ChAd/ChAd, 1.4 (95% CI: 1.1, 1.8) for ChAd/BNT, 1.5 (95% CI: 1.2, 1.8) for BNT/BNT and 1.6 (95% CI: 1.2 ,2.0) for BNT/ChAd (**Figure 65**). The consistency of the higher neutralising titre with all long interval regimens in the absence of a binding antibody titre difference for ChAd/BNT, suggests that there is a qualitative difference in the nature of antibody response produced between short and long interval regimes. To investigate this qualitative difference further, the pseudo-typed virus neutralisation titre was divided by the anti-SARS-CoV2 spike binding IgG titre to produce a measure of the proportion of neutralising antibodies in relation to the total antibody response (**Figure 66**). For all schedules, longer interval regimens produced a greater proportion of neutralising antibodies than shorter interval regimens. The ratios between long and short intervals of neutralisation/binding titres all increased: ChAd/ChAd 1.4 (95% CI: 1.2, 1.7), ChAd/BNT 1.4 (95% CI: 1.2, 1.5), BNT/BNT 1.2 (95% CI: 1.1, 1.3) and BNT/ChAd 1.1 (95% CI: 1.0, 1.3).

4.4.2.1.2.1 Paracetamol sub-study sensitivity analysis

Sensitivity analysis revealed qualitatively and quantitatively similar results (**Supplementary Figure 13** & **Supplementary Figure 14**).

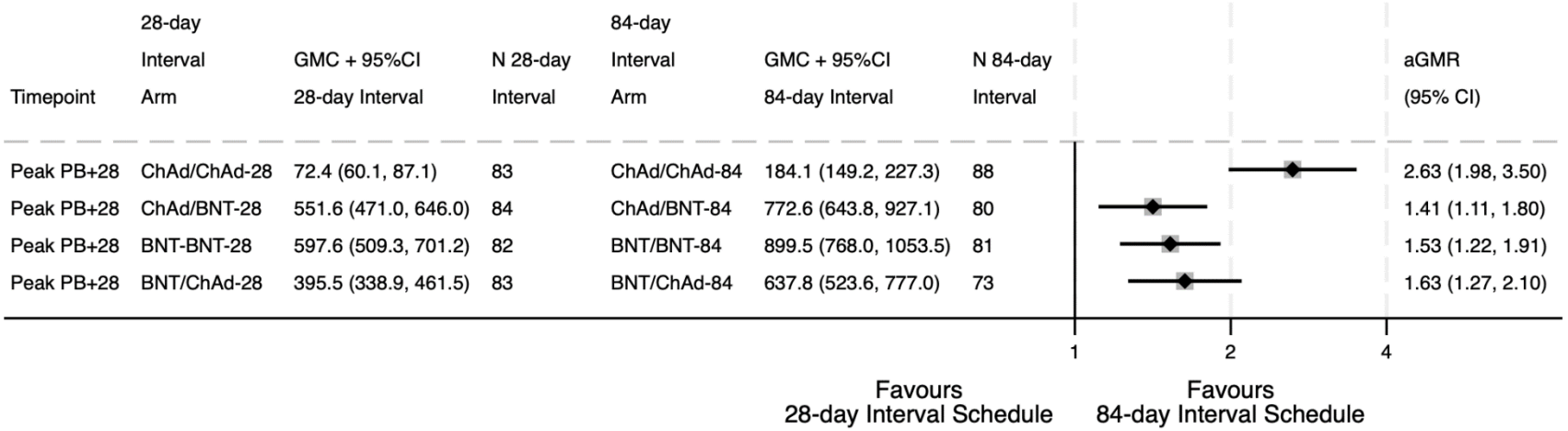

Figure 65 *– Com-COV1 Forest plot comparing peak (28 days post second dose) pseudotype virus neutralisation 50 titres between 4-week and 12-week interval schedules . Left sided columns represent the raw, unadjusted GMC ± 95% CI. The forest plot represents the right sided aGMR derived from a multivariate linear regression.*

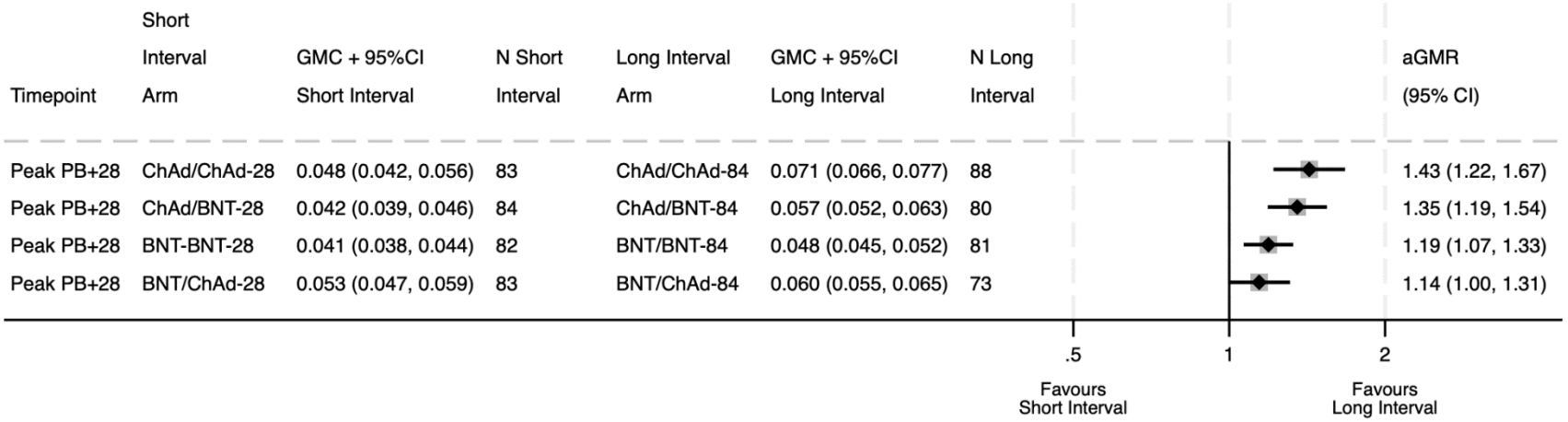

Figure 66 *– Com-COV1 Forest plot comparing the ratio between pseudotype virus neutralisation 50 titre and anti-SARS-CoV2 spike IgG at peak timepoint (28 days post second dose) between 4-week and 12-week interval schedules . Left sided columns represent the raw, unadjusted GMC ± 95% CI. The forest plot represents the right sided aGMR derived from a multivariate linear regression.*

4.4.2.1.3 T-cell ELISpot

Increasing interval universally reduced the peak T-cell ELISpot count across all schedules, although this was not statistically significant for ChAd/ChAd. This lower T-cell response is maintained at 5 months post second dose for all schedules apart from BNT/BNT (**Figure 67**). On analysis of rates of wane, there is no clear difference between long and short interval schedules (**Figure 68**).

On review of the kinetics diagram **Figure 18**, which plotted short and long interval regimens together, aligning them by the timing of their first dose, it is clear that longer interval schedules had a lower peak T-cell response. However, the second dose brought the peak long-interval T-cell level in line with the waning level of T-cells from the 4-week schedules that had already been decaying for two months – this was the case for ChAd/ChAd, ChAd/BNT and BNT/BNT, but not BNT/ChAd.

4.4.2.1.3.1 Paracetamol sub-study sensitivity analysis

Sensitivity analysis revealed qualitatively and quantitatively similar results (**Supplementary Figure 15** & **Supplementary Figure 16**)

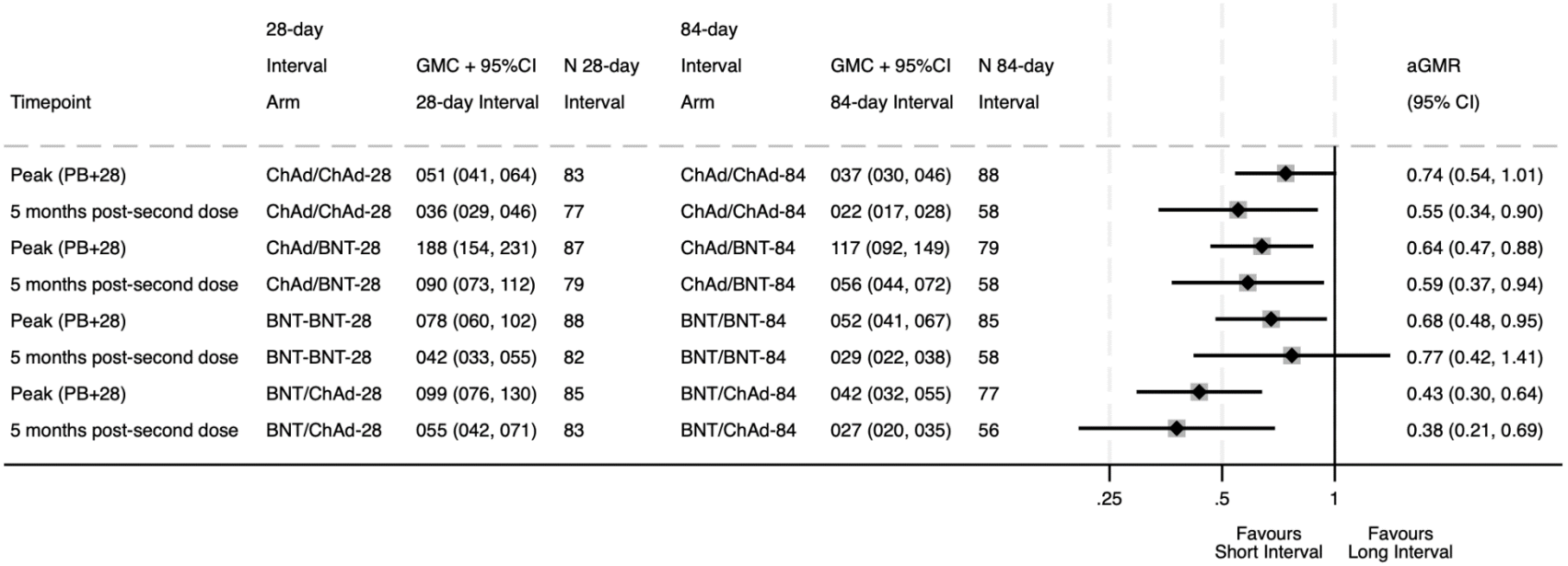

Figure 67 *– Com-COV1 Forest plot comparing peak (28 days post second dose) and 5-months post second dose IFNγ-SFC between 4-week and 12-week interval schedules . Peak response for the 4 week schedules was at 56 days, whilst for 12-week this was at 112 days. The 5-month timepoint was at day 182 for 4-week schedules, and at day 255 for the 12-week schedules. Left sided columns represent the raw, unadjusted GMC $\pm$ 95% CI. The forest plot represents the right sided aGMR derived from a multivariate linear regression.*

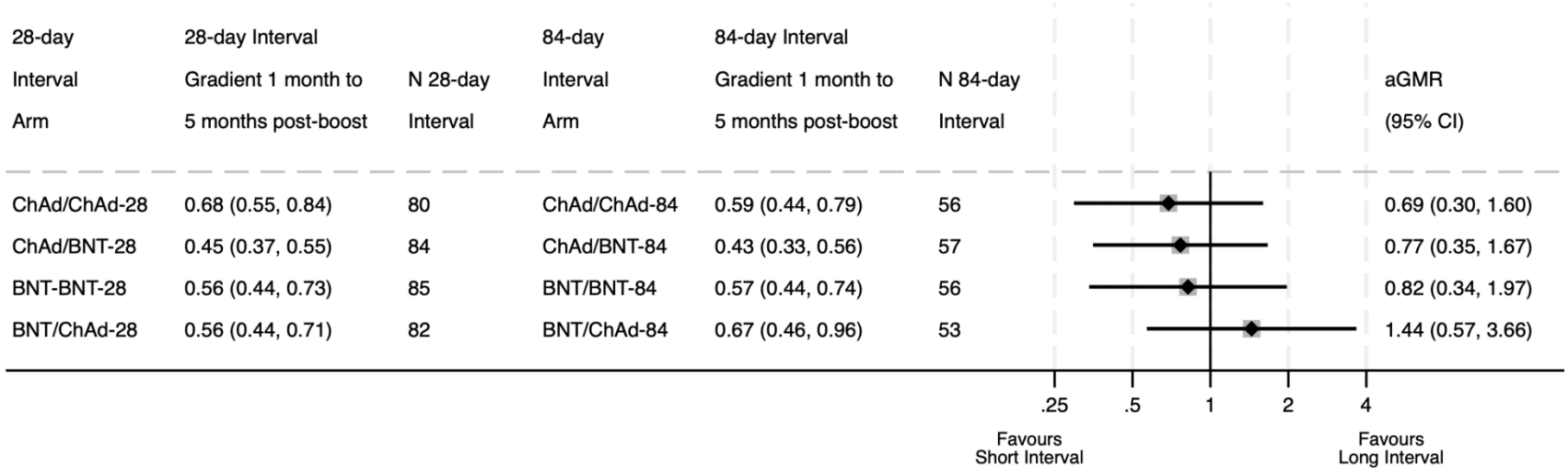

Figure 68 *– Com-COV1 Forest plot comparing rates of IFNγ-SFC wane between 4-week and 12-week interval schedules . Left sided columns represent the raw, unadjusted GMC ± 95% CI of the ratio of the 5-month antibody level over the peak T-cell response. The forest plot represents the right sided aGMR derived from a multivariate linear regression*

4.4.2.2 Exploratory Com-COV2 subgroup analysis by interval

As interval was not randomised at baseline, the interval analysis here is a post-hoc exploratory one. The permissible interval of 8-12 weeks was divided in two at the middle (10 weeks) although this left a slightly unequal division, as the majority of participants were vaccinated with a less than 10-week interval.

4.4.2.2.1 Anti-SARS-CoV2 spike IgG

Only BNT/BNT and BNT/NVX have a clearly improved humoral response with increased interval: aGMRs 1.2 (95%CI: 1.0, 1.5) and 1.7 (95%CI: 1.2, 2.3) respectively, although BNT/Mod and ChAd/Mod have non-significant trends in the same direction 1.1 (95%CI: 0.9, 1.3) and 1.2 (95%CI: 1.0, 1.4) respectively. (**Figure 69**).

Timepoint	Schedule	Interval	GMC + 95%CI Shorter Interval	N Shorter Interval	Interval	GMC + 95%CI Longer Interval	N Longer Interval	aGMR (95% CI)
28	ChAd/ChAd	56-69 days	2069.1 (1773.1, 2414.5)	126	70-84 days	1711.4 (1243.1, 2356.1)	45	0.95 (0.71, 1.28)
196	ChAd/ChAd	56-69 days	502.2 (394.6, 639.1)	79	70-84 days	473.0 (281.3, 795.4)	29	0.85 (0.55, 1.32)
28	ChAd/Mod	56-69 days	19068.2 (16794.3, 21650.0)	113	70-84 days	22767.5 (19368.6, 26762.8)	54	1.18 (0.96, 1.43)
196	ChAd/Mod	56-69 days	2939.4 (2515.0, 3435.3)	96	70-84 days	3804.4 (2955.7, 4897.0)	45	1.30 (0.98, 1.72)
28	ChAd/NVX	56-69 days	5728.5 (4682.6, 7008.1)	106	70-84 days	5224.4 (3993.7, 6834.3)	59	0.91 (0.66, 1.26)
196	ChAd/NVX	56-69 days	1060.6 (812.3, 1384.8)	72	70-84 days	1038.0 (741.4, 1453.4)	43	1.02 (0.67, 1.56)
28	BNT/BNT	56-69 days	16134.7 (13948.0, 18664.3)	114	70-84 days	18310.0 (15199.3, 22057.3)	53	1.24 (1.01, 1.53)
196	BNT/BNT	56-69 days	2072.0 (1703.0, 2520.9)	71	70-84 days	2672.0 (2126.0, 3358.4)	43	1.20 (0.89, 1.62)
28	BNT/Mod	56-69 days	23682.1 (20892.6, 26844.0)	112	70-84 days	21830.1 (18023.6, 26440.4)	53	1.08 (0.88, 1.33)
196	BNT/Mod	56-69 days	3539.0 (3044.7, 4113.6)	81	70-84 days	3679.1 (2820.6, 4798.9)	44	1.33 (0.95, 1.87)
28	BNT/NVX	56-69 days	8291.9 (6605.1, 10409.6)	118	70-84 days	10504.6 (8072.4, 13669.6)	54	1.65 (1.18, 2.29)
196	BNT/NVX	56-69 days	1206.2 (873.3, 1666.0)	83	70-84 days	1663.0 (1102.3, 2509.0)	38	1.55 (0.91, 2.65)

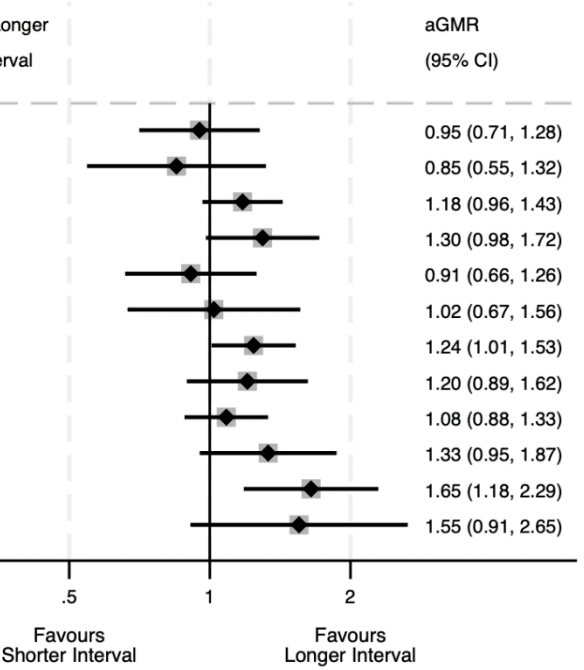

Figure 69 *– Com-COV2 Exploratory interval subgroup analysis forest plot comparing peak (28 days post second dose) and 5.5-months post second dose antibody responses between shorter and longer interval schedules . Left sided columns represent the raw, unadjusted GMC ± 95% CI. The forest plot represents the right sided aGMR derived from a multivariate linear regression.*

4.4.2.2.2 Pseudotype-virus neutralisation titre

BNT/BNT and BNT/NVX both demonstrate a larger response with longer intervals with aGMRs of 1.4 (95% CI: 1.1, 1.9) and 1.6 (95% CI: 1.1, 2.5) respectively. However, ChAd/NVX has a higher response with a shorter interval with an aGMR of 0.7 (95% CI: 0.5, 1.0). The remaining schedules ChAd/ChAd, ChAd/Mod and BNT/Mod do not have statistically significant differences between short and long interval schedules (**Figure 70**).

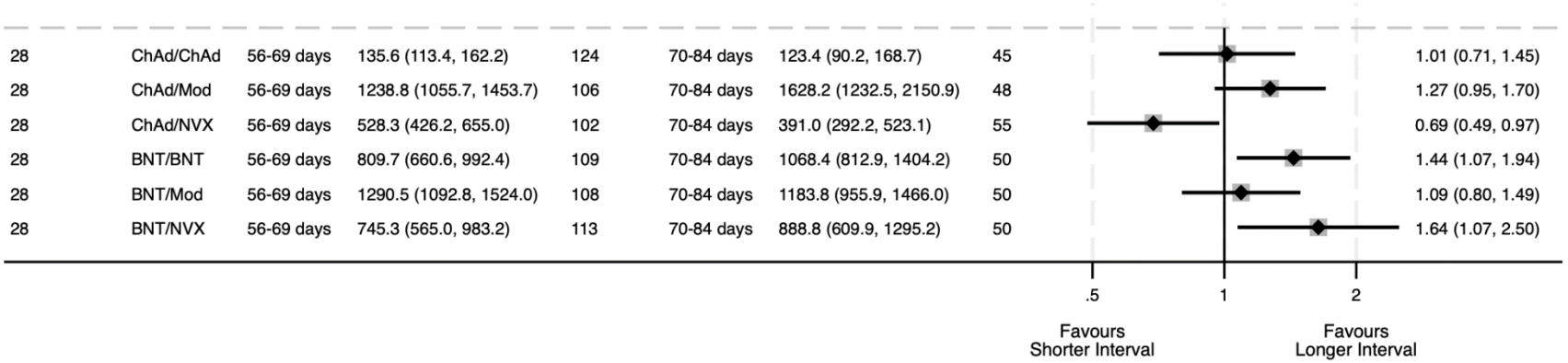

Figure 70 *– Com-COV2 Exploratory interval subgroup analysis forest plot comparing peak pseudotype virus neutralisation responses between shorter and longer interval schedules . Left sided columns represent the raw, unadjusted GMC ± 95% CI. The forest plot represents the right sided aGMR derived from a multivariate linear regression.*

4.4.2.2.3 T-cell ELISpot

Increasing interval had no discernible effect on T-cell responses at peak timepoint or later on at 5.5 months post-second dose (**Figure 71**).

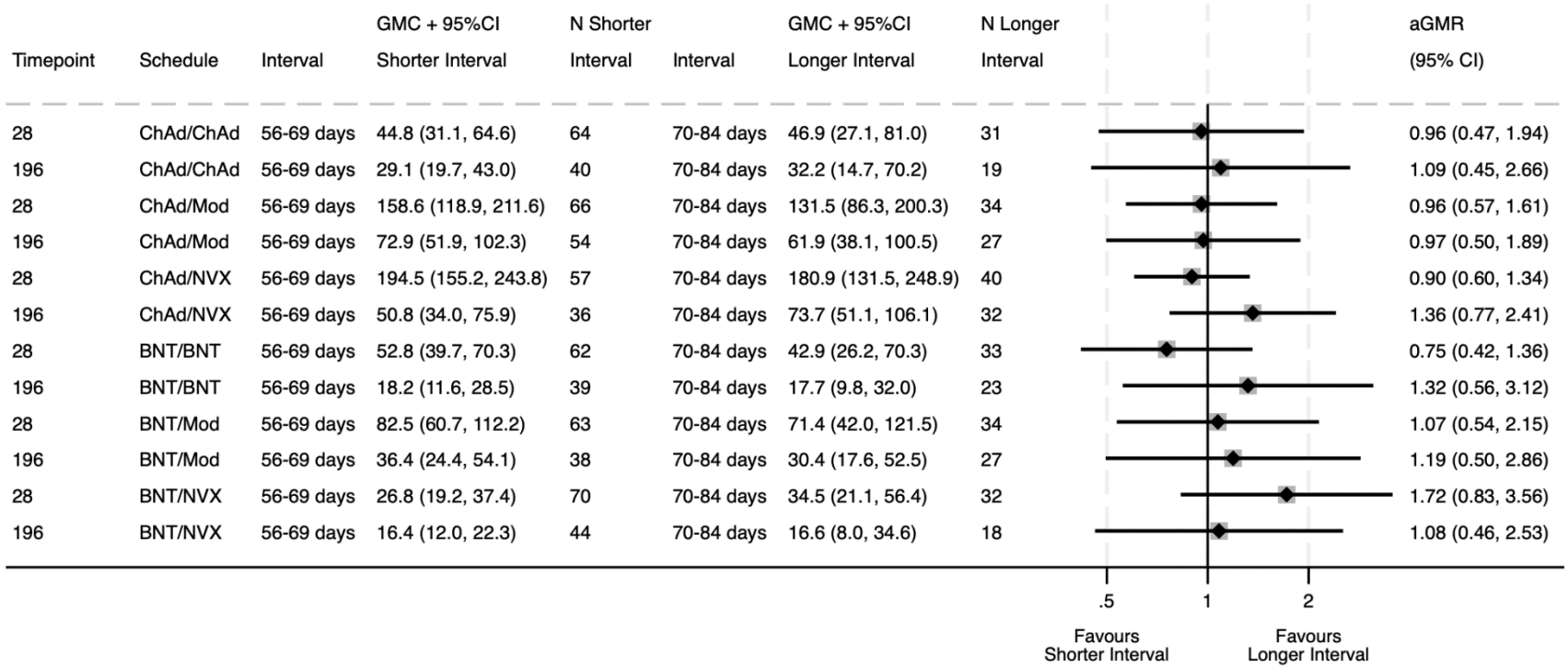

Figure 71 *– Com-COV2 Exploratory interval subgroup analysis forest plot comparing peak (28 days post second dose) and 5.5-months post second dose IFNγ-SFC between shorter and longer interval schedules . Left sided columns represent the raw, unadjusted GMC ± 95% CI. The forest plot represents the right sided aGMR derived from a multivariate linear regression.*

4.4.3 Reactogenicity in Com-COV1 only

Although not all symptom comparisons reach statistical significance, there are emerging trends (**Figure 72**). For BNT-boosted regimens (homologous BNT/BNT and heterologous ChAd/BNT) prolonging interval reduces the frequency of second dose systemic reactogenicity, as well as the duration for which severe symptoms were suffered, but severity itself was unaffected. For homologous ChAd/ChAd, prolonging interval increased frequency of systemic reactogenicity, but did not affect severity or duration of symptoms. For heterologous BNT/ChAd, the effect of prolonging interval was less clear, with no clear increase in frequency or duration, but a slight increase in severity (**Figure 73**, **Figure 74** & **Figure 75**).

These described effects were seen with systemic symptoms, however, with local symptoms, the above trends were only seen with BNT/BNT (**Supplementary Figure 17**, **Supplementary Figure 18** & **Supplementary Figure 19**).

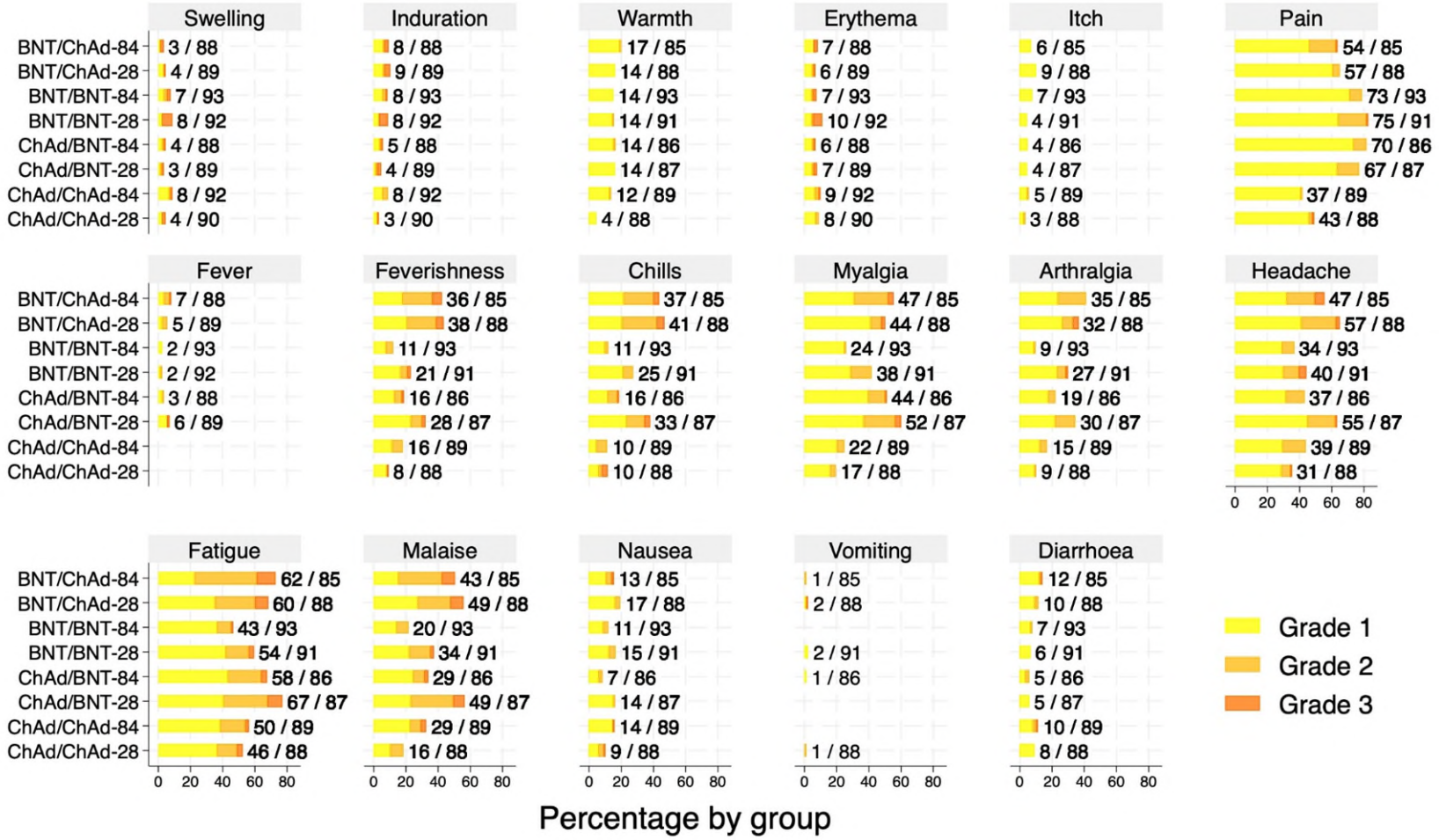

Figure 72 *– Com-COV1 Stacked bar chart showing the total number of participants suffering each local and systemic reactogenicity symptom after the second dose for all schedules . The severity presented is each participant's highest severity across 7 days after vaccination. Seronegative and seropositive participants are grouped together*

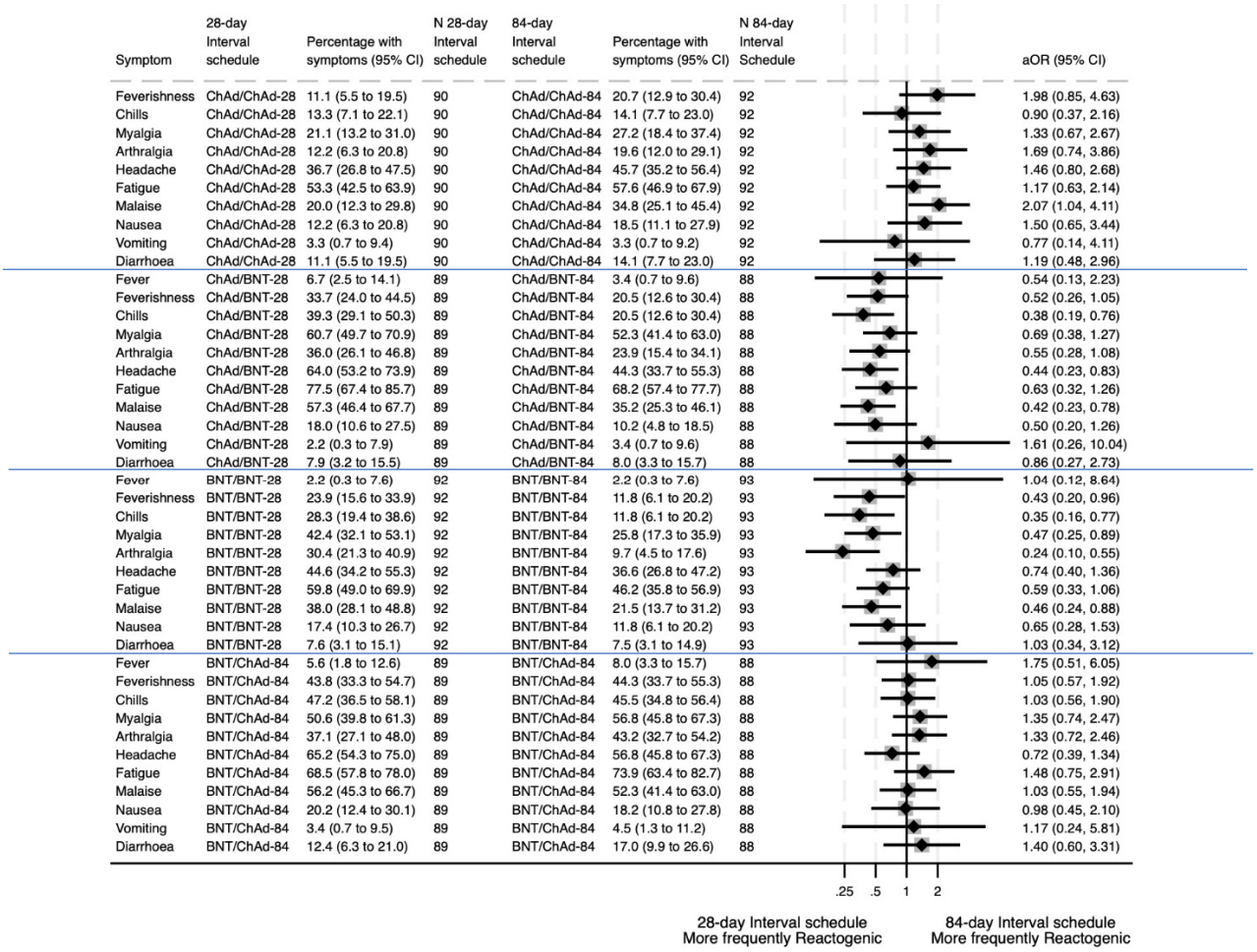

Figure 73 *– Com-COV1 Forest plot comparing the proportions of participants (seronegative and seropositive combined) suffering systemic reactogenicity symptoms in the first 7 days after the second COVID-19 vaccination between 4-week and 12-week interval schedules "Were you more likely to suffer a symptom of any grade after the second dose if the schedule had a short or long interval?"*

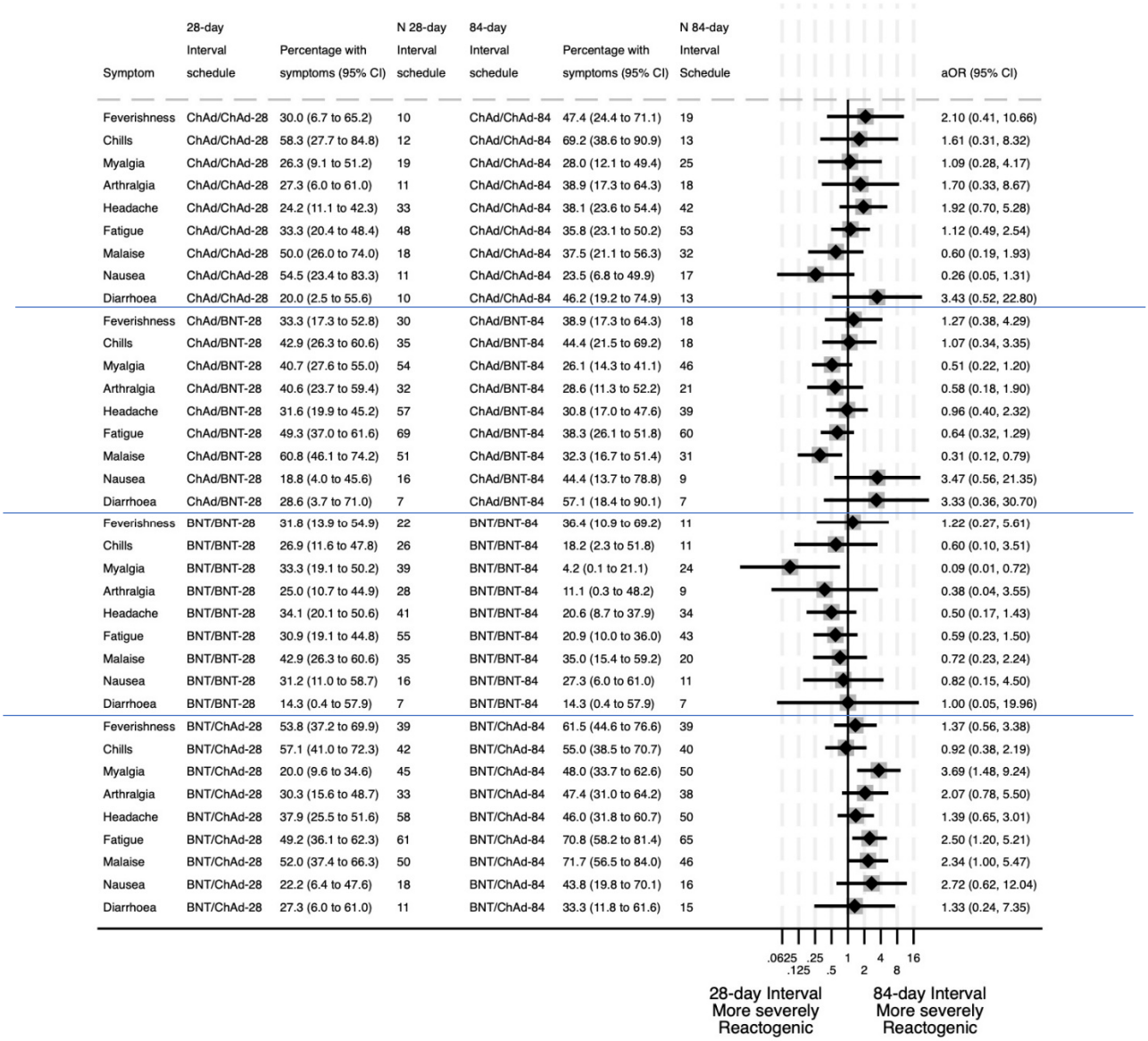

Figure 74 *– Com-COV1 Forest plot comparing proportions of participants (seronegative and seropositive combined) suffering moderate-severe (Grade 2 or above) systemic reactogenicity symptoms out of the total number of participants suffering any grade symptoms, in the first 7 days after a second COVID-19 vaccination between 4-week and 12-week interval schedule. "If you did suffer a symptom of any grade after the second dose, was it more likely to be moderate/severe if the schedule had a short or long interval?"*

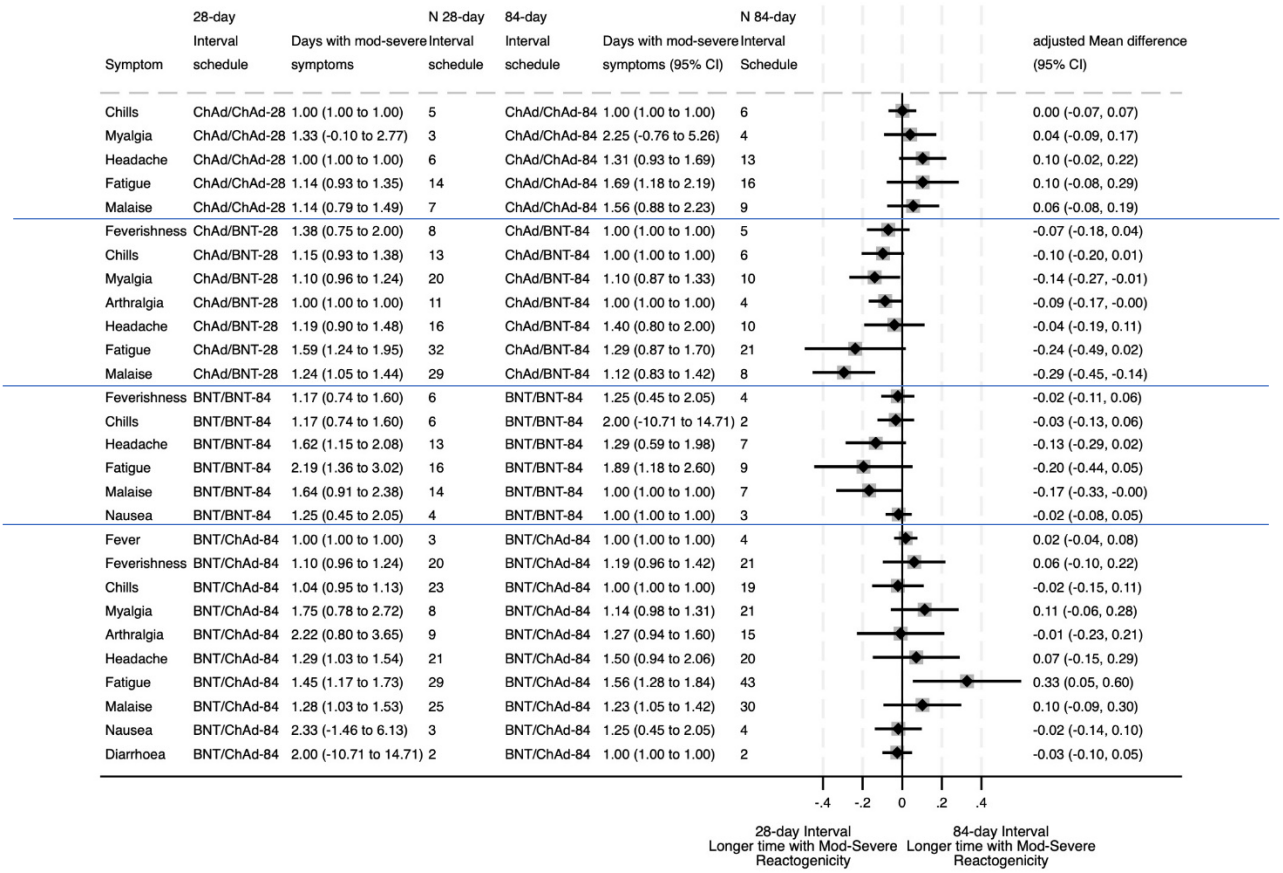

Figure 75 *– Com-COV1 Forest plot comparing durations of time spent with grade 2 or higher systemic reactogenicity symptoms after the second COVID-19 vaccination between participants (seronegative and seropositive combined) receiving schedules with 4-week or 12-week intervals a. "If you did suffer with moderate/severe symptoms after the second dose, were the symptoms likely to last longer after a short or long interval schedule?"*

4.4.3.1 Paracetamol sub-study sensitivity analysis

Results for frequency, severity and symptom duration for both local and systemic symptoms were broadly unaffected by adjusting for the paracetamol sub-study (**Supplementary Figure 20**, **Supplementary Figure 21**, **Supplementary Figure 22**, **Supplementary Figure 23**, **Supplementary Figure 24** & **Supplementary Figure 25**)

4.5 Discussion & Conclusions

4.5.1 Reactogenicity

It should again be noted here that the reactogenicity analysis for Com-COV1 is confounded by the 'advice for paracetamol at second dose' sub-study amendment, which impacted only on the second dose of the 12-week interval schedules and has been previously outlined. This sub-study will likely have resulted, overall, in an increased amount of paracetamol being taken in the trial at the time of the second dose in the 12-week arms only. Paracetamol is known to reduce typical reactogenicity symptoms. Therefore any results where the 12-week interval schedules were more reactogenic is likely to be true, but the magnitude of the effect may have been larger than demonstrated. For results where the 12-week interval is less reactogenic, it is not clear how much of this effect might be due to the potential increased paracetamol usage.

The second dose in ChAd/ChAd-84 is more reactogenic than the second dose in ChAd/ChAd-28. 12-week interval schedules with BNT as a second dose are less reactogenic than their 4-week interval counterpart schedules. Although this findings may, in part, be due to the confounding of the paracetamol sub-study, similar findings were obtained after adjustment for paracetamol study assignation in the sensitivity analysis giving confidence that this is a true result.

These data add complementary support to the hypotheses of how reactogenicity may be mediated, described in Section 3.5.1.2. If reactogenicity to BNT is mediated by typical IFNγ producing cells such as CD4 and CD8 T cells against the spike portion of the vaccine, then allowing a longer period for the T cell response to wane before administering the second dose of vaccine may mean reduced second dose reactogenicity with a longer interval.

In the case of homologous ChAd/ChAd, humorally mediated anti-vector immunity has been well described (141,152). Pre-second dose levels of anti-vector neutralising antibody have been noted to be inversely correlated with anti-SARS-CoV2 spike antibody response, but not T cell response. The hypothesis here is that anti-vector immunity (mediated by either the anti-vector antibody response or anti-vector T cell response) neutralises some of the vaccine and therefore the effective dose is reduced, resulting in lower immunogenicity. Increasing interval may allow the anti-vector response to wane, causing the effective long-interval second dose to be larger than the effective short-interval dose, thereby increasing reactogenicity that is mediated by atypical IFNγ producing cells, such as MAIT cells, which respond to the adenoviral vector backbone.

The lack of clear effect of interval on the reactogenicity of BNT/ChAd is in keeping with the above described model. Reactogenicity mediated by atypical IFNγ producing cells responding to the adenoviral vector backbone should be the same regardless of interval as the second dose is always the first time the individual's immune system will have seen the adenoviral backbone. The typical IFNγ producing cells which respond to the spike antigen should be reduced with a longer interval due to T cell waning, however, it may be that the dominant reactogenicity mediator may be the atypical IFNγ producing cells and so any drop in reactogenicity due to waning typical IFNγ producing cells may not be clearly seen.

4.5.2 Immunogenicity

A longer interval led to a larger humoral response including a disproportionately larger neutralising response. Conversely, a longer interval led to a lower T cell response. Both these effects were seen predominantly in the Com-COV1 analysis, where interval was randomised and included a larger range of intervals for comparison, including the shorter 4-week interval.

The lack of clear signal in the antibody response seen in Com-COV2 might be due to the limited range of interval examined, which is likely to blunt any effect seen. Importantly, the EBOVAC2 trial (117) demonstrated that most of the advantage in terms of antibody response was seen by increasing the interval from 28 to 56 days, with little change beyond this. If a similar pattern holds true for COVID vaccines then the varying interval from 56 to 84 days is significantly less likely to show an effect.

In this chapter's introduction, the underlying biology of why a longer interval might result in a larger binding antibody response is discussed. There is a biologically plausible hypothesis that by allowing a more complete maturation of the priming response with a longer interval, before initiating the anamnestic response, one might expect an incremental benefit to delaying the second dose up to the point that the priming response has fully matured. Avidity testing, although not performed, might be a further way of evaluating this hypothesis – one might therefore expect a higher affinity humoral response with longer interval schedules.

An alternative hypothesis, which is not mutually exclusive, would be related to the 'blunting effect' seen in infant responses. Maternal vaccination and subsequent transmission of these antibodies to infants via the placenta may reduce the infants' abilities to produce a humoral response to vaccines subsequently administered to the child directly (153). In the case of COVID-19 vaccines, it may be that the presence of antibodies from the priming dose might cause an 'auto-blunting' effect, whereby a higher antibody titre at the time of the second dose, would bind the vaccine antigen, reducing the effective vaccine dose delivered and therefore reducing the peak response.

An experiment to delineate these effects would be to inoculate a cohort of vaccine-naïve mice with anti-SARS-CoV2 spike IgG and subsequently vaccinate them and compare their response against mice who had not been inoculated with anti-SARS-CoV2 spike IgG . A similar experiment has already been performed by a colleague (Cameron Bissett,

DPhil thesis, unpublished work), where mice who have received wild-type SARS-CoV2 vaccines were bled and their serum transferred to naïve mice. These recipient mice then received an Omicron-SARS-CoV2 vaccine. Mice who had higher levels of transferred antibody had lower levels of vaccine response.

I have demonstrated no difference in wane between different intervals for any schedule. Analyses that I have previously published suggested that there was a statistically significant difference in rate of wane between BNT/BNT-84 and BNT/BNT-28, with the long interval schedule waning more slowly. However, the absolute difference in wane was small 18% vs 19% (134), of the peak antibody titre remaining, which is unlikely to be of clinical significance. This divergence in conclusions is likely to have arisen from small differences in the analysis population (**Figure 64**).

The drop in T cell ELISpot count with extended interval is of uncertain significance, especially in the context of proven improved efficacy in the setting of a clinical trial. Further investigation to delineate whether there are differences in T cell populations via flow cytometry would be of value to determine whether there is a cellular component that could be contributing to the difference in vaccine efficacy and what the significance of the difference in IFNγ SFC frequencies may be.

4.5.3 Caveats

The analysis of interval in Com-COV2 is a post-hoc one, as interval was not a randomised parameter. Although post-hoc exploratory analyses have their place and are of use in generating hypotheses that may subsequently be tested through experimentation or further trials, they must be viewed circumspectly. The lack of effect of interval seen in some of the Com-COV2 schedules, including homologous ChAd/ChAd where this has been demonstrated in Com-COV1 and the original ChAdOx1 nCoV-19 efficacy trials is a warning to interpret these results with caution, although other

reasons, such as an insufficient range of intervals in Com-COV2 may explain this lack of response.

4.5.4 Final Comment

Although there was an improvement in antibody response (and efficacy against symptomatic infection in the ChAdOx1 nCoV-19 trial (87)), more recent developments in the understanding of protection against SARS-CoV2 suggest that protection against symptomatic infection is short-lived regardless of vaccine administered, whilst protection against severe disease has not been seen reliably to wane. Purposefully to delay a second dose of vaccine purely for an improved humoral response might not be advisable from this point of view. The programmatic decision for interval selection is a complicated one, based not just on immunogenicity data, but also on the availability and supply of vaccine, the levels of transmission in the community, the vulnerability of the population in question and the degree to which non-pharmaceutical public health measures are being enforced. However, what these data do provide is evidence to support a flexible approach towards interval, rather than a rigid 3-4 weeks as originally recommended by the manufacturer.

Chapter 5 Mucosal Immune Responses

5.1 Background

5.1.1 Anatomy & Structure of the Mucosal Immune System

Mucosal surfaces are the first line of defence against the vast majority of pathogens, as most pathogens gain access to the human body via the respiratory, gastrointestinal or genitourinary mucosae. The mucosal immune system has evolved to protect these surfaces from invasive pathogenic organisms, as well as to tolerise and regulate the immune response to a diverse range of non-pathogenic stimuli such as food proteins and commensal bacteria.

The mucosal immune system, including the skin, covers a huge area and forms the largest part of the body's immune tissues containing approximately three quarters of the body's lymphocytes and the majority of the body's immunoglobulins (154,155). Lymphocytes and other immune cells are found throughout the mucosa, both scattered throughout the epithelium and sub-epithelial lamina propria, as well as in more organised immune structures. These more organised secondary immune structures are generically known as mucosa-associated lymphoid tissues (MALT). Some of the major secondary lymphoid structures exist in the oropharynx (the palatine tonsils, adenoids and lingual tonsils), which form an anatomical ring (Waldeyer's ring) at the entrance to the aerodigestive tract, but there are also isolated follicles in other parts of the upper respiratory tract including the bronchus (bronchus-associated lymphoid tissue, BALT) and the nasal mucosa (nasally associated mucosal tissue, NALT). The lymphoid cells outside of these organised structures comprise the effector T cells and plasma cells of the mucosal immune system.

The systemic and mucosal immune systems are similarly structured in terms of their constituent humoral and cellular components, with the major antibody isotypes in relation

to mucosal bacterial and viral infection being IgG, IgA and IgM (107). The mucosal work presented in this chapter investigates only antibody responses and so this background will similarly focus on the humoral aspect of the mucosal immune response.

There are broad, crucial differences between the systemic and mucosal immune systems. The antibody isotype that predominates in the blood is monomeric IgG. In the mucosa, the predominant immunoglobulin is secretory IgA (sIgA), a dimeric form of IgA covalently bound to a protein called the secretory component (SC), in contrast to the monomeric form of IgA found in serum. IgA does not mediate the same kind of pro-inflammatory responses that systemic IgG is capable of, such as activation of the classical complement pathway, opsonisation or NK cell activation. Instead IgA predominantly acts via neutralisation and prevention of adherence (also known as immune exclusion) (156). Mucosal IgA has been shown to have more potent and broader neutralisation capabilities than monomeric mucosal IgG in the case of influenza (157) and has been shown to confer protection alongside serum IgG in the case of RSV (158).

The more potent neutralisation capability of sIgA may be mediated by differences in both affinity and avidity. Experiments which have constructed dimeric clones of plasma-derived monomeric IgA have demonstrated an average 15 fold (range 4 to 113) increase in neutralisation, supporting the notion that it is sIgA's dimeric nature that is responsible for a large portion of its neutralising capability (159). This broader neutralisation capability may, in part, be due to atypical non-Fab mediated binding such as by Fc-mediated binding, which itself may depend on glycosylation characteristics (160).

The mucosal immune system is further set apart from the systemic system by the presence of populations of non-antigen-specific natural effector cells such as MAIT cells (mucosal associated invariant T cells) that play an increasingly described role in defence (161). Finally, the follicular dendritic cells that present antigen are specialised. They, like macrophages, plasma cells and germinal cells of the intestine, display the Fcα/μR

receptor, which binds IgA and IgM. There is speculation that they may have a role in antigen capture for presentation to B cells in follicles (162). These dendritic cells can be divided broadly into intraepithelial and lamina propria populations. They co-stimulate lymphocytes with anatomically site-restricted proteins that allow targeting of immune responses by a homing or trafficking mechanism mediated by anatomically restricted vascular adhesion molecules.

As one component of the body's wider immune system, the mucosal immune system functions in a reasonably compartmentalised fashion, largely independently of the systemic immune system. The concept of the 'common mucosal immune system' encompasses the idea that antigenic exposure at one mucosal surface, may result in induction of an immune response at anatomically distinct mucosal surfaces (163). However, more recent work suggests that, whilst this may, in part, be true, there is internal compartmentalisation within the mucosal immune system (155), likely dependent on the sharing of anatomically restricted vascular endothelial adhesion proteins. This results in the gut and mammary glands being separated from the respiratory mucosa and urogenital tracts.

Despite its numeric advantage over the systemic immune system in terms of number of immune cells contained within it, the mucosal immune system remains relatively understudied in comparison to the systemic immune responses.

As the first line of defence against many pathogens, the mucosal immune system is critical in terms of the prevention of pathogen invasion and the development of clinical disease and is likely key in determining pathogen transmission characteristics.

5.1.2 Respiratory mucosal sample types

There is a variety of different respiratory mucosal sample types. This includes sample types acquired from relatively small anatomical compartments such as the nasopharynx:

Nasal lining fluid, saliva and gingival crevicular fluid (GCF). It also includes samples from the lower respiratory tract, such as bronchoalveolar lavage (BAL). Each sample type is distinct and has its own advantages and disadvantages.

Lower respiratory tract antibody responses are more invasive and more technically difficult to achieve. They do, however, give an idea of the immune response deeper in the lungs, which is typically dominated by systemically derived IgG, a finding supported by the presence of monomeric IgA (164).

Upper respiratory tract antibody responses are much easier to acquire and are typically dominated by locally produced dimeric IgA. Additionally, saliva and oral fluid samples contain significant contributions from GCF, which is a rich source of transudate from the systemic circulation. This makes interpretation of the origin of immunoglobulin in these samples more challenging (165). Nasal epithelial lining fluid, on the other hand, is not affected by GCF.

The choice of mucosal sample in any piece of research has profound implications on the interpretation of the results.

5.1.3 Mucosal antibody origins

The majority of mucosal IgG is thought to enter from the systemic circulation by various mechanisms including passive paracellular diffusion, receptor-mediated transepithelial transport (via FcRn) and fluid-phase endocytosis. FcRn, whilst abundant in the placenta and responsible for the active transport of maternal IgG, has only relatively recently been found in adult bronchial tissue and been considered to contribute to mucosal IgG levels (166).

The notion that most mucosal IgG is systemically derived is supported by various pieces of evidence including the presence of similar ratios of total vs antigen-specific IgG in both mucosal secretions and in blood, and also the presence of similar relative

proportions of IgG subclasses. There are, however, some mucosal surfaces, such as the female genital tract, whose IgG appears to be predominantly derived from local production (156).

The evidence that mucosal IgG can be derived through local production comes from various studies showing differences in antigen-specific IgG titres between mucosal secretions from different anatomical locations (167). These include differences in respiratory pathogen specificity (168), gastrointestinal pathogen specificity (169) and HIV antigen specificity (170). Additionally, there is evidence that IgA deficient individuals produce higher levels of local IgG- and IgM-producing cells, which helps ensure total immunoglobulin amounts are maintained, compensating for the lack of IgA (171).

5.1.4 Quantifying Mucosal Immune responses

The quantification of mucosal antibody titres requires some consideration, as the sample types described are subject to some degree of variation, unlike serum. Absolute titres of antigen-specific mucosal IgG and IgA are sometimes reported as values normalised against the total non-antigen specific IgG and IgA respectively (172). The rationale for this is two-fold:

Firstly, to account for unmeasured variability in sampling volume. One study performed sampling using nasosorption strips in a laboratory environment, using pre- and post-sampling weights to estimate average volume absorption (107).

Secondly, to account for intra-individual variation in immunoglobulin concentration. Salivary IgA increases with mastication due to increased epithelial cell transcytosis (173). Diurnal variation of IgA in salivary samples can be up to 4-fold (174). Most of this variability was seen to be due to increased titres at night, with there being relatively little variation between 0800-1600 (when most clinical trial samples are taken), a finding supported by another study, which found no significant difference between morning and

afternoon samples (175). Diurnal variation is also observed in nasal samples (176,177) and appears mediated by changes in volume of mucous secretion rather than in levels of antibody secretion.

Normalisation as a strategy to account for these unknowns appears justifiable, however, there is ongoing debate as to the magnitude of benefit: One study's results suggests that normalisation by total IgA or total IgG does not impact significantly on analysis results when compared to non-normalised analyses (107).

5.1.5 Mucosal immune responses

5.1.5.1 Responses to SARS-CoV2 infection

The SARS-CoV2 virus is a respiratory pathogen that is transmitted predominantly via airborne routes (such as via droplets or aerosol) and, to a lesser extent, via fomite contact (178). The respiratory mucosa is the first point of contact with the immune system and likely plays a pivotal role in early infection control and transmission reduction (172,179).

The magnitude of mucosal antibody responses to SARS-CoV2 infection, much like serum IgG, has been variably reported to correlate with disease severity in the unvaccinated (180), but this finding has not been universally reported (159,181).

In participants who developed SARS-CoV2 infection during the SARS-CoV2 challenge study (182), nasal IgG and IgA antibody levels lagged behind serum levels by about a day, but subsequently peaked more rapidly, before a more rapid initial wane, although antibody was still detectable at levels above baseline at 90 days. Data were insufficient to delineate differences in rates of long-term antibody decay between serum and mucosal antibody.

Waning of oral fluid and nasal antibodies post-infection have been reported to be rapid, with individual's titres of mucosal anti-SARS-CoV2 spike IgA being significantly reduced by three months (180,183), but still raised 8-9 months post-infection and 6 months post-vaccination) (184–186). Oral fluid and nasal IgG appear to be more persistent, with a closer correlation to serum IgG over time.

Cohort studies examining the household contacts of diagnosed cases found that those with mildly symptomatic infection (including both those who were PCR negative and PCR positive) did not always demonstrate a serum antibody response. Small numbers of participants in another study also failed to demonstrate a mucosal antibody response (187), but did demonstrate a plasma T cell response (188,189). The implications of the presence of this T cell response are potentially confounded by pre-existing cross-reactive T cell populations against seasonal coronaviruses. Hypothesised seasonal coronavirus cross-reactive T cell responses have been observed in undiagnosed, asymptomatic participants (190). Increases in mucosal IgA in the absence of seropositivity have been reported in a handful of patients (191). In combination, these studies suggest that not all aspects of the immune response in all immune compartments are activated in the same way in different people.

Post-infection mucosal anti-SARS-CoV2 spike IgA levels have been associated with protection against further infection by both Omicron and BQ.1 strains (192,193). Anti-SARS-CoV2 spike mucosal IgA levels from previous infection have additionally been demonstrated to provide protection against symptomatic infection over and above the protection provided by serum anti-SARS-CoV2 spike IgG levels, when investigated in a nested Poisson regression analysis (194).

The functionality of mucosal lgA post SARS-CoV2 infection is predominantly neutralising (187). IgA contributes more to neutralisation more than IgG in salivary and nasal lining fluid samples in convalescent patients (164), but IgG and IgA contribute more equally in

oral fluid samples. IgG appears to be the predominant neutralising isotype in oral fluid and nasal lining fluid in those without previous infection (184,194,195). These differences suggest that mucosal exposure is key to developing a mucosal IgA response.

The functionality of IgG in different anatomical compartments has been observed to vary with mucosal lgG having greater monocyte dependent phagocytosis than serum IgG post-infection (187). This supports the notion that mucosal IgG may be a mixed population of both locally produced and systemically derived antibody.

The cross-protective neutralising response against variants of concern (VOC) in those previously infected, appears to be mediated predominantly by nasal IgA, again supporting the notion that the higher valence of dimeric IgA imbues a broader neutralisation capacity.

5.1.5.2 Responses to non-COVID-19 immunisation

Although pre-clinical animal and human studies have investigated whether intramuscular vaccination can stimulate a de novo local mucosal antibody response, further investigation is needed as to the nature of such responses and their potential contribution to overall immunity (196).

An important caveat of the studies which used animal models to investigate mucosal responses to intramuscular vaccination is the fundamental difference between the immune systems of humans and other mammals (197). These include differences in immune cell population make up, antibody transporters (198), the absence of a ‘hepatic pump’ system in humans which secretes secretory IgA into the bile in rats (167) as well as differences in antibodies themselves – mice have a single IgA isotype, whereas humans have two: IgA1 and IgA2 (160). Inference from animal studies must be made cautiously.

In humans, various parenteral routes of immunisation have been investigated for their impact on mucosal immunity. Mucosal delivery of antigen via oral, nasal or inhalational systems is very different and is discussed in the context of SARS-CoV2 in Chapter 6 .

Intraperitoneal immunisation has been variably described to produce mucosal lgA in mice (199,200), but has failed to produce a mucosal IgA response in humans (201). Although unlikely to become a viable route for mass immunisation in humans, it is an attractive thought that, as a mucosal surface, the peritoneum may generate a de novo local mucosal immune response. The difference in response between mice and humans may be due to fundamental differences between murine and human B cells. Two types of murine B cell exist: locally produced IgA-producing B1 cells and bone-marrow produced B2 cells. Human B-cells are all B2-like, without a B1 equivalent (202).

Human mucosal responses to subcutaneous and intradermal vaccines have not been well studied. Subcutaneous vaccination in mice does not induce a mucosal IgA response, although mucosal IgG has been detected in several studies (203,204). Intradermal vaccination in murine studies have focussed on deep respiratory samples, whose antibody titres have heavy contribution from the systemic circulation (205–207). Studies which took upper respiratory tract samples did not detect mucosal IgA responses in nasal or lung compartments (208,209) or in faecal samples (209,210). Intradermal vaccination in pigs and mice may, however produce an IgA response in vaginal secretions (211–213).

Intramuscular vaccination is by far the most commonly used route of immunisation, however, the literature is inconsistent with respect to sample type used and antibody isotype assayed.

One murine study reports measurement of increased mucosal IgG, but not IgA following IM immunisation in lung washing samples (214), a sample type more likely to contain transudated systemic IgG (215–217). Nasal sampling in murine studies investigating E.

coli and Group B Streptococcus subunit protein vaccines found that IM vaccination did not produce a nasal mucosal IgA response (218,219).

In contrast, a study in bovine calves found IM immunisation with recombinant E. coli proteins did produce mucosal IgA and IgG responses, which was associated with reduced faecal shedding of bacteria after oral challenge (220).

A study conducted outside of influenza season in humans comparing oral, intranasal and IM trivalent inactivated influenza vaccine, or intranasal live attenuated, cold-adapted reassortant influenza A virus showed a lack of nasal IgA response after IM immunogen exposure, although there was a small salivary IgA response (221).

In contrast again, other older studies in humans have shown two doses of IM pneumococcal conjugate vaccine produce salivary IgA responses (222,223) and that IM pneumococcal polysaccharide immunisation gives a minor increase in both salivary and lacrimal IgA (224).

Overall, there is no strong evidence that any kind of non-mucosally delivered vaccination strategy elicits a local mucosally derived immune response.

5.1.5.3 Responses to COVID-19 immunisation

Immunological analysis of both COVID and non-COVID vaccines has focussed on responses in the blood. Whilst this may give partial insight into the mechanisms of protection against both severe disease and symptomatic infection, alone it does not give a complete understanding of the body's immune response – an analysis of the mucosal responses is also required.

Anti-SARS-CoV2 spike nasal mucosal IgG and oral fluid IgG levels correlate well with systemic binding IgG and are boosted by IM vaccination. Neutralising activity has also been shown to increase in both systemic and mucosal compartments in response to

booster doses of vaccine (225). However, reports of how IM immunisation affects nasal mucosal IgA have varied significantly (180,195).

One study found no significant salivary anti-SARS-CoV2 spike IgA response following first or second doses of mRNA vaccine (226), whilst another study reported oral fluid anti-SARS-CoV2 spike mucosal IgA response to first doses of BNT162b2 in previously infected participants. However, in this second study there was a concurrent *rise* in anti-nucleocapsid oral fluid IgA responses, suggesting that the anti-SARS-CoV2 spike mucosal IgA rise might have been induced by mucosal SARS-CoV2 exposure (195). Other data suggest that mRNA vaccines might induce a low level of locally produced salivary (227) or nasal anti-SARS-CoV2 spike IgA responses after both first and second BNT doses (228–230), however, levels of measured mucosal IgA were low and inconsistent.

Some of the studies reporting mucosal IgA responses postulate the mechanism for local mucosal IgA production may be through the dissemination of mRNA lipid nanoparticles from the site of vaccination with the possibility of antigen production at distant sites. This theory is supported by a murine study, describing broad, but low level biodistribution of an influenza mRNA vaccine (231) as well as by work in humans showing S1 subunit and subsequently spike protein being detectable in blood for up to 8 days and 9-28 days respectively after mRNA vaccination (232).

An important caveat for many of the studies reporting a potential anti-SARS-CoV2 spike IgA increase in response to IM vaccination, is that they were not always able also to measure mucosal anti-nucleocapsid responses, as a way to check for confounding mucosal SARS-CoV2 exposure.

The neutralisation boost seen in one study, which also reported a mucosal IgG boost (225) was seen to be effective only against closely related variants, suggesting that IgG-mediated mucosal neutralisation may have a narrower spectrum than the neutralisation

mediated by secreted dimeric mucosal IgA (192,193). An alternative interpretation is that immune imprinting has narrowed the antibody repertoire such that VOC are not effectively neutralised (233,234).

Another analysis of mucosal responses (235) measured salivary IgA, using a secondary antibody specific against the secretory component rather than the Fc component of IgA. This theoretically allows differentiation between local produced dimeric IgA from transudated monomeric IgA. The number of participants was small (18 baseline seropositive participants and 11 baseline seronegative participants), but importantly the authors measured salivary anti-nucleocapsid IgA, which allowed them to account to some degree for concurrent mucosal SARS-CoV2 exposure. They investigated the possibility of concurrent mucosal exposure by visual inspection of the pooled anti-nucleocapsid mucosal IgA graphed data, deeming it unlikely. However, they stopped short of a more formal statistical analysis on an individual participant level. They do report mucosal IgA increases apparently in response to IM vaccination, but these responses are inconsistent between participants. The two seronegative participants with the largest apparent IM-vaccine-induced anti-SARS-CoV2 spike mucosal IgA responses did, in fact, have an increase in their anti-nucleocapsid mucosal IgA response at the point of vaccination, as did a large number of the seropositive participants.

The same group analysed mucosal responses to third doses of vaccine in a separate paper, but did not measure mucosal anti-nucleocapsid IgA. They drew similar conclusions to their work on two doses, however salivary sIgA was not consistently increased post-vaccination. Additionally there was evidence that sIgA increases tended to occur after breakthrough infection (236).

It is vitally important that studies clearly report what aspects of immunity are measured, how they are measured and what the sampling method and location are. As the relatively new field of mucosal immunology has developed and matured, an appreciation for these

aspects has increased. Biological and linguistic oversimplifications have been unhelpful leading to increased confusion in this field (160).

The conclusions of the papers discussed are varied, with some suggesting that non-mucosally delivered vaccines are able to induce mucosal immune responses. However, by being more granular in one's approach to the interpretation of the reported results, it is apparent that non-mucosally delivered vaccines may well induce an IgG response that is detectable in both the upper and lower respiratory mucosae, but this response is likely to originate from the serum via active and passive transport. Upper respiratory mucosal dimeric IgA does not appear to be induced by non-mucosally delivered vaccines, however, some of the more recent studies looking at COVID, challenge this view. In order to investigate this further, future studies, including the work presented in this chapter, will have to account for confounding events such as inter-current natural exposure or infection. This is especially critical when there is active community transmission such as in an endemic or pandemic setting. Mucosally-delivered vaccines are the obvious next step in terms of trying to elicit a de novo locally derived mucosal response and this has been recognised by the US government, who have dedicated significant funds to develop this 'next generation' of vaccines as well as investigate their mucosal and systemic immune responses (237).

5.2 Introduction

The design of the Com-COV1 trial was such, as to aid rapid decision making to UK immunisation policy makers. The infrastructure of this trial, was an excellent setting on which to conduct exploratory analyses of additional mucosal samples. The schedules investigated in this mucosal work are:

- ChAd/ChAd-84 – 12 week interval homologous AstraZeneca/AstraZeneca
- ChAd/BNT-84 – 12 week interval heterologous AstraZeneca/Pfizer
- BNT/BNT-84 – 12 week interval homologous Pfizer/Pfizer
- BNT/ChAd-84 – 12 week interval heterologous Pfizer/AstraZeneca

5.3 Initial hypotheses

1) Mucosal IgG responses, as a proportion of Serum IgG responses, will not vary between schedules
2) Mucosal IgA responses will not be induced by IM vaccination

5.4 Results

5.4.1 Pre-pandemic baseline

Interpretation of both serum and mucosal antibody titres requires defining a pre-pandemic baseline for full contextualisation. This was achieved by conducting the MSD assay (Section 2.16.2) on 28 pre-pandemic serum samples and 25 pre-pandemic mucosal lining fluid samples. The geometric mean concentration (GMC) plus two standard deviations (SD) of these samples was taken as the upper limit of reactivity. For antigens which would not have been encountered by the general population before (SARS-CoV2 spike, SARS-CoV2 RBD, SARS-CoV2 nucleocapsid, SARS-CoV1 spike, MERS-CoV spike), this level represents the limit of non-specific reactivity and can be interpreted as 'seronegativity' or 'muconegativity'. For antigens where previous exposure is highly likely (the seasonal coronaviruses HCoV-OC43 spike, HCoV-HKU1 spike, HCoV-NL63 spike and HCoV-229E spike), this value represents pre-pandemic levels of antigen-specific reactivity (**Figure 76**).

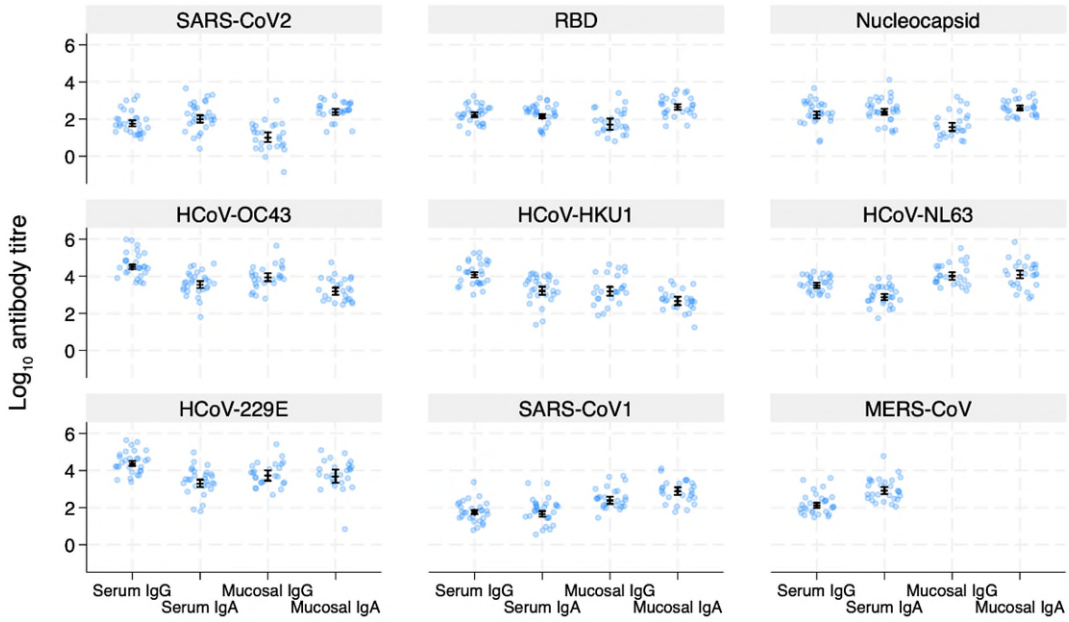

Figure 76 *– Scatter plots of log_{10} transformed IgG and IgA antibody titres from both serum and mucosal samples from pre-pandemic samples.*

5.4.2 IgG and IgA responses in both serum and mucosa

The mucosal and serum anti-SARS-CoV2 spike IgA and IgG responses were measured in each of the four 12-week schedules. 25 samples per schedule were analysed at pre-prime (day 0), 28-days post-second dose (day 112) and a persistence timepoint (day 182) (**Figure 77**).

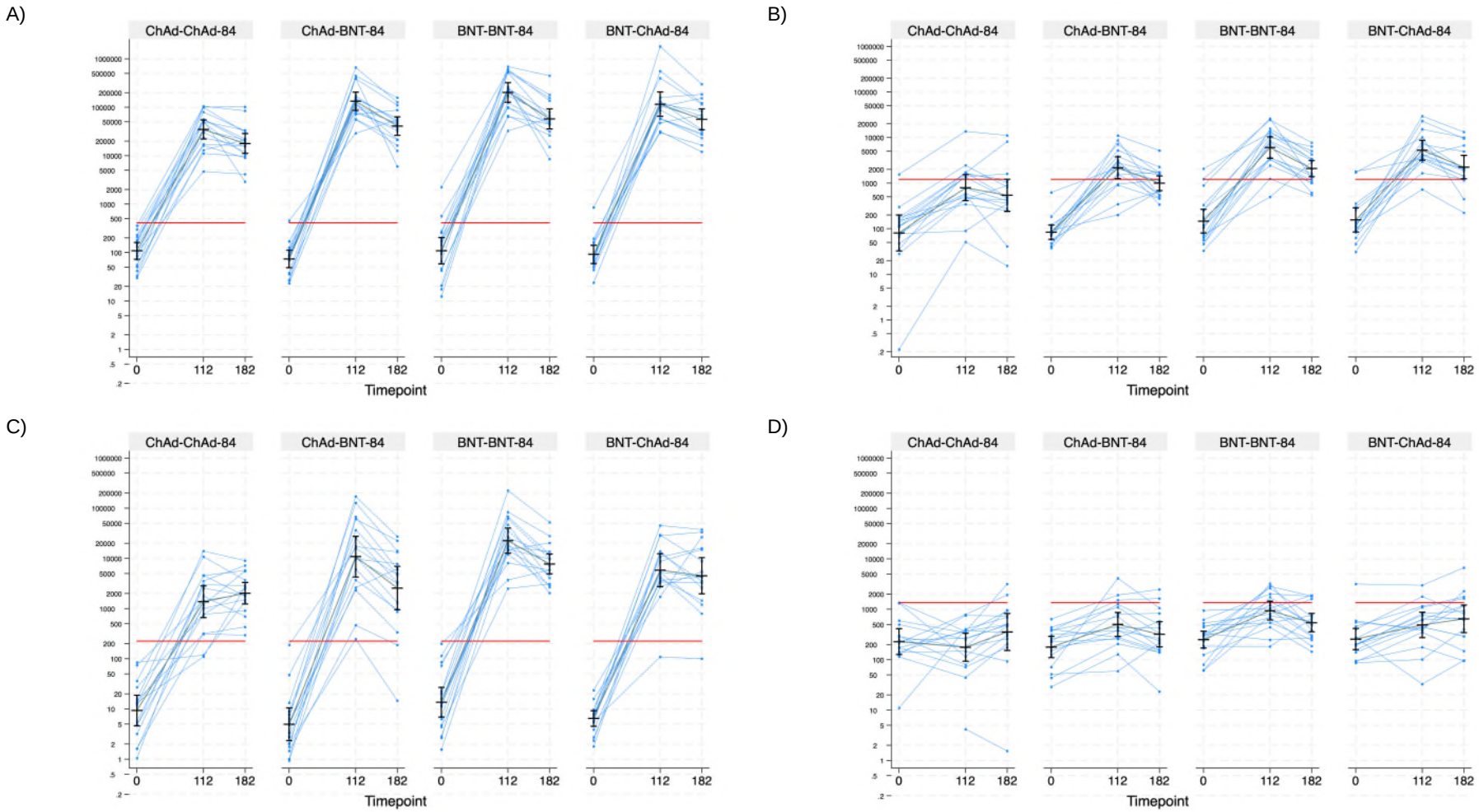

Figure 77 *– Kinetics of anti-SARS-CoV2 spike antibody response in seronegative participants (blue) with GMC ± 95% CI (grey/black) by 12-week vaccine schedule for A) Serum IgG, B) Serum IgA, C) Mucosal IgG, D) Mucosal IgA. Timepoints: D0 (pre-prime), D112 (28 days post second dose), D182 (2 months post second dose). Red horizontal line: Pre-pandemic threshold*

Serum anti-SARS-CoV2 spike IgG showed a consistent clear response at Day 112 in seronegative participants (serostatus defined in Section 2.11.1) with subsequent waning at Day 182. Serum IgA and Mucosal IgG showed qualitatively similar responses to Serum IgG. Serum IgA titres were 25-56 times less in magnitude than serum IgG, the variability of which, suggests that serum IgG and serum IgA are not always produced in the same ratio between vaccine schedules (**Table 37**).

Table 37 *– GMC (95% CI) for Serum IgG & Serum IgA per schedule at D112*

Arm	Serum IgG GMC (95% CI)	Serum IgA GMC (95% CI)
ChAd/ChAd-84	34,798 (23,258, 52,063)	989 (505, 1,934)
ChAd/BNT-84	143,863 (94,925, 218,031)	2,557 (1,478, 4,422)
BNT/BNT-84	226,341 (144,493, 354,554)	6,474 (3,642, 11,508)
BNT/ChAd-84	119,882 (79,490, 180,799)	4,847 (3,043, 7,719)

Mucosal IgG was 11-25 times less in magnitude than serum IgG. This variability in mucosal lgG as a proportion of serum IgG suggests that mucosal IgG may not be entirely due to passive transudation from serum (**Table 38**).

Table 38 *– GMC (95% CI) for Serum IgG & Mucosal IgG per schedule at D112*

Arm	Serum IgG GMC (95% CI)	Mucosal IgG GMC (95% CI)
ChAd/ChAd-84	34,798 (23,258, 52,063)	1,410 (740, 2,690)
ChAd/BNT-84	143,863 (94,925, 218,031)	13,600 (5,620, 32,902)
BNT/BNT-84	226,341 (144,493, 354,554)	20,392 (11,757, 35,372)
BNT-ChAd-84	119,882 (79,490, 180,799)	5,471 (2,814, 10,639)

Mucosal IgA unexpectedly demonstrated a significant 2.8-3.1 fold increase at Day 112 in comparison to Day 0 in the ChAd/BNT and BNT/BNT schedules with a non-significant 1.5 fold increase in BNT/ChAd (**Figure 78**). Values were approximately 5-13 times less than those in serum IgA and were frequently below the pre-pandemic threshold (**Table 39**).

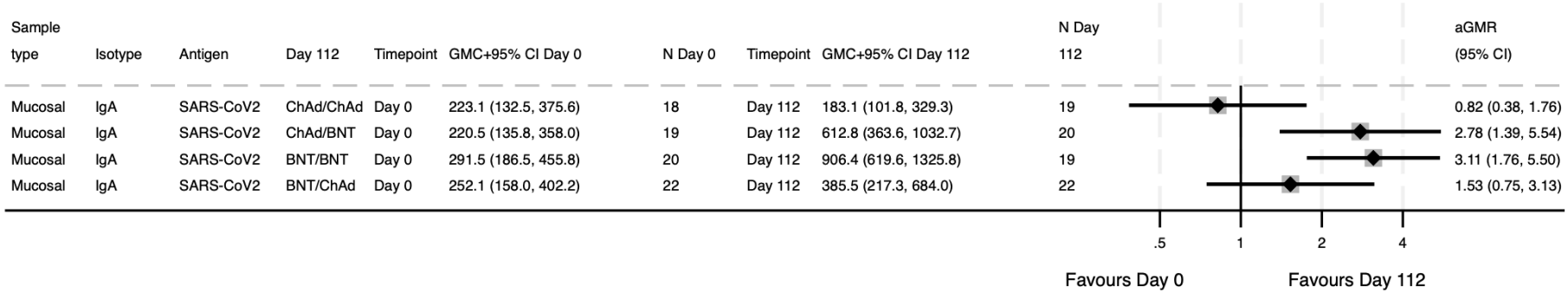

Figure 78 *– Forest plot comparing anti-SARS-CoV2 spike Mucosal IgA titres at Day 112 to Day 0 per schedule*

Table 39 *- GMC (95% CI) for Serum IgA & Mucosal IgA per schedule at D112*

Arm	Serum IgA GMC (95% CI)	Mucosal IgA GMC (95% CI)
ChAd/ChAd-84	989 (505, 1,934)	183 (102, 329)
ChAd/BNT-84	2,557 (1,478, 4,422)	613 (364, 1033)
BNT/BNT-84	6,474 (3,642, 11,508)	906 (620, 1326)
BNT-ChAd-84	4,847 (3,043, 7,719)	386 (217, 684)

Additionally in BNT/ChAd and ChAd/ChAd schedules there was an increase in titre from Day 112 to Day 182. These results are not in keeping with current knowledge regarding the ability of intramuscularly delivered vaccines to induce a local mucosal IgA response.

An alternative cause of the mucosal anti-SARS-CoV2 spike IgA increase could be incidental asymptomatic infection. However, the analysis, thus far displayed, contains only those who were serum anti-nucleocapsid IgG negative at baseline and throughout the study. Anti-nucleocapsid serum IgA, mucosal IgG and mucosal IgA do demonstrate some immunological conversion from ‘negative’ to ‘positive’ over the course of the study, which might suggest that there has been some degree of asymptomatic systemic infection, or simply, ‘mucosal exposure’ to SARS-CoV2 (**Figure 79**).

In order to delineate this response further, day 56 (56 days post first dose) and day 84 (pre-second dose) timepoints were also analysed (**Figure 80**).

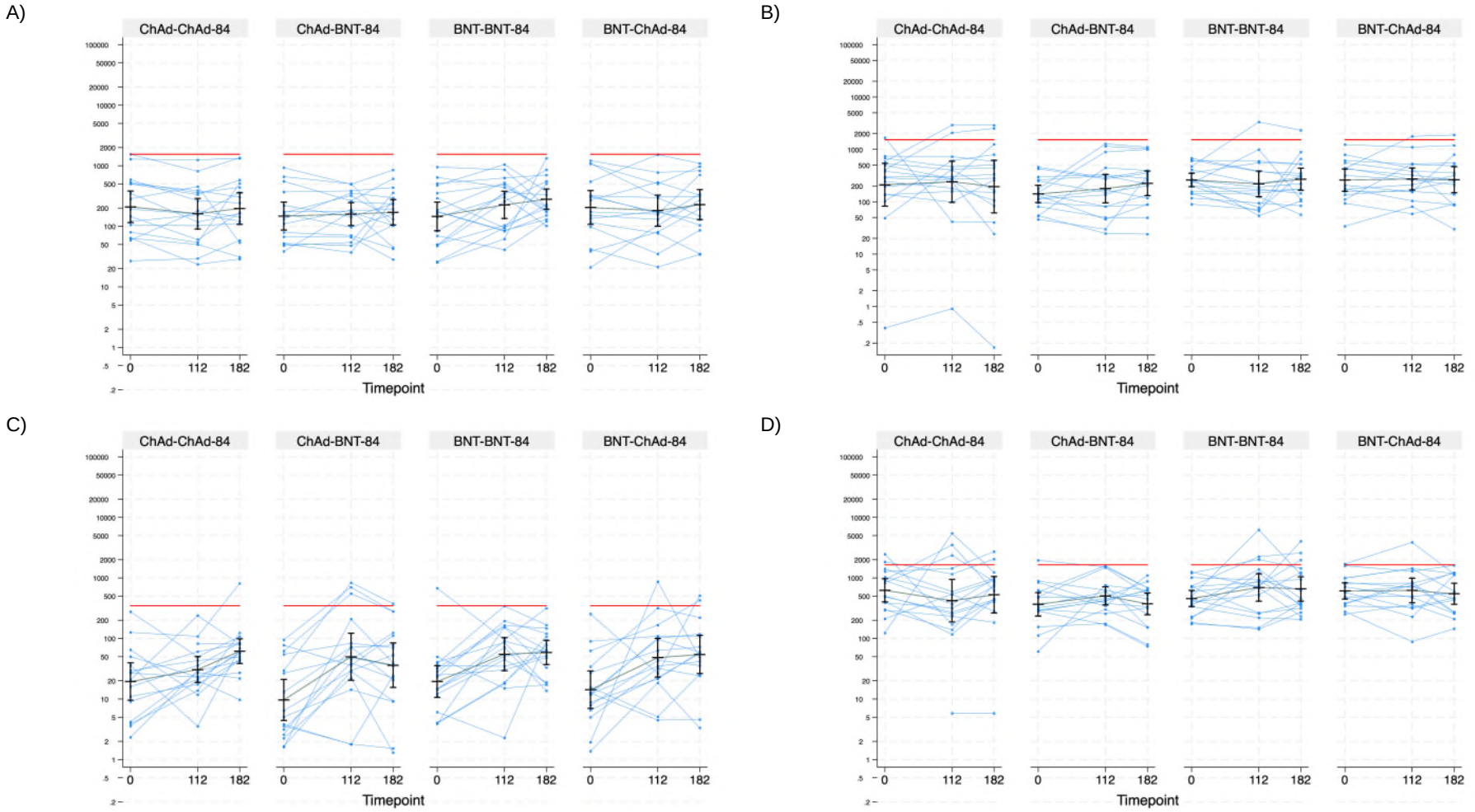

Figure 79 *– Kinetics of anti-nucleocapsid antibody response in seronegative participants (blue) with GMC ± 95% CI (grey/black) by 12-week vaccine schedule for A) Serum IgG, B) Serum IgA, C) Mucosal IgG, D) Mucosal IgA. Timepoints: D0 (pre-prime), D112 (28 days post second dose), D182 (2 months post second dose). Red horizontal line: Pre-pandemic threshold*

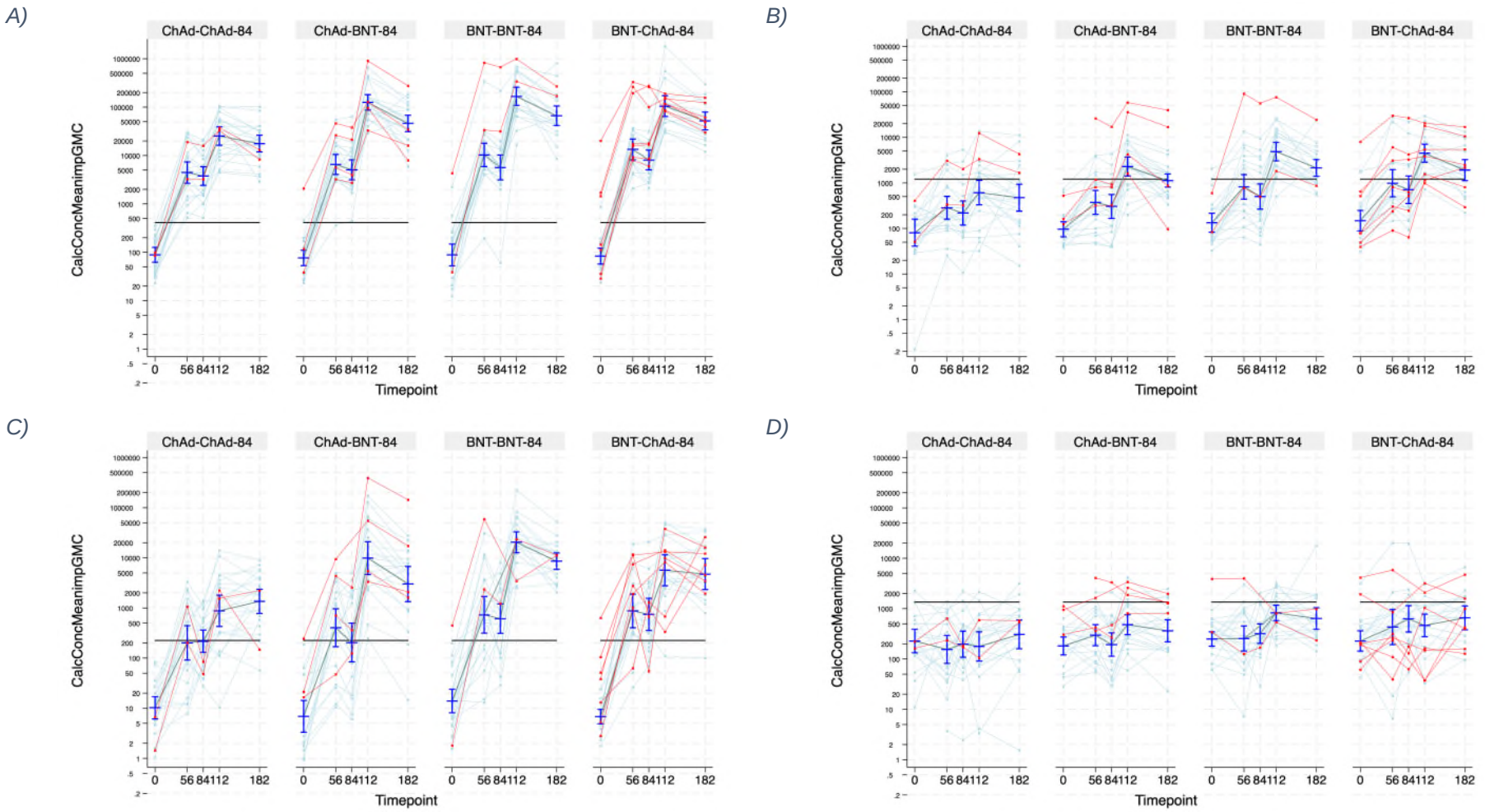

Figure 80 *– Kinetics of anti-SARS-CoV2 spike antibody response in seronegative participants vaccinated at D0 and D84 (light blue) with GMC ± 95% CI (grey/blue) and seropositive participants (red) by 12-week vaccine schedule for A) Serum IgG, B) Serum IgA, C) Mucosal IgG, D) Mucosal IgA. D0 (pre-prime), D56, D84 (pre-second dose), D112 (28 days post second dose), D182 (2 months post second dose). Horizontal black line: Pre-pandemic threshold*

The kinetics of individuals' serum IgG are all predictable with responses increasing after prime, falling pre-second dose, increasing post-second dose and falling again subsequently. Similar qualitative patterns are seen with serum IgA and mucosal IgG.

However, the mucosal IgA response differs qualitatively with no consistent, discernible pattern across individuals, and is not obviously consistent with a causative association with vaccine doses. The statistically significant increases in titre (**Figure 77** & **Figure 78**) warrant further investigation.

Proportions of participants who had a mucosal IgA level at D112 above the pre-pandemic threshold 28 days post second dose were low (**Table 40**).

Table 40 *– Proportions of 'mucopositive' participants at peak vaccine response timepoint (D112)*

Schedule	Proportion (%) with a 'positive' mucosal IgA at peak vaccine response time
ChAd/ChAd-84	2/25 (8%)
ChAd/BNT-84	6/25 (24%)
BNT/BNT-84	5/24 (21%)
BNT/ChAd-84	6/26 (23%)

Although numbers of seropositive participants are small, a similar pattern can be seen in the serum IgG panel (**Figure 80A**) to that described in Chapter 3 Seropositive participants have a higher response than seronegative participants after the first dose, but this difference decreases with the administration of the second dose. The serum IgA and mucosal IgG patterns for seropositive participants are qualitatively similar to seronegative participants, possibly with slightly higher responses. However, the mucosal IgA panel's seropositive responses are much more variable without a consistently stronger response than the seronegative participants.

5.4.3 New Hypothesis: Mucosal SARS-CoV2 exposure drives mucosal IgA increase without systemic activation: “Nasal mucoconversion without seroconversion”

The increases in mucosal anti-SARS-CoV2 spike IgA titres seem to be independent of the timing of IM vaccination. A possible explanation is that the significant rises in mucosal anti-SARS-CoV2 spike IgA may be driven by mucosal SARS-CoV2 exposure without systemic immunological activation. Given that the trial period (Feb 2021 – Jan 2022) overlapped with the ‘Delta wave’ (July-Dec 2021), a period of high community transmission, this is epidemiologically and biologically plausible (**Figure 81**).

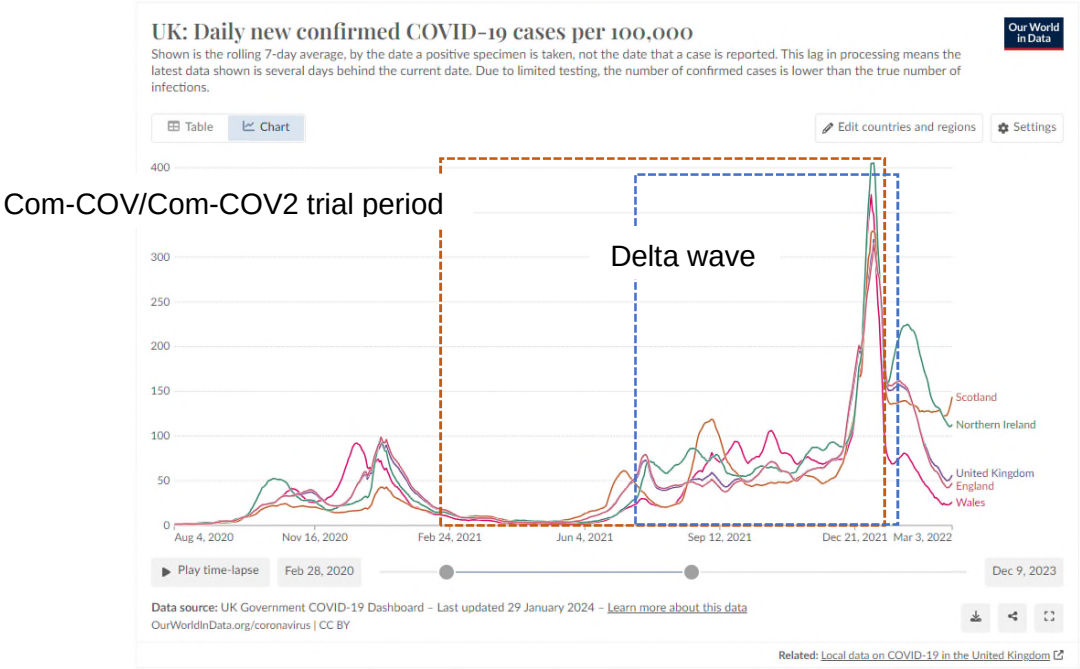

Figure 81 *– OurWorldInData.org – The rolling 7-day average of number of positive SARS-CoV2 PCR tests taken in the UK and devolved nations : “Daily new confirmed COVID-19 cases per 100,000” Modified screenshot* (238)

As a first measure of whether the syndrome of ‘nasal mucoconversion without seroconversion’ syndrome exists, one can create a 2x2 table of seroconversion against nasal mucoconversion for the participants who have mucosal data (**Table 41**). Seroconversion and muconversion are defined in Section 2.11.1.

***Table 41** – 2x2 table of seroconversion vs nasal mucoconversion.*

	No Nasal Mucoconversion	**Nasal Mucoconversion**
No seroconversion	72	23
Seroconversion	10	5*

**3 out of 5 mucoconverted during the same time period as their seroconversion. This means that two participants seroconverted and mucoconverted 'asynchronously'*

The 23 participants who demonstrate nasal mucoconversion, but who show no evidence of a systemic humoral response suggest that the concept of "nasal mucoconversion without seroconversion" may exist.

Of these 23 participants, 15 had a concurrent rise in T cell response, whilst eight showed no evidence of a concurrent T cell response. This further supports the idea that in at least some participants with "nasal mucoconversion without seroconversion" there was no detectable systemic response of any form (humoral or cellular) occurring concurrently with the mucosal response.

5.4.4 Mucosal SARS-CoV2 Spike IgA associations

Figure 82 shows the timecourse of eight immune markers of interest in one participant, to demonstrate a key point regarding the relationship of these markers.

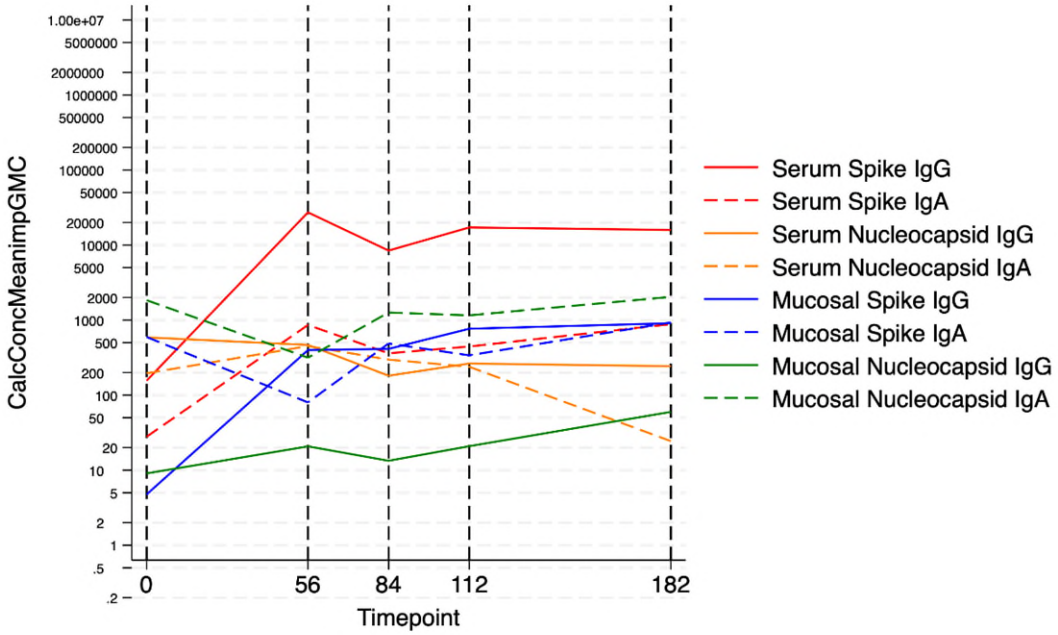

***Figure 82** – Exemplar participant with antibody time courses for Serum anti-SARS-CoV2 Spike IgG, Serum anti-SARS-CoV2 Spike IgA, Serum anti-Nucleocapsid IgG, Serum anti-Nucleocapsid IgA, Mucosal anti-SARS-CoV2 Spike IgG, Mucosal anti-SARS-CoV2 Spike IgA, Mucosal anti-Nucleocapsid IgG and Mucosal anti-Nucleocapsid IgA*

Serum spike IgG, serum spike IgA and mucosal spike IgG (solid red line, dotted red line and solid blue line respectively) are the clearest markers of immune response to immunisation, and give a classical response: Rising after the first dose, falling after this, rising after the second dose and falling again. It is not clear why this participant had a larger serum anti-SARS-CoV2 spike IgG response to their first dose than to their second.

Conversely, the rise and fall of markers hypothesised to reflect mucosal SARS-CoV2 exposure only (mucosal spike IgA [dashed blue line] and mucosal nucleocapsid IgA [dashed green line]), completely oppose that which would be expected of an immune marker associated with vaccine response: They fall directly after the vaccine doses, and rise outside of these timepoints.

To further analyse this, the gradient of the logarithmically transformed immunoglobulin titres from each pair of timepoints (0-56, 56-84, 84-112, 112-182) for each individual was taken and a correlation analysis conducted between these as a measure of how closely the movement of each pair of immune markers was matched over time (**Figure 83**). **Figure 84**, **Figure 85**, & **Figure 86** replicate **Figure 83** with varying annotations to aid interpretation. The correlation plot incorporates seropositive and seronegative participants, but excludes those who showed virological or systemic immunological evidence of infection.

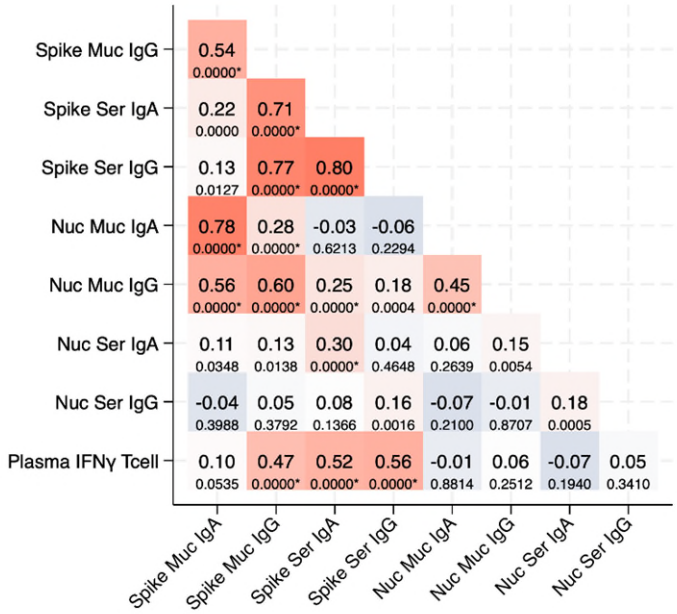

Figure 83 *– Correlation heatplot matrix of immune measure gradients . Seropositive and seronegative 12-week interval participants (Seroconverted excluded). The larger upper number in each square is the correlation coefficient, which varies from +1 to -1. +1 indicates a perfect positive correlation, -1 indicates a perfect negative correlation with 0 indicating no correlation. Furthermore red represents a positive correlation, whilst blue represents a negative correlation. The deeper the colour, the stronger the correlation with white indicating no correlation. The lower number in each square represents the crude p-value, but statistical significance (as indicated by a *) is reached only if this is less than the Bonferroni-corrected threshold for significance. 100 participants (up to 384 datapoints) for each correlation*

The six-way correlations between serum anti-SARS-CoV2 spike IgG, serum anti-SARS-CoV2 spike IgA, mucosal anti-SARS-CoV2 spike IgG and the IFNγ T cell ELISpot, are all in keeping with a response to IM vaccination (six black lines) (**Figure 84**). IM vaccination drives serum anti-SARS-CoV2 IgG, serum anti-SARS-CoV2 IgA and IFNγ T cell ELISpot (solid orange lines), and may also drive Spike Mucosal IgG (although this may be indirectly driven due to transudation from serum [dashed orange line]).

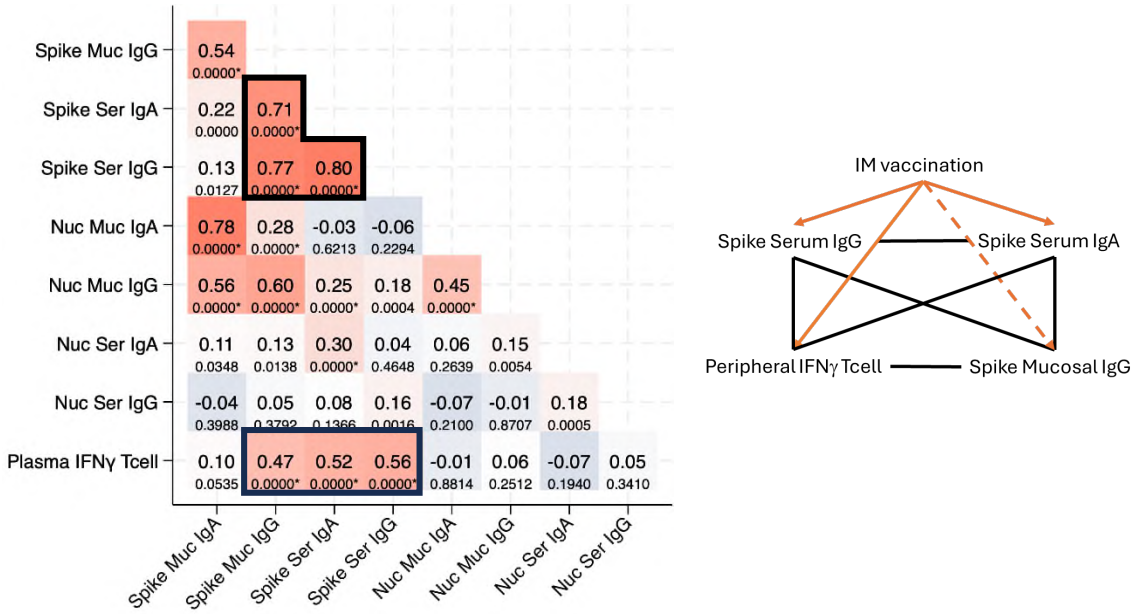

Figure 84 *– 6-way correlation for markers directly and indirectly induced by IM vaccination (serum anti-SARS-CoV2 spike IgG, serum anti-SARS-CoV2 spike IgA, mucosal anti-SARS-CoV2 spike IgG and IFNγ T cell ELISpot). Orange arrows represent causative association (solid) and possible/probably causative association (dotted). Black lines show the 6-way correlation*

The six-way correlation between the mucosal markers – mucosal anti-SARS-CoV2 spike IgG, mucosal anti-SARS-CoV2 spike IgA, mucosal anti-nucleocapsid IgG and mucosal anti-nucleocapsid IgA are in keeping with all being driven by a mucosal stimulus such as SARS-CoV2 exposure (**Figure 85**).

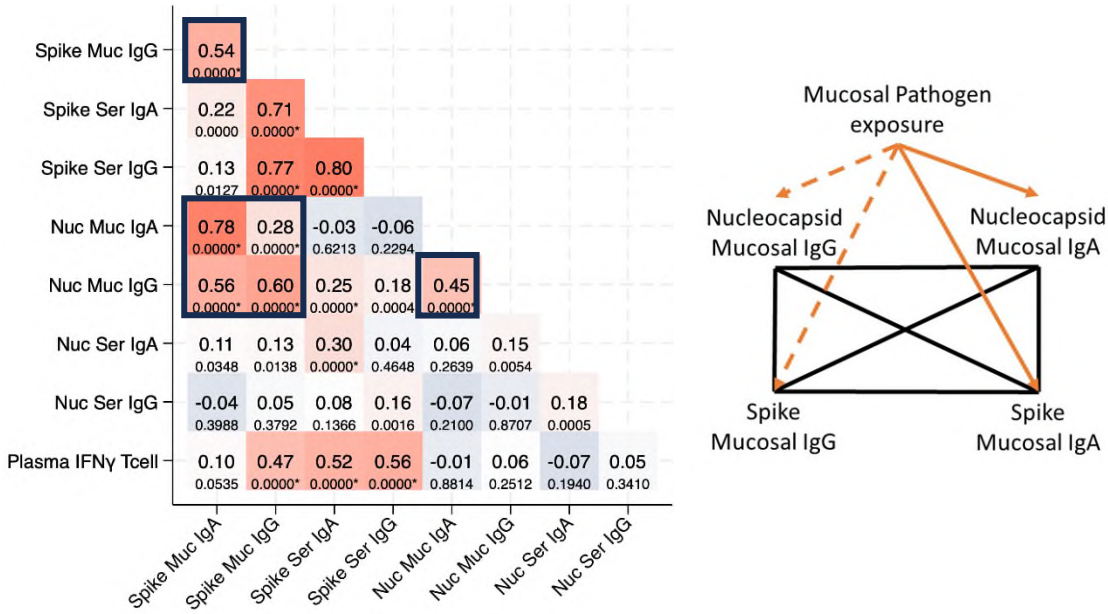

Figure 85 *– 6-way correlation for markers induced by mucosa exposure (mucosal anti-SARS-CoV2 spike IgG, mucosal anti-SARS-CoV2 spike IgA, mucosal anti-nucleocapsid IgG and mucosal anti-nucleocapsid IgA). Orange arrows represent causative association (solid) and possible/probably causative association (dotted). Black lines show the 6-way correlation*

The correlations of mucosal anti-SARS-CoV2 spike IgA (the only immune marker which did not clearly associate with IM vaccination) with the other immune markers are in the first column of the heatplot. Anti-SARS-CoV2 spike IgA correlates well with the markers of mucosal exposure (red box: mucosal anti-nucleocapsid IgG, mucosal anti-nucleocapsid IgA and mucosal anti-SARS-CoV2 spike IgG), but not with the markers of IM vaccination (orange boxes: serum anti-SARS-CoV2 spike IgG, serum anti-SARS-CoV2 spike IgA, IFNγ T cell ELISpot), nor with the markers of systemic infection (blue box: serum anti-nucleocapsid IgG, serum anti-nucleocapsid IgA) (**Figure 86**).

These patterns indicate mucosal anti-SARS-CoV2 spike IgA is likely mediated by SARS-CoV2 mucosal exposure. Mucosal anti-SARS-CoV2 spike IgA also correlates with mucosal anti-SARS-CoV2 spike IgG (an indirect measure of response to IM vaccination [green box]), but not with the other markers of IM vaccination, suggesting that mucosal anti-SARS-CoV2 spike IgG may be due to a combination of both mucosal SARS-CoV2 exposure as well as IM vaccination.

Local mucosal IgG production is further supported by the presence of a correlation between mucosal anti-nucleocapsid IgG and mucosal anti-nucleocapsid IgA, but a lack of correlation between mucosal anti-nucleocapsid IgG and serum anti-nucleocapsid IgG (purple boxes).

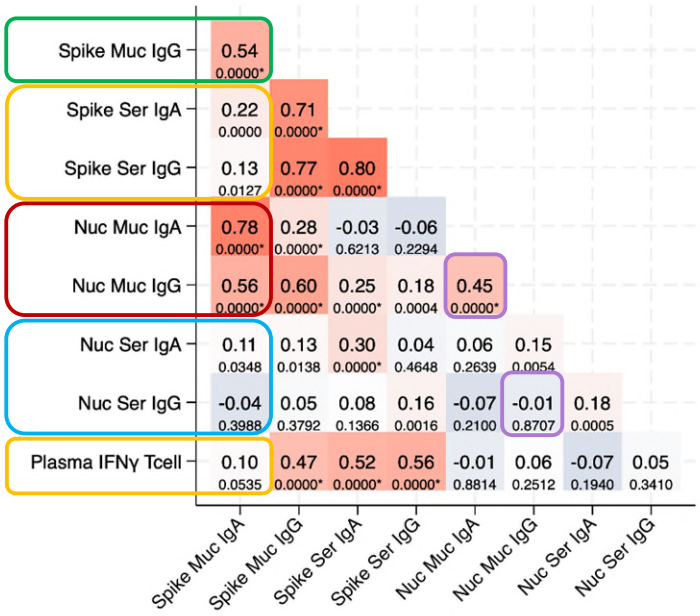

Figure 86 *– Correlations with mucosal anti-SARS-CoV2 spike IgA . Blue boxes: Markers of systemic infection (serum anti-nucleocapsid IgG, serum anti-nucleocapsid IgA); Orange boxes: Markers of IM vaccination (serum anti-SARS-CoV2 spike IgG, serum anti-SARS-CoV2 spike IgA, IFNg Tcell); Red box: Markers of mucosal SARS-CoV2 exposure (mucosal anti-nucleocapsid IgG, mucosal anti-nucleocapsid IgA); Green box: Mucosal Spike IgG (potentially due to a combination of both IM vaccination and mucosal SARS-CoV2 exposure); Purple boxes: Additional evidence of local mucosal IgG production (correlation of mucosal anti-nucleocapsid IgG and mucosal anti-nucleocapsid IgA, alongside an absence of correlation between serum anti-nucleocapsid IgG and mucosal anti-nucelocapsid IgG)*

The above-described analysis includes both seropositive and seronegative participants from all four vaccine schedules, covering time periods both after the first dose (but before the second dose), and after the second dose. Participants showing immunological or virological evidence of seroconversion were excluded.

There are insufficient numbers of participants to investigate the effect of serostatus, schedule and first or second vaccine dose, simultaneously. However, these factors may be investigated independently, in turn, to gauge some idea of their effects.

5.4.4.1 Vaccine schedule by dose

5.4.4.1.1 First dose

For both ChAd-primed and BNT-primed seronegative participants there is a qualitatively similar pattern of response to the main analysis. Some of the previously described correlations no longer reach statistical significance. Notably, there is no dramatic change to the correlations between mucosal spike IgA and the systemic markers of IM vaccination [black boxes] (**Figure 87**).

For seropositive participants there appears to be a statistically non-significant increase in correlation for ChAd-primed, but not BNT-primed participants of mucosal anti-SARS-CoV2 spike IgA with the markers of vaccine response, although numbers are small (**Figure 87C**).

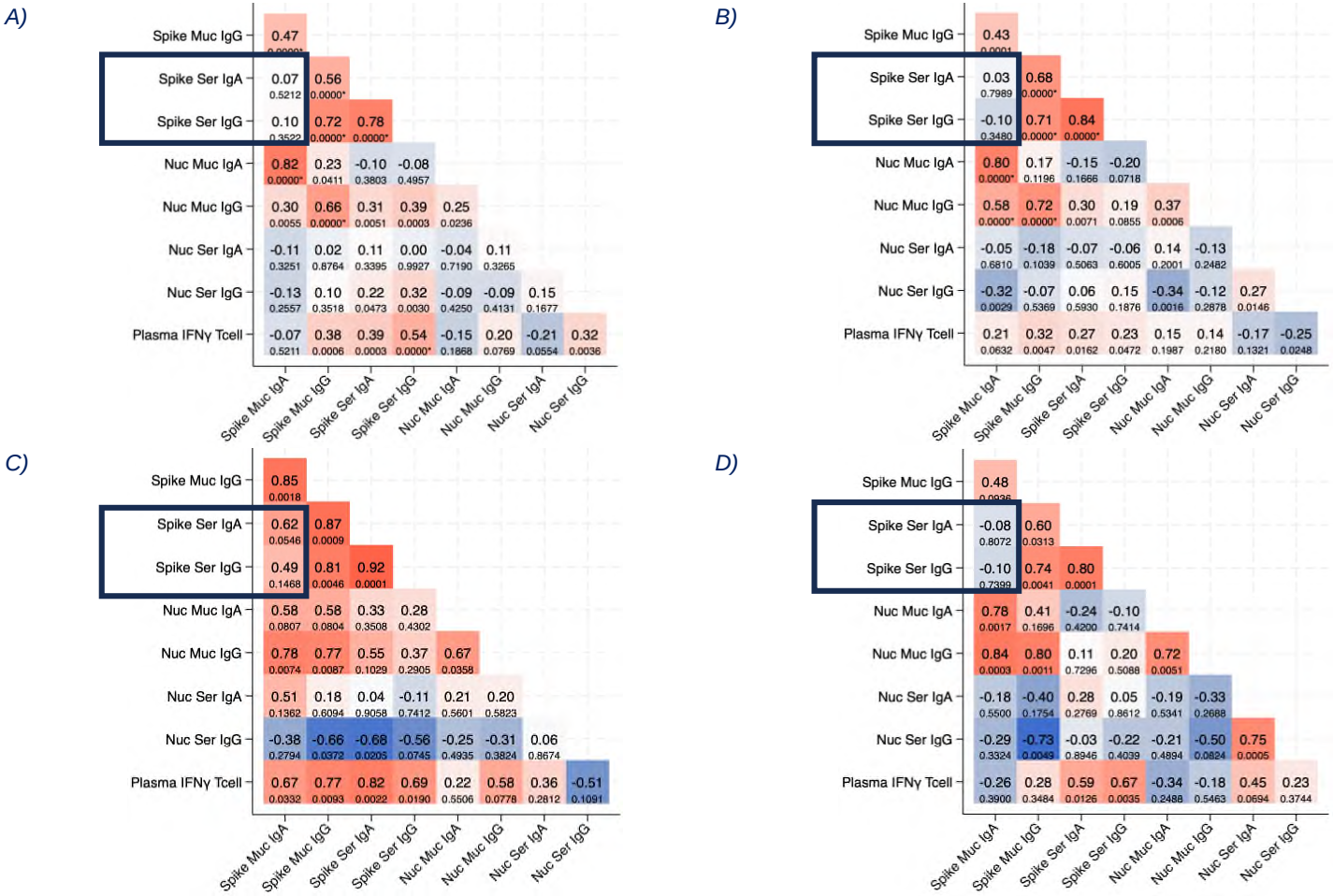

Figure 87 *– Correlation heatplot matrices 0-56 & 56-84 timepoints . A) Seronegative ChAd prime (43 participants, up to 83 datapoints), B) Seronegative BNT prime (42 participants, up to 84 datapoints) C) Seropositive ChAd prime (6 participants, up to 13 datapoints), D) Seropositive BNT prime (9 participants, up to 15 datapoints)*

5.4.4.1.2 Second dose

For seronegative participants receiving ChAd/ChAd and BNT/ChAd, there is again a similar quantitative pattern of response to the main analysis above, with no change to the correlations between mucosal spike IgA and the systemic markers of IM vaccination (**Figure 88A** & **Figure 88D**).

For ChAd/BNT and BNT/BNT, there has been a non-statistically significant increase in the size of the correlations between mucosal spike IgA and the systemic markers of IM vaccination (**Figure 88B** & **Figure 88C**).

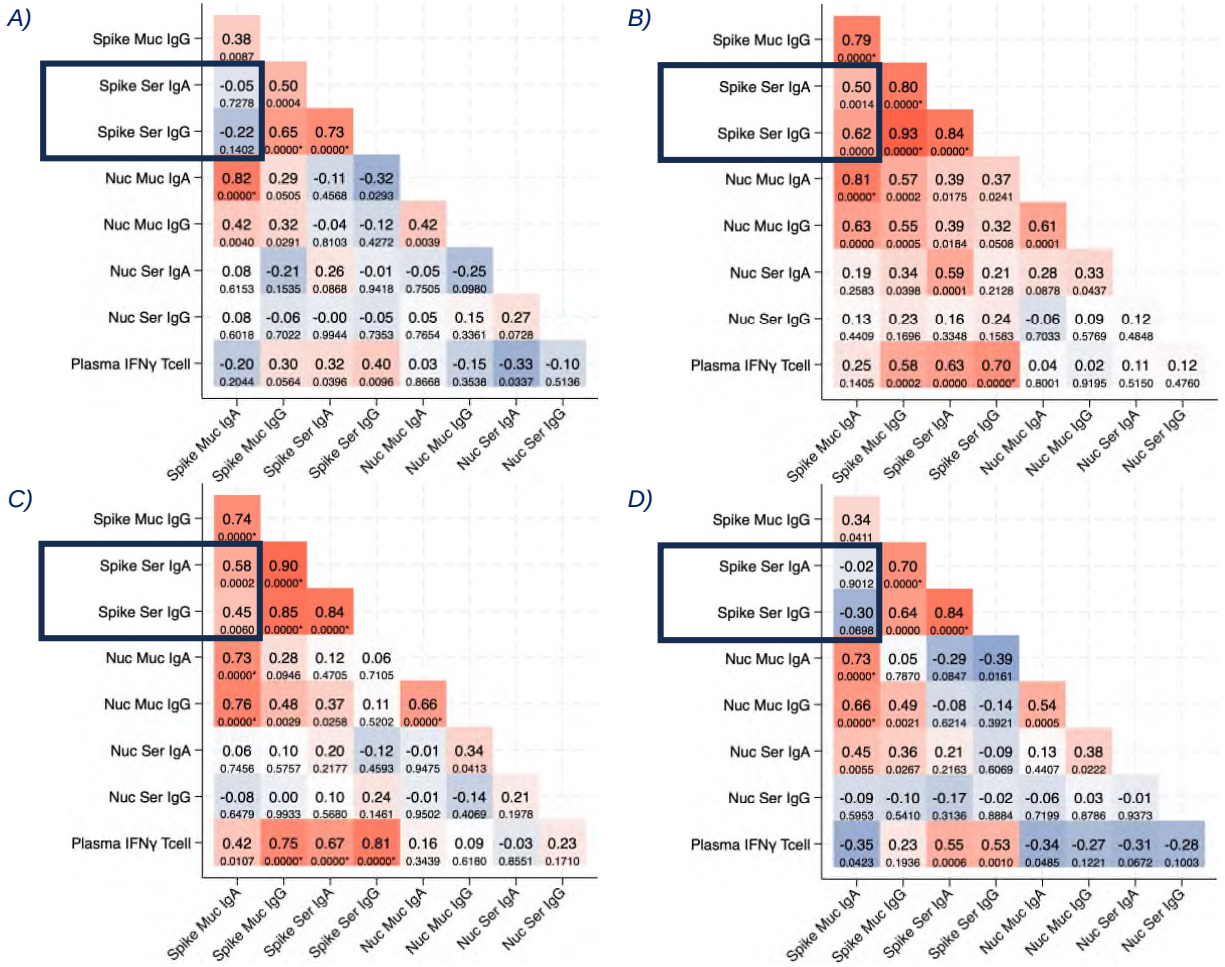

Figure 88 *– Correlation heatplot matrices, second dose (84-112, 112-182), seronegative participants . A) ChAd/ChAd (23 participants, up to 46 datapoints), B) ChAd/BNT schedule (19 participants, up to 37 datapoints), C) BNT/BNT (21 participants, up to 38 datapoints), D) BNT/ChAd (19 participants, up to 37 datapoints)*

5.4.4.2 Baseline serostatus effect on second dose

Both the seronegative and seropositive analyses, grouped across all schedules, appear to be qualitatively similar to the main analysis. The seropositive analysis has a slightly greater statistically non-significant correlation between mucosal spike IgA and the systemic markers of IM vaccination (**Figure 89**).

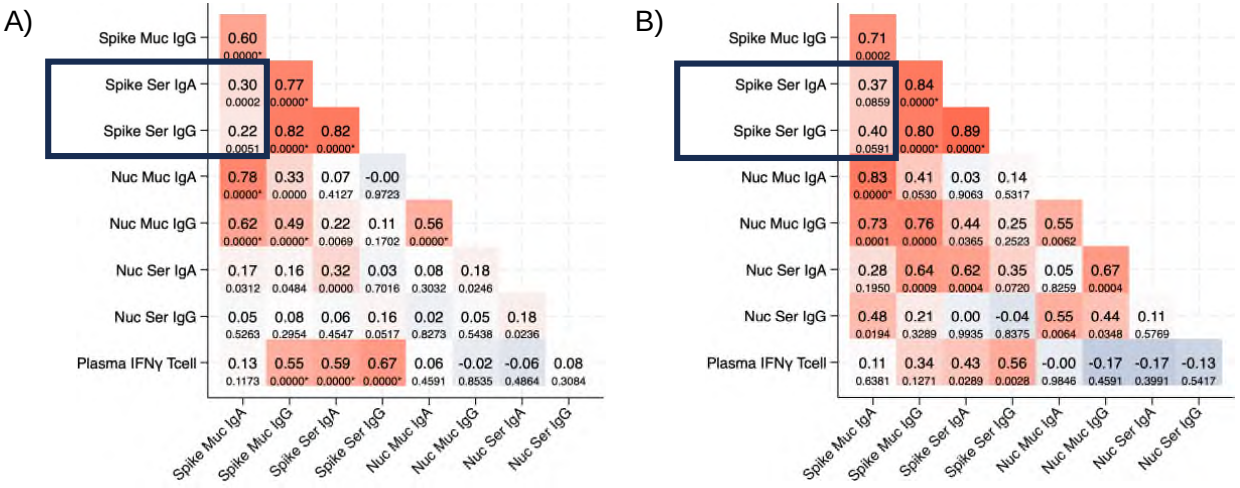

Figure 89 *– Correlation heatplot matrices. Second dose (84-112 & 112-182) timepoints . A) Seronegative (82 participants, up to 158 datapoints), B) Seropositive (14 participants, up to 28 datapoints). All vaccine schedules combined*

5.4.4.3 Confirmatory multivariate regression analysis of IM immunisation induced mucosal anti-SARS-CoV2 spike IgA

The main interpretation of the initial combined main analysis is that intramuscular vaccination does not induce a locally produced mucosal IgA response. There are no statistically significant results which contradict this result in the subgroup analyses, although there are some trends that warrant further attention. Each of these (ChAd prime in seropositive participants, BNT/BNT second dose in seronegative participants and ChAd/BNT in seronegative participants) are further explored in turn in the following sub-sections.

5.4.4.3.1 ChAd prime in seropositive participants

Univariate regression modelling predicting the gradient of mucosal anti-SARS-CoV2 spike IgA using the gradient of serum anti-SARS-CoV2 spike IgG shows a coefficient of 0.344, with a non-significant p-value, likely reflecting the small number of datapoints (**Table 42**).

Repeating the analysis as a multivariate regression including covariates of serum anti-SARS-CoV2 spike IgA (a marker of IgA transudation from serum), mucosal nucleocapsid IgG and mucosal nucleocapsid IgA (markers of mucosal SARS-CoV2 exposure), the coefficient for serum spike IgG drops from 0.344 to -0.092, with an even less significant p-value (**Table 43**). This dramatic reduction in magnitude of coefficient suggests that the potential correlation between serum spike IgG and mucosal spike IgA seen in the correlation plot is likely spurious and may well be due to coincidental nasal SARS-CoV2 exposure.

Table 42 *– Univariate regression analysis examining the relationship between serum spike IgG (marker of vaccine response) and mucosal spike IgA for ChAd prime . Population seropositive participants recieving ChAd as their first dose, time intervals 0-56, 56-84 only. 13 datapoints*

Gradient Mucosal Spike IgA	**Coef.**	**St.Err.**	**t-value**	**p-value**	**[95% Conf**	**Interval]**
Gradient Serum Spike IgG	**.344**	.186	1.85	**.092**	-.066	.754
Constant	-.004	.005	-0.79	.445	-.014	.007

Mean dependent var	0.002	SD dependent var	0.013
R-squared	0.237	Number of obs	13
F-test	3.415	Prob > F	0.092
Akaike crit. (AIC)	-76.148	Bayesian crit. (BIC)	-75.018

Table 43 *– Multivariate regression analysis examining the relationship between between serum spike IgG (marker of vaccine response) and mucosal spike IgA for ChAd prime , with serum spike IgA (marker of transudating systemic IgA), mucosal nucleocapsid IgG and IgA (markers of mucosal SARS-CoV2 exposure) as other explanatory covariates.. Population seropositive participants recieving ChAd as their first dose, time intervals 0-56, 56-84 only. 13 datapoints*

Gradient Mucosal Spike IgA	**Coef.**	**St.Err.**	**t-value**	**p-value**	**[95% Conf**	**Interval]**
Gradient Mucosal Nucleocapsid IgA	.341	.357	0.96	.367	-.482	1.165
Gradient Mucosal Nucleocapsid IgG	.213	.219	0.97	.36	-.293	.719
Gradient Serum Spike IgA	.628	.272	2.30	.05	0	1.255
Gradient Serum Spike IgG	**-.092**	.167	-0.55	**.596**	-.478	.293
Constant	-.003	.004	-0.75	.472	-.011	.005

Mean dependent var	0.002	SD dependent var	0.013
R-squared	0.237	Number of obs	13
F-test	3.415	Prob > F	0.092
Akaike crit. (AIC)	-76.148	Bayesian crit. (BIC)	-75.018

5.4.4.3.2 BNT/BNT second dose in seronegative participants

A similar set of univariate and multivariate analyses can be performed to interrogate the results seen for seronegative participants receiving a BNT second dose in BNT/BNT (**Table 44** & **Table 45**)

The coefficient for serum spike IgG drops from 0.223 (p-value 0.006) in the univariate analysis to 0.085 (p-value 0.3) in the multivariate analysis, again suggesting that this low level non-statistically significant correlation seen earlier is spurious.

Table 44 *– Univariate linear regression analysis examining the relationship between serum spike IgG (marker of vaccine response) and mucosal spike IgA for BNT/BNT . Population seronegative BNT-primed participants recieving BNT as their second dose, time intervals 84-112, 112-182 only. 21 participants, 36 datapoints*

Gradient Mucosal Spike IgA	**Coef.**	**St.Err.**	**t-value**	**p-value**	**[95% Conf**	**Interval]**
Gradient Serum Spike IgG	**.223**	.076	2.93	**.006***	.068	.377
Constant	0	.003	0.11	.91	-.006	.007

Mean dependent var	0.006	SD dependent var	0.017
R-squared	0.201	Number of obs	36
F-test	8.574	Prob > F	0.006
Akaike crit. (AIC)	-197.917	Bayesian crit. (BIC)	-194.750

Table 45 *– Multivariate linear regression analysis examining the relationship between serum spike IgG (marker of vaccine response) mucosal spike IgA for BNT/BNT , with serum spike IgA (marker of transudating systemic IgA), mucosal nucleocapsid IgG and IgA (markers of mucosal SARS-CoV2 exposure) as other explanatory covariates. Population seronegative BNT-primed participants recieving BNT as their second dose, time intervals 84-112, 112-182 only. 21 participants, 36 datapoints*

Gradient Mucosal Spike IgA	**Coef.**	**St.Err.**	**t-value**	**p-value**	**[95% Conf**	**Interval]**
Gradient Mucosal Nucleocapsid IgA	0.574	0.136	4.20	0.000	0.295	0.852
Gradient Mucosal Nucleocapsid IgG	0.312	0.111	2.80	0.009	0.085	0.539
Gradient Serum Spike IgA	0.168	0.12	1.39	0.174	-0.078	0.413
Gradient Serum Spike IgG	**0.085**	0.081	1.05	**0.300**	-0.080	0.251
Constant	-0.001	0.002	-0.68	0.499	-0.004	0.002

Mean dependent var	0.006	SD dependent var	0.017
R-squared	0.819	Number of obs	36
F-test	34.956	Prob > F	0.000
Akaike crit. (AIC)	-245.260	Bayesian crit. (BIC)	-237.343

5.4.4.3.3 ChAd/BNT second dose in seronegative participants

Univariate and multivariate analyses can also be performed for ChAd/BNT (**Table 46** & **Table 47**).

The coefficient for serum anti-SARS-CoV2 spike IgG starts at 0.303 (p-value 0.000) and drops only to 0.281 (p-value 0.000) in the multivariate analysis. Although there has been some reduction in the magnitude of this coefficient by accounting for mucosal SARS-CoV2 exposure, this reduction is incomplete in the multivariate analysis and the coefficient remains significant.

Table 46 *– Univariate linear regression analysis examining the relationship between serum spike IgG (marker of vaccine response) and mucosal spike IgA for ChAd/BNT . Population seronegative ChAd-primed participants recieving BNT as their second dose, time intervals 84-112, 112-182 only. 19 participants, 37 datapoints*

Gradient Mucosal Spike IgA	**Coef.**	**St.Err.**	**t-value**	**p-value**	**[95% Conf**	**Interval]**
Gradient Serum Spike IgG	**.303**	.064	4.71	**0.000***	.173	.434
Constant	-.002	.002	-0.65	.523	-.007	.003

Mean dependent var	0.006	SD dependent var	0.015
R-squared	0.388	Number of obs	37
F-test	22.202	Prob > F	0.000
Akaike crit. (AIC)	-220.408	Bayesian crit. (BIC)	-217.186

Table 47 *– Multivariate linear regression analysis examining the relationship between serum spike IgG (marker of vaccine response) and mucosal spike IgA for ChAd/BNT , with serum spike IgA (marker of transudating systemic IgA), mucosal nucleocapsid IgG & IgA (markers of mucosal SARS-CoV2 exposure) as other covariates. Population seronegative ChAd-primed participants recieving BNT as their 2nd dose, time intervals 84-112, 112-182. 19 participants, 37 datapoints*

Gradient Mucosal Spike IgA	**Coef.**	**St.Err.**	**t-value**	**p-value**	**[95% Conf**	**Interval]**
Gradient Mucosal Nucleocapsid IgA	0.901	0.155	5.81	0.000	0.585	1.217
Gradient Mucosal Nucleocapsid IgG	0.129	0.064	2.02	0.052	-0.001	0.260
Gradient Serum Spike IgA	-0.174	0.089	-1.96	0.059	-0.354	0.007
Gradient Serum Spike IgG	**0.281**	0.068	4.13	**0.000**	0.142	0.420
Constant	0.000	0.001	-0.30	0.769	-0.003	0.003

Mean dependent var	0.006	SD dependent var	0.015
R-squared	0.813	Number of obs	37
F-test	34.692	Prob > F	0.000
Akaike crit. (AIC)	-258.191	Bayesian crit. (BIC)	-250.137

5.4.5 Immune responses in participants with virologically confirmed infection but no serum anti-nucleocapsid IgG seroconversion

Within Com-COV1, there were 39 PCR- or LFA-diagnosed cases of SARS-CoV2 infection (**Table 48**), of whom 35 occurred at least two weeks after the second dose of vaccine.

Table 48 *– 2x2 table comparing numbers of participants who seroconverted as defined by serum anti-nucleocapsid IgG vs those who were diagnosed with SARS-CoV2 infection by PCR or LFA*

830 participants	**No SARS-CoV2 infection**	**SARS-CoV2 infection***
No seroconversion	777	10 (1 with mucosal data)
Seroconversion	13 (8 with mucosal data)	24

** 1 participant had no anti-nucleocapsid data following infection to determine whether or not they had seroconverted.*

Of the ten who had virologically confirmed SARS-CoV2 infection, but who showed no evidence of seroconversion at their subsequent visit, the responses of their other immune markers just subsequent to infection are displayed in **Table 49**.

Table 49 *– 3x3 frequency table displaying the numbers of participants who had rises, falls or missing data following SARS-CoV2 infection for their serum anti-SARS-CoV2 spike IgG and IFNγ-SFC*

		Serum anti-SARS-CoV2 spike IgG response		
		Rise	**Fall**	**Missing**
T cell ELISpot response	**Rise**	2	1	2
	Fall	-	-	-
	Missing	-	1	4

The single participant with mucosal data showed no increase in serum anti-SARS-CoV2 spike IgG, but did show an increase in T cell ELISpot response. The mucosal markers, mucosal anti-SARS-CoV2 spike IgA and mucosal anti-nucleocapsid IgA showed a shallow decrease in titre (**Figure 90**).

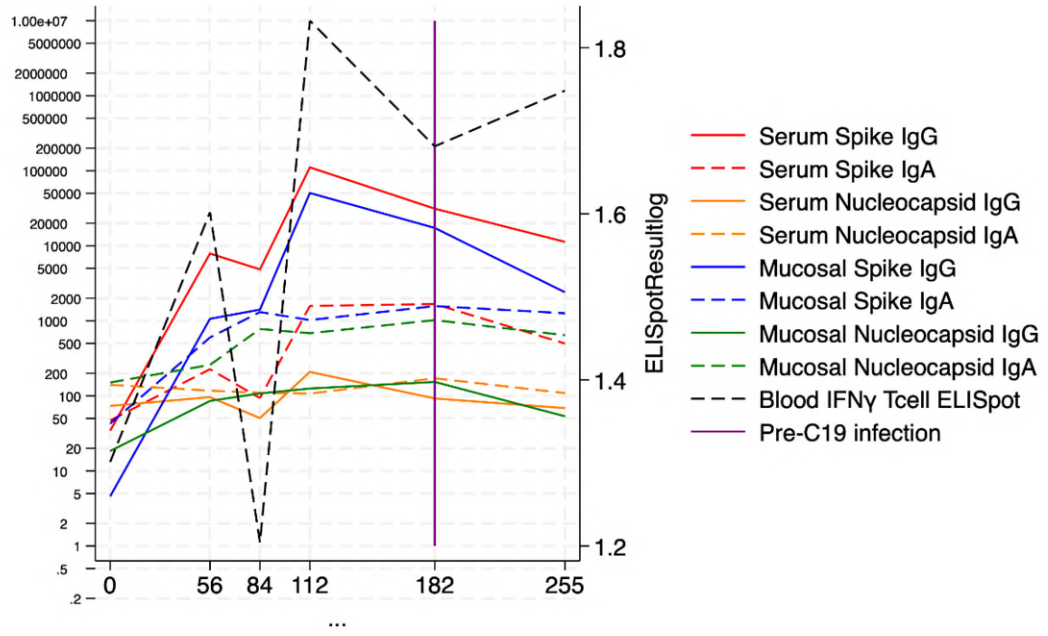

Figure 90 *– Immune marker time course for the single participant with mucosal data who had virological confirmation of SARS-CoV2 infection without a serum anti-SARS-CoV2 spike IgG increase . The vertical purple line labelled Pre-C19 infection represents the last measured timepoint pre-infection. SARS-CoV2 infection occurred 14 days after this point.*

Amongst the 24 participants who demonstrated seroconversion with virologically confirmed infection, 14 showed concurrent increases in both serum anti-SARS-CoV2 spike IgG and T cell ELISpot, 7 showed an increase in serum anti-SARS-CoV2 spike IgG but not T cell ELISpot and finally 3 showed an increase in serum anti-SARS-CoV2 spike IgG, but had missing T cell data.

5.4.6 Heterologous vs homologous serum comparison by MSD

Similar analyses to those conducted in Chapter 3 comparing immune responses of homologous vs heterologous schedules using anti-SARS-CoV2 spike ELISA data are presented.

Serum anti-SARS-CoV2 spike IgG aGMR for ChAd/ChAd vs ChAd/BNT using MSD data is 5.3 (95% CI: 2.9, 9.6), which is extremely close to the ELISA data value of 5.2 (134). Similarly serum anti-SARS-CoV2 IgG aGMR for BNT/BNT vs BNT/ChAd using MSD data is 0.59 (95% CI: 0.29, 1.18), again extremely close to the 0.57 from the ELISA data (**Figure 91**).

The serum IgA response has been previously less well described. There is no ELISA data on this from the main trial. Broadly, the results are similar qualitatively to the serum IgG response, however, confidence intervals are wide and it is difficult to draw out definitive conclusions from this data (**Figure 92**).

Timepoint	Sample type	Isotype	Antigen	Homologous Arm	GMC+95% CI Homologous	N Homnologous	Heterologous Arm	GMC+95% CI Heterologous	N Heterologous	aGMR (95% CI)
112	Serum	IgG	SARS-CoV2	ChAd/ChAd	34797.9 (23258.4, 52062.6)	19	ChAd/BNT	143863.2 (94925.0, 218031.1)	20	5.27 (2.91, 9.56)
112	Serum	IgG	RBD	ChAd/ChAd	18894.6 (12198.2, 29267.0)	19	ChAd/BNT	75552.4 (49823.8, 114567.1)	20	5.24 (2.80, 9.81)
112	Serum	IgG	Nucleocapsid	ChAd/ChAd	193.6 (106.8, 351.1)	19	ChAd/BNT	259.0 (132.3, 506.8)	20	1.28 (0.62, 2.64)
112	Serum	IgG	SARS-CoV2	BNT/BNT	226341.5 (144492.6, 354554.2)	19	BNT/ChAd	119882.6 (79490.6, 180799.2)	22	0.59 (0.29, 1.18)
112	Serum	IgG	RBD	BNT/BNT	132337.4 (81961.5, 213675.6)	19	BNT/ChAd	66329.8 (44182.5, 99578.8)	22	0.59 (0.29, 1.23)
112	Serum	IgG	Nucleocapsid	BNT/BNT	324.9 (155.7, 677.9)	19	BNT/ChAd	377.8 (182.0, 784.1)	22	0.60 (0.27, 1.36)

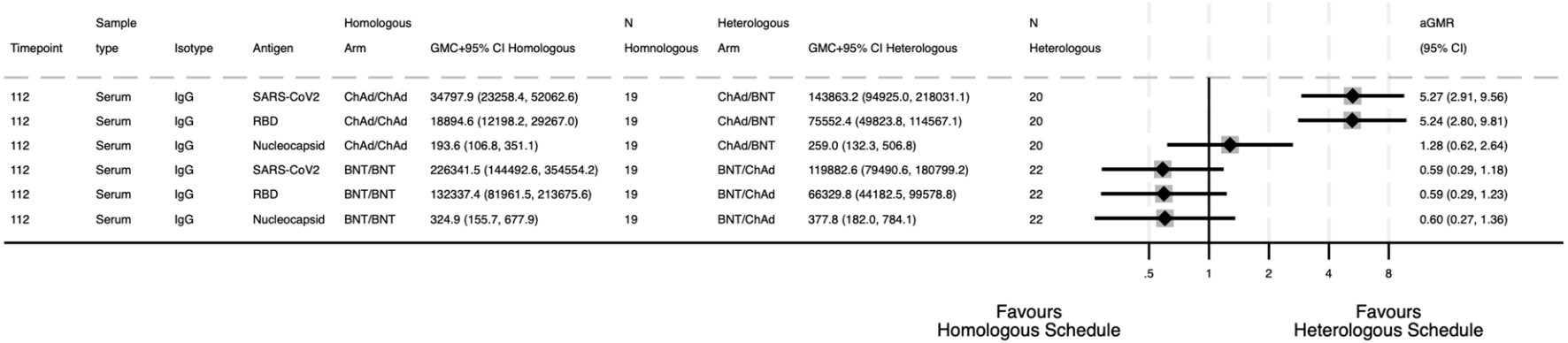

Figure 91 *– Forest plot comparing homologous to heterologous 12-week vaccine schedules. Serum IgG*

Timepoint	Sample type	Isotype	Antigen	Homologous Arm	GMC+95% CI Homologous	N Homnologous	Heterologous Arm	GMC+95% CI Heterologous	N Heterologous	aGMR (95% CI)
112	Serum	IgA	SARS-CoV2	ChAd/ChAd	988.5 (505.2, 1934.2)	19	ChAd/BNT	2556.6 (1478.0, 4422.2)	20	2.26 (0.92, 5.53)
112	Serum	IgA	RBD	ChAd/ChAd	544.6 (286.1, 1036.5)	19	ChAd/BNT	1286.7 (712.2, 2324.7)	20	2.10 (0.80, 5.49)
112	Serum	IgA	Nucleocapsid	ChAd/ChAd	286.1 (116.1, 704.7)	19	ChAd/BNT	207.4 (115.2, 373.2)	20	0.73 (0.23, 2.27)
112	Serum	IgA	SARS-CoV2	BNT/BNT	6474.1 (3642.1, 11508.3)	19	BNT/ChAd	4846.5 (3042.8, 7719.2)	22	0.72 (0.30, 1.74)
112	Serum	IgA	RBD	BNT/BNT	2727.4 (1281.1, 5806.6)	19	BNT/ChAd	2265.0 (1324.7, 3872.7)	22	0.68 (0.23, 2.05)
112	Serum	IgA	Nucleocapsid	BNT/BNT	246.2 (144.5, 419.6)	19	BNT/ChAd	303.1 (175.2, 524.3)	22	1.04 (0.45, 2.39)

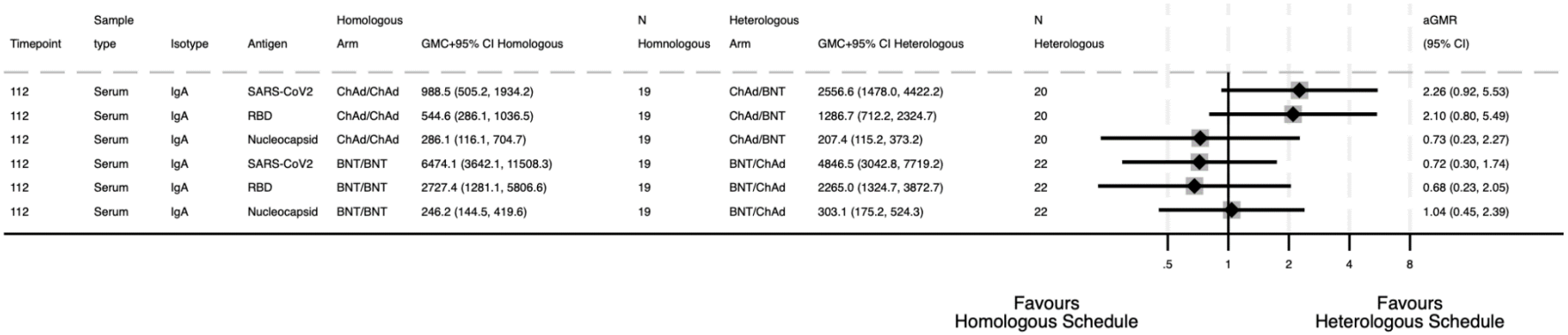

Figure 92 *– Forest plot comparing homologous to heterologous 12-week vaccine schedules. Serum IgA*

5.4.7 Heterologous vs homologous mucosal comparison by MSD

5.4.7.1 Mucosal IgG

Mucosal anti-SARS-CoV2 spike IgG aGMR for ChAd/ChAd vs ChAd/BNT is 11.38 (95% CI: 3.5, 36.8) (compared to 5.27 for serum), whilst the mucosal anti-SARS-CoV2 spike IgG aGMR for BNT/BNT vs BNT/ChAd is 0.47 (95% CI: 0.18, 1.21) (compared to 0.59 for serum) (**Figure 93**).

The significant difference in ChAd/ChAd vs ChAd/BNT aGMRs between mucosal and serum IgG implies ChAd/BNT recipients had proportionally more mucosal IgG than ChAd/ChAd recipients.

This analysis was repeated accounting for the D112 mucosal anti-nucleocapsid IgG and IgA levels as markers of mucosal SARS-CoV2 exposure (**Figure 94**). The mucosal anti-SARS-CoV2 spike IgG aGMR for ChAd/ChAd vs ChAd/BNT drops from 11.38 (95% CI: 3.5, 36.8) to 5.9 (95% CI: 2.5, 13.8) (very similar to the serum IgG aGMR of 5.3).

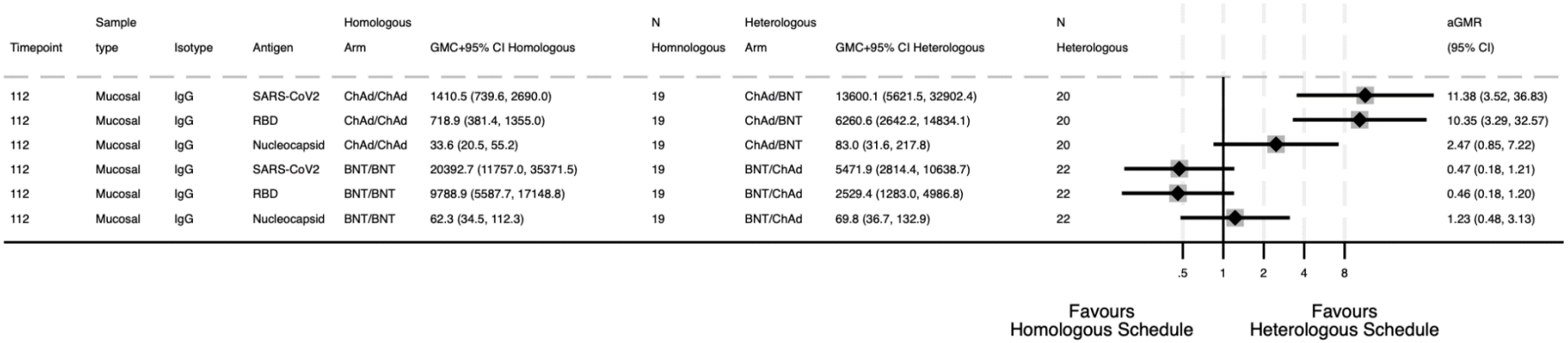

Figure 93 *– Forest plot comparing homologous to heterologous 12-week vaccine schedules. Mucosal IgG*

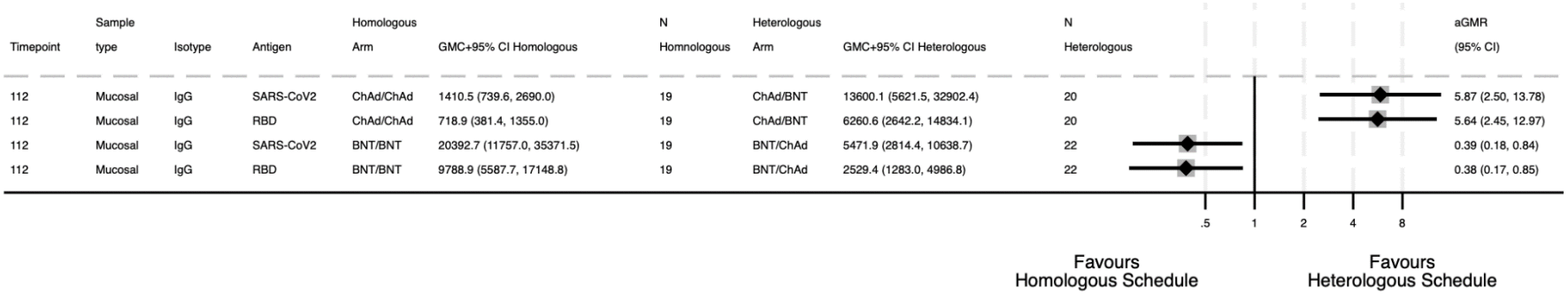

Figure 94 *– Forest plot comparing homologous to heterologous 12-week vaccine schedules. Mucosal IgG, accounting for mucosal anti-nucleocapsid IgG and IgA levels at D112*

5.4.7.2 Mucosal IgA

Mucosal anti-SARS-CoV2 spike IgA aGMR for ChAd/ChAd vs ChAd/BNT is 3.2 (95% CI: 1.4, 7.6) (**Figure 95**). This analysis was repeated accounting for the D112 mucosal anti-nucleocapsid IgG and IgA levels as markers of mucosal SARS-CoV2 exposure. The mucosal anti-SARS-CoV2 spike IgA aGMR for ChAd/ChAd vs ChAd/BNT drops from 3.2 (95% CI: 1.4, 7.6) to 1.8 (95% CI: 0.9, 3.8) (**Figure 96**).

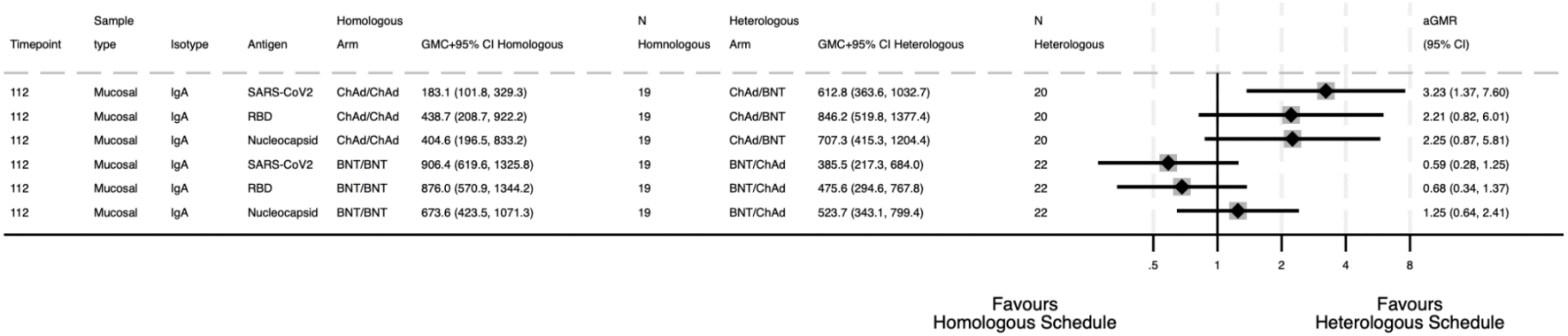

Figure 95 *– Forest plot comparing homologous to heterologous 12-week vaccine schedules. Mucosal IgA*

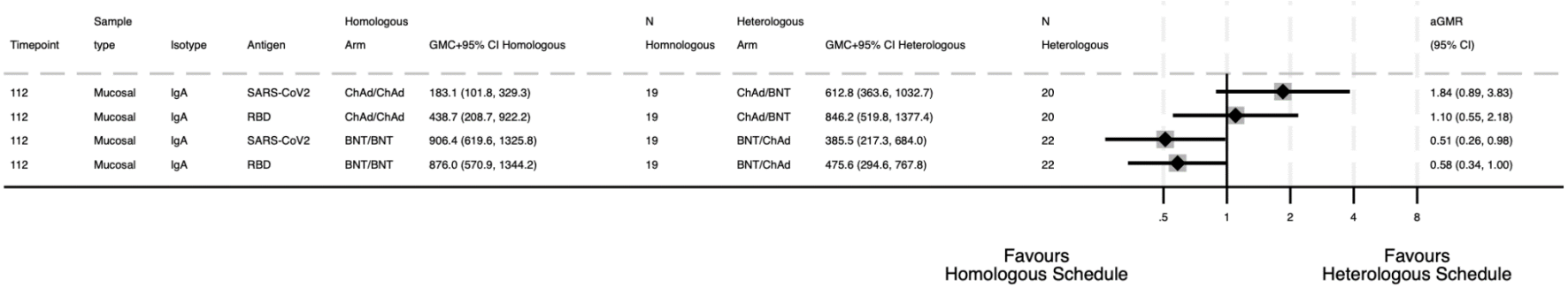

Figure 96 *– Forest plot comparing homologous to heterologous 12-week vaccine schedules. Mucosal IgA, accounting for mucosal anti-nucleocapsid IgG and IgA levels at D112*

5.4.8 Seasonal coronavirus cross-reactivity

The individual fold change (D112/D0) for each 'spike' antigen is demonstrated for serum IgG (**Figure 97**) and serum IgA (**Figure 98**). The magnitude of the fold increase (implying cross reactivity of the SARS-CoV2 vaccine-induced immune response) semi-quantitatively follows the degree of spike primary sequence homology (**Table 50**).

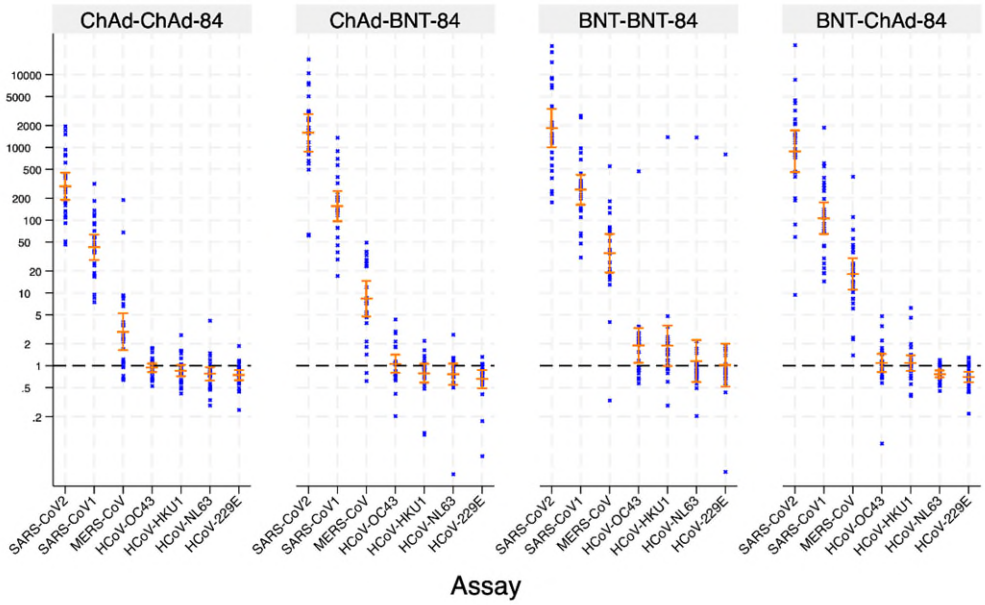

Figure 97 *– Serum IgG fold changes (D112/D0) of all spike antigens . Blue crosses represent individuals, with the group GMC + 95% CI in orange*

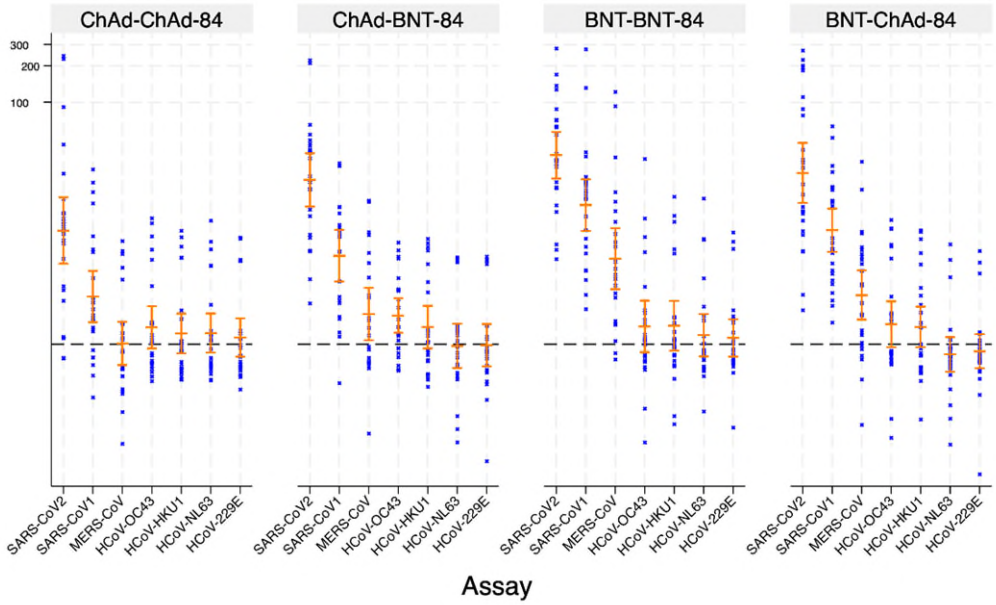

Figure 98 *– Serum IgA fold changes (D112/D0) of all spike antigens . Blue crosses represent individuals, with the group GMC + 95% CI in orange*

***Table 50** – Percentage primary sequence homology between full spike proteins for the seven members of the coronavirus family which can cause human infection . Alignment comparison performed on https://benchling.com/ using archetypal amino acid sequences for the spike of each coronavirus from the NCBI virus database (https://www.ncbi.nlm.nih.gov/labs/virus/vssi/#/virus?SeqType_s=Nucleotide)*

Full Spike protein	SARS-CoV2 WT Spike	SARS-CoV1 Spike	MERS-CoV spike	HCoV-OC43 spike	HCoV-HKU1 spike	HCoV-229E spike	HCoV-NL63 spike
SARS-CoV2 WT Spike		81%	39%	36%	35%	28%	23%
SARS-CoV1 Spike	81%		40%	36%	36%	30%	23%
MERS-CoV spike	39%	40%		36%	36%	26%	23%
HCoV-OC43 spike	36%	36%	36%		67%	22%	23%
HCoV-HKU1 spike	35%	36%	36%	67%		22%	22%
HCoV-229E spike	28%	30%	26%	22%	22%		48%
HCoV-NL63 spike	23%	23%	23%	23%	22%	48%	

5.4.9 Cross-protection of Seasonal coronavirus Mucosal IgA – PITCH sub-study

Pre-existing seasonal coronaviruses serum IgG has been associated with poorer clinical outcomes in unvaccinated patients, unwell enough to be admitted to ITU (239). How pre-existing mucosal responses against seasonal coronaviruses impact on SARS-CoV2 infection risk is unknown.

The mucosal samples of a subset of 36 medical students from the PITCH cohort study were analysed. At the point of enrolment, none had had clinical or virological evidence of SARS-CoV2 infection. Of these, 23/36 (64%) then went on to get symptomatic SARS-CoV2 infection whilst 13/36 (36%) did not.

Baseline mucosal anti-SARS-CoV2 spike IgG titres were higher in the students who subsequently went on to develop SARS-CoV2 infection (**Figure 99**).

Anti-SARS-CoV2 spike IgA against both alphacoronavirus, have a higher titre at baseline in those who did not go on to develop SARS-CoV2 infection (although only HCoV-NL63 reaches statistical significance) (**Figure 100**).

Assay	Isotype	GMC+95%CI - No future SARS-CoV2 infection	N - No future SARS-CoV2 infection	GMC+95%CI - Future SARS-CoV2 infection	N - Future SARS-CoV2 infection	GMR (95% CI)
SARS-CoV2	IgG	33.81 (13.68, 83.58)	12	386.87 (108.16, 1383.68)	19	11.4 (2.1, 62.9)
RBD	IgG	46.50 (23.96, 90.25)	10	196.74 (85.10, 454.84)	17	4.2 (1.3, 13.5)
Nucleocapsid	IgG	122.77 (48.31, 312.01)	11	239.45 (85.97, 666.91)	19	2.0 (0.4, 8.5)
HCoV-OC43	IgG	5633.28 (3040.15, 10438.23)	11	12764.61 (7811.55, 20858.27)	20	2.3 (1.0, 4.9)
HCoV-HKU1	IgG	3118.77 (1449.14, 6712.07)	11	3316.86 (1673.03, 6575.82)	18	1.1 (0.4, 2.9)
HCoV-NL63	IgG	491.69 (169.55, 1425.88)	11	957.50 (578.70, 1584.25)	19	1.9 (0.7, 5.2)
HCoV-229E	IgG	5396.28 (2599.84, 11200.65)	11	6555.56 (3923.38, 10953.65)	21	1.2 (0.5, 2.8)
SARS-CoV1	IgG	30.58 (14.59, 64.11)	11	181.10 (72.78, 450.64)	19	5.9 (1.6, 21.4)
MERS-CoV	IgG	59.37 (29.28, 120.39)	12	256.33 (90.90, 722.84)	17	4.3 (1.2, 16.2)

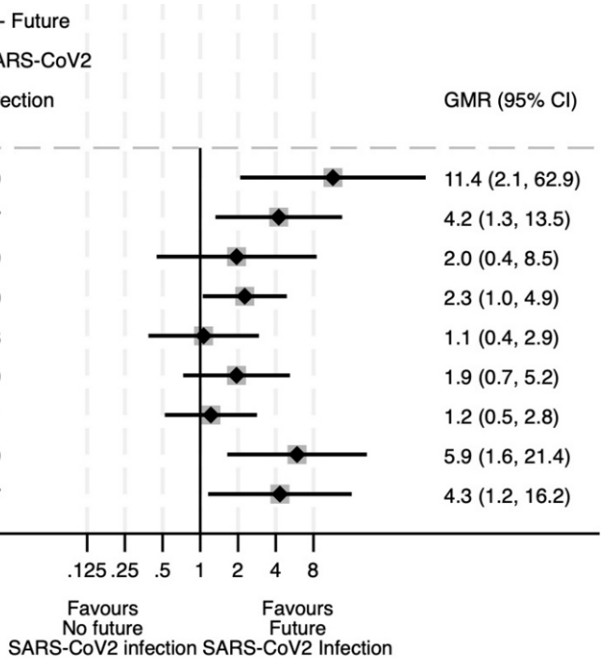

Figure 99 *– Forest plot comparing baseline mucosal IgG titres of participants who subsequently did and did not develop SARS-CoV2 infection*

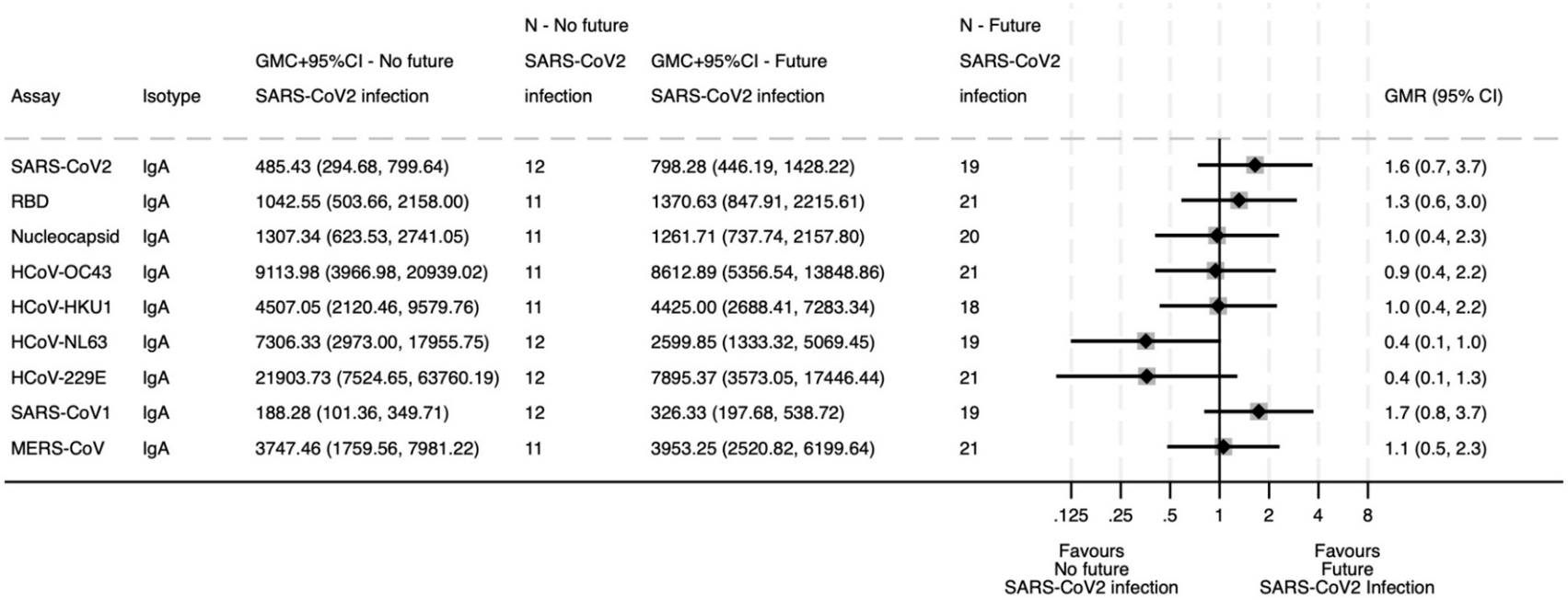

Figure 100 *– Forest plot comparing baseline mucosal IgA titres of participants who subsequently did and did not develop SARS-CoV2 infection*

5.4.10 Mucosal effects on IM vaccination response

In Chapter 3 , I outlined how seropositivity at baseline affected the systemic immune response to IM vaccination. It is, however, unknown how mucosal anti-SARS-CoV2 spike immunoglobulin levels or how serum and mucosal levels of seasonal coronavirus immunoglobulin affect IM vaccination response.

Multivariate regression models (covariates described in Section 2.11) are used to investigate, in turn, each predictive parameter's (Baseline mucostatus and seromucostatus as well as baseline values for Mucosal IgG, Mucosal IgA, Serum IgG and Serum IgA for SARS-CoV2, HCoV-OC43, HCoV-HKU1, HCoV-NL63 and HCoV-229E) effect on the archetypal markers of vaccine response (Serum anti-SARS-CoV2 spike IgG, serum anti-SARS-CoV2 spike IgA, mucosal anti-SARS-CoV2 spike IgG) (**Supplementary Table 1**).

- Baseline serum anti-nucleocapsid IgG was significantly associated with peak serum anti-SARS-CoV2 spike IgG levels.
- Baseline serum anti-nucleocapsid IgA was significantly associated with peak serum anti-SARS-CoV2 spike IgA.
- Baseline serum anti-SARS-CoV2 spike IgA was significantly associated with peak serum anti-SARS-CoV2 spike IgA.

These three associations are in keeping with the fact that seropositive participants produce a larger peak anti-SARS-CoV2 spike IgG and IgA response, although notably Baseline serum anti-SARS-CoV2 spike IgG was not significantly predictive of vaccine response.

Additionally baseline mucosal anti-HCoV-HKU1 spike IgG was significantly associated with peak mucosal anti-SARS-CoV2 spike IgG.

In summary, beyond evidence to support seropositivity being associated with a stronger vaccine response, there was no other significant effect on any of the markers of vaccine response.

5.4.11 Mucosal IgA as a candidate for epidemiological monitoring

To explore the potential use of mucosal anti-SARS-CoV2 spike IgA as a diagnostic or epidemiological tool, each value of mucosal anti-SARS-CoV2 spike IgA was labelled as 'inferred recent mucosal exposure' if the gradient of the preceding time interval was positive or 'no inferred recent mucosal exposure' if the gradient was negative (**Figure 101**).

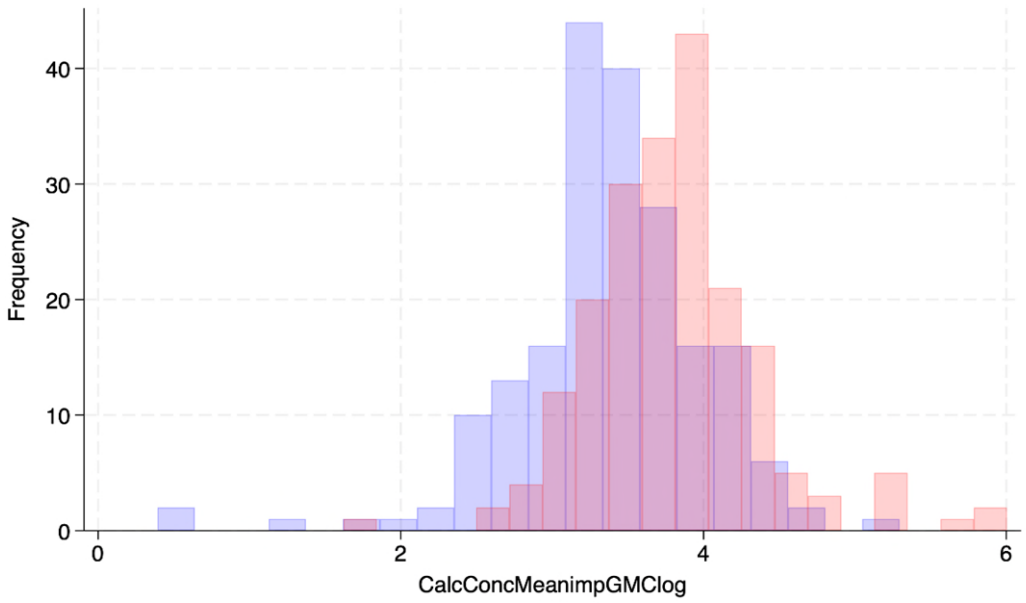

Figure 101 *– Histogram of Mucosal Spike IgA value . Blue represents no recent inferred mucosal exposure. Red represents recent inferred mucosal exposure*

As expected, the populations of 'inferred recently exposed participants' and 'inferred non-recently exposed participants' appear different, but overlap significantly.

In order to investigate mucosal IgA's performance more thoroughly 6 mucosal parameters (mucosal anti-SARS-CoV2 spike IgG, mucosal anti-SARS-CoV2 spike IgA, mucosal anti-RBD IgG, mucosal anti-RBD IgA, mucosal anti-nucleocapsid IgG, mucosal

anti-nucleocapsid IgA) were assessed in an 'all against all' fashion by including all combinations of between one and six parameters in a binary logistic regression model to predict recent inferred mucosal exposure.

Models were grouped by how many terms they had (one to six) and for each of these, the model with the lowest Akaike information criterion (AIC) was taken forwards (**Table 51**). Each of these models was then put through a cross-validated area under the curve analysis to estimate the ROC characteristics. The cross validation analysis for the best performing logistic regression model with one parameter is shown (**Figure 102**)

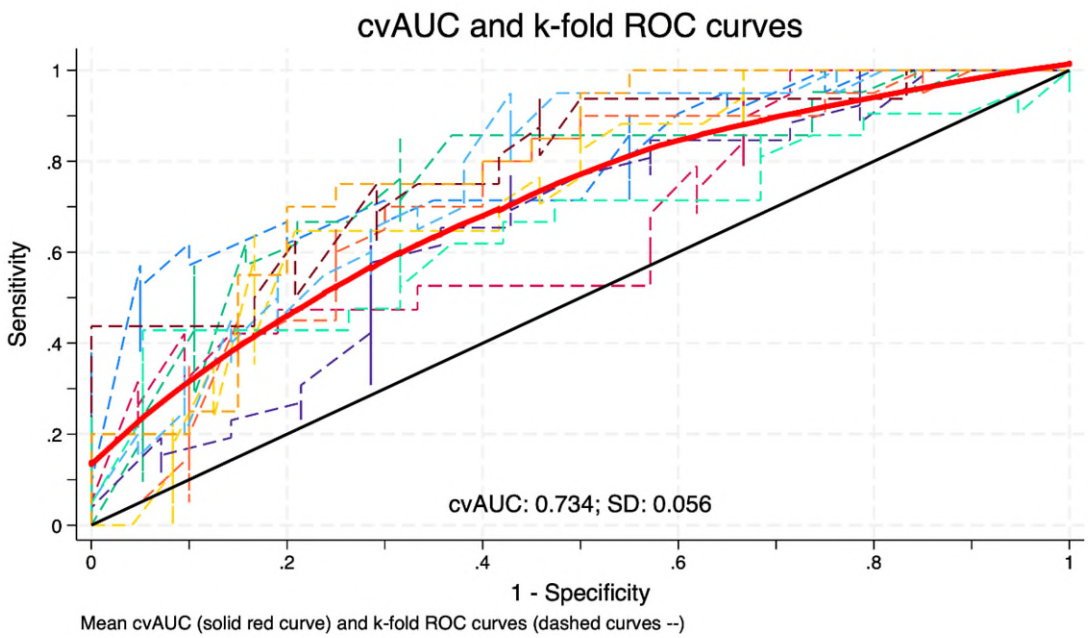

Figure 102 *– 10 k-fold cross-validated area under the curve ROC analysis for single term model including mucosal anti-RBD IgA*

Table 51*- Top performing models with 1-6 covariates in k-fold cross-validation for ROC analysis*

Covariates	Immunological measures	AIC	cvAUC
1	RBD Muc IgA	491	0.734
2	RBD Muc IgA, Nuc Muc IgA	483	0.751
3	RBD Muc IgA, Nuc Muc IgG , Nuc Muc IgA	485	0.747
4	Spike Muc IgG, RBD Muc IgA , Nuc Muc IgG , Nuc Muc IgA	485	0.753
5	Spike Muc IgG, Spike Muc IgA, RBD Muc IgA , Nuc Muc IgG , Nuc Muc IgA	486	0.755
6	Spike Muc IgG, Spike Muc IgA, RBD Muc IgG, RBD Muc IgA , Nuc Muc IgG , Nuc Muc IgA	488	0.754

5.5 Discussion & Conclusions

5.5.1 Highlights

5.5.1.1 IM vaccination does not induce local mucosal IgA production in seronegative participants

Normally, to assess the immunogenicity of an IM vaccination, the change examined is from pre-vaccination until the desired peak post-vaccination point (eg 28 days post-vaccination), with the expectation that the measured immunological measure may rise. Implicit in this, is the understanding that the immune marker will likely subsequently decline. However, in the context of mucosal SARS-CoV2 exposures, this single measure alone, over one time interval, is insufficient to tease apart response to IM vaccination from confounding mucosal exposure. Instead, it is necessary to look at the kinetic course over several timepoints.

Inter-timepoint gradients, rather than absolute antibody titres were correlated, as it is the dynamic change and therefore evidence of exposure, that is of interest, with the gradient itself representing the change in titre as a proportion per day (If the proportion is less than 1, it indicates a drop between the two times, if greater than 1, it indicates an increase). A correlation coefficient close to zero means it is unlikely that those two immunological markers are being driven by the same underlying event. However, the presence of correlation coefficients closer to 1, but in the absence of a significant p-value should not be over-interpreted.

The main analysis in Section 5.4.4 shows it is highly unlikely that IM vaccination is capable of producing a de novo local mucosal IgA response.

The purpose of the subgroup analyses in the same section were hypothesis-generating to see if there were specific instances where IM vaccination might produce a mucosal IgA response. The responses to a) a first dose of ChAd in seropositive participants, b)

the second dose of ChAd/BNT in seronegative participants, and c) the second dose of BNT/BNT in seronegative participants all had increases in correlation with vaccine marker responses. However, when verifying this relationship through univariate and multivariate linear regression models accounting for mucosal SARS-CoV2 exposure, the apparent association disappeared for first dose ChAd in seropositive participants and second dose BNT/BNT in seronegative participants.

The association for second dose ChAd/BNT in seronegative participants remained after accounting for mucosal SARS-CoV2 exposure and this is worthy of future exploration.

However, despite this, in both the ChAd/BNT and BNT/BNT multivariate regression models, mucosal nucleocapsid IgA had the largest coefficient with a significant p-value, suggesting that that incidental mucosal SARS-CoV2 exposure is important in driving at least part of the mucosal spike IgA response.

If there were a true mucosal anti-SARS-CoV2 spike IgA response, it would be biologically most plausible that this would occur in those with prior mucosal exposure (i.e. seropositive participants) and that this would most likely be detected in the most immunogenic schedules (i.e those with BNT as a second dose). However, numbers of seropositive participants per schedule were insufficient to perform this analysis. The combined seropositive analysis over both vaccine doses and all schedules failed to lend any convincing support to this idea however.

The importance of analysing data not just at a group level, but also at a participant level is clear. Additionally the use of carefully considered multivariate analyses, where appropriate, are key to demonstrate true relationships in complex biological systems.

5.5.1.2 Nasal mucoconversion without systemic immune activation

This study also highlights the limitations of the current paradigms used to define the immunological states in response to exposure to a pathogen. Convention is to describe

those with previous exposure as seropositive based upon their serum antibody response and those without previous exposure as seronegative. There may well be a number of other immunological states relating to infection that are worthy of definition, including some which are already recognised – seronegative, but with a detectable T cell response. In fact the importance of T cells for rapid resolution of SARS-CoV2 infection has been previously described in other studies (240,241).

However, more of interest to this body of work, are those who showed evidence of nasal mucoconversion with no systemic markers of immune response (antibody or T cell), of which there were 8 amongst the 23 participants who mucoconverted without seroconversion. None of these participants had virologically confirmed SARS-CoV2 infection, possibly due to a paucity or lack of symptoms and therefore no testing being sought. However, this finding suggests that nasal mucoconversion without seroconversion (possibly caused by transient mucosal SARS-CoV2 exposure) may be an important immunological state from both an individual and public health point of view and thus far has been inadequately characterised. One might additionally postulate that nasal mucoconversion without systemic activation may be more likely to occur in situations where the viral mucosal exposure or inoculum was small.

There are two counter arguments to the existence of the immunological state of “nasal mucoconversion without seroconversion”. The first is that immunological sampling was insufficiently frequent to detect a small systemic immunological response, before it started waning again by the next measured timepoint, although typically mucosal responses have been reported to wane faster than serum ones, making this less likely (182).

The second counter argument is a related one: That if the systemic response was sufficiently high, its ongoing wane over time might be greater than any uptick caused by transient asymptomatic infection or mucosal exposure and so the overall downward

trend would mask any systemic activation. The datapoints are insufficiently frequent to detect changes in rates of wane to gauge this.

For the single participant for who had evidence of virologically confirmed infection, and for whom there was mucosal data available, there was no clear rise in anti-SARS-CoV2 spike mucosal IgA or mucosal anti-nucleocapsid IgA (i.e nasal mucoconversion). This single participant's data does not support nasal mucoconversion without seroconversion following virologically confirmed infection. However, in the context of the flat trajectory of these mucosal immune markers, between the pre-infection and post-infection timepoints, it would be interesting to measure the mucosal response of a sample taken at the point of infection or just afterwards. Such a sample exists and has yet to be tested due to biosafety level restrictions, but has the theoretical potential to demonstrate a transient rise in mucosal IgA responses.

The concept of nasal mucoconversion without systemic immunological activation naturally leads to the conclusion that asymptomatic infection may be a heterogeneous syndrome that includes both asymptomatic seroconversion and nasal mucoconversion without systemic immunological activation.

Further to this, the conclusion that mucosal immunological activation can occur secondary to transient pathogen exposure, one must be especially cautious in the interpretation of rises in mucosal anti-SARS-CoV2 spike IgA as a response to IM vaccination. In the context of active community transmission in an endemic/pandemic/epidemic setting, it becomes easy to conflate vaccine response (measured over the course of several weeks) with time spent in pandemic, during which time there will be a high risk of mucosal exposure to the pathogen in question.

5.5.1.3 Variability in immune response

Of those participants who developed virologically confirmed infection (regardless of seroconversion), not all had measurable increases in both humoral and cellular compartments. The numbers showing this phenomenon of seemingly unlinked responses are small (one anti-nucleocapsid non-seroconverting participant who showed a T cell response without an anti-SARS-CoV2 spike IgG rise; seven anti-nucleocapsid seroconverting participants who showed an anti-SARS-CoV2 spike IgG rise without a T cell response). In cases where there was no seroconversion, where data was available, there was always a measurable T cell response.

There are several possibilities for this spectrum of heterogenous responses – one hypothesis is that there might be some degree of redundancy within the human immune system, where a response is not always required from all components in all circumstances, although it is not clear what would drive the preferential stimulus of one aspect of the immune response over another.

5.5.1.4 Cross-protection by pre-existing mucosal immunity against seasonal coronaviruses

In the PITCH study, baseline mucosal anti-SARS-CoV2 spike IgG response was greater in those who experienced subsequent SARS-CoV2 infection. It is not clear why this is the case, however, one explanation could be that the students had been assigned roles that differed in their risk of exposure to SARS-CoV2. Those who had been assigned roles with a greater risk of SARS-CoV2 exposure may have therefore silently mucoconverted, but still remained at higher risk of future symptomatic SARS-CoV2 infection.

Conversely, baseline mucosal IgA responses against the spike proteins of the alphacoronaviruses (HCoV-229E, HCoV-NL63) were greater in those who did not

experience subsequent SARS-CoV2 infection. The implication of this is that pre-existing alphacoronavirus mucosal IgA levels offer some degree of protection against WT SARS-CoV2 infection. This would be in keeping with the possibility of a previously described 'heterologous' protective effect of mucosal IgA against SARS-CoV2 induced by previous infection with a non-Omicron SARS-CoV2 variant, protecting against Omicron variant infection (185).

Given the closer genetic resemblance in general (**Figure 103**), and in the primary sequence homology of the spike protein in specific (**Table 50**), of seasonal betacoronaviruses to SARS-CoV2, one might expect that mucosal IgA against seasonal betacoronaviruses were more likely to give protection against SARS-CoV2 infection.

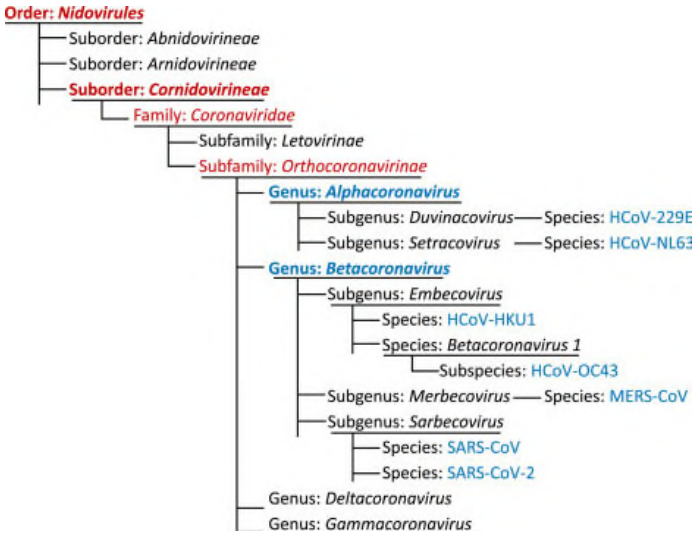

Figure 103 *– Phylogenetic tree of relevant human coronavirus pathogens demonstrating that SARS-CoV1 and MERS-CoV are most closely related to SARS-CoV2, followed by HCoV-OC43 and HCoV-HKU1, and finally by HCoV-NL63 and HCoV-229E* (242)

One could hypothesise that despite differences in the whole spike primary sequence, it may be similarities in the RBD region of the spike protein and similarities in cellular receptor requirement (**Table 52**) that are driving this counter-intuitive differential protective effect of alphacoronaviruses over seasonal betacoronaviruses.

HCoV-NL63 gains entry to human cells via ACE2, much like SARS-CoV2. It is perhaps initially biologically plausible that HCoV-NL63, specifically, may be mediating protection. The similar HCoV-229E response might be a result of cross-reactivity with HCoV-NL63 antibodies, given the close primary sequence homology between these two alphacoronaviruses. However, when comparing primary sequence homology of established or putative RBD regions of the spike proteins of these viruses, the levels of similarity are not supportive of this hypothesis (**Table 53**).

Table 52 *– Cellular receptor entry targets for the 7 members of the coronavirus family known to cause human infection*

HCoV	Cellular Receptor
HCoV-229E	Aminopeptidase N
HCoV-NL63	ACE2
SARS-CoV1	ACE2
SARS-CoV2	ACE2
MERS-CoV	DPP4
HCoV-OC43	? with 9-O-Ac-sialic acid
HCoV-HKU1	TMPRSS2 with 9-O-Ac-sialic acid

Table 53 *– Percentage primary sequence homology between confirmed or putative RBD sections of spike proteins for the seven members of the coronavirus family which can cause human infection . Alignment comparison performed on https://benchling.com/ using archetypal amino acid sequences for the spike of each coronavirus from the NCBI virus database (https://www.ncbi.nlm.nih.gov/labs/virus/vssi/#/virus?SeqType_s=Nucleotide)*

RBD	SARS-CoV2 WT Spike	SARS-CoV1 Spike	MERS-CoV spike	HCoV-OC43 spike	HCoV-HKU1 spike	HCoV-229E spike	HCoV-NL63 spike
SARS-CoV2 WT Spike		68%	30%	35%	18%	30%	10%
SARS-CoV1 Spike	68%		40%	37%	21%	30%	26%
MERS-CoV spike	30%	40%		38%	7%	26%	28%
HCoV-OC43 spike	35%	37%	38%		17%	30%	29%
HCoV-HKU1 spike	18%	21%	7%	17%		23%	26%
HCoV-229E spike	30%	30%	26%	30%	23%		7%
HCoV-NL63 spike	10%	26%	28%	29%	26%	7%	

An alternative explanation would be that mucosal IgA against any seasonal coronaviruses might be able to mediate protection against SARS-CoV2 infection, but either one or both alphacoronaviruses had circulated most recently in this population and therefore this mucosal response had been more recently boosted and therefore had

a larger, more measurable effect. What little epidemiological data on seasonal coronaviruses that exists, however, does not support this theory: An 8 year retrospective molecular analysis of respiratory infections in Scotland suggest that HCoV-OC43 is the most frequently diagnosed seasonal coronavirus and is about twice as common as HCoV-NL63 and HCoV-229E (no data for HCoV-HKU1) (243).

5.5.1.5 Mucosal IgG is a mixed population

If mucosal IgG were due only to transudation from serum, one would expect different absolute quantities of IgG to transudate through to the mucosa from the serum dependent on the serum IgG level (and therefore schedule). However, one would expect the same proportion of serum IgG to filter through to the mucosa regardless of schedule. Therefore the homologous/heterologous aGMRs for mucosal IgG should be the same as for serum IgG.

The initial analysis in Section 5.4.7 shows that there are clear differences in homologous/heterologous aGMR between serum and mucosa, particularly for ChAd/ChAd vs ChAd/BNT. This implies that there is proportionally a greater amount of mucosal IgG present for ChAd/BNT than one would expect purely from transudation.

There are two interpretations for this. The first is that there is a difference between schedules in terms of how mucosal lgG responses are produced (ie there may be another mechanism other than transudation from serum, such as if antibody producing cells were able to migrate to the lamina propria below the mucosa and produced antibodies locally). However, before one can consider such an interpretation, a second interpretation needs to be excluded: That the mucosal IgG has been supplemented by local production secondary to incidental mucosal SARS-CoV2 exposure. For this second interpretation to be feasible, there would have had to have been a difference between schedules in mucosal exposure (**Figure 79**).

One must be cautious suggesting that there may have been an imbalance in environmental exposure within a randomised control trial. However, on repeating the analysis accounting for mucosal SARS-CoV2 exposure, the significantly greater mucosal heterologous/homologous aGMR drops to a level in keeping with the serum heterologous/homologous aGMR.

This change in aGMR on accounting for mucosal SARS-CoV2 exposure gives further support to the idea that mucosal IgG is a mixture of transudate from the serum as well as local production. Based on previous literature (187), these antibody populations may be hypothesised to have different antigenic specificities as well as different immune functions, including Fc-mediated function and neutralisation.

5.5.1.6 Insufficient evidence to suggest use as an epidemiological marker

Given mucosal IgA's apparent sensitivity for mucosal pathogen exposure regardless of the presence of virologically or clinically confirmed infection, the next important clinical question that may be asked, is whether measurement may hold some value as an epidemiological marker of community or hospital transmission.

The main potential advantage that it has over nucleic acid amplification tests is that in asymptomatic infection, mucosal IgA is likely to be raised for a longer period than a PCR test may remain positive for. Thus far, it has been the gradients between timepoints of mucosal immune markers that have been investigated. However, to be useful as an epidemiological tool, a one-off absolute measurement ideally should be usable rather than a trend between two sampling times.

Although there is a clear difference between the 'recent inferred mucosal exposure' and 'no recent inferred mucosal exposure' populations in terms of the mucosal anti-SARS-CoV2 spike IgA response, there is no clear cut off value of anti-SARS-CoV2 spike

mucosal IgA which would clearly separate these two populations with high sensitivity and specificity.

Overall, none of the models performed sufficiently well to demonstrate the use of mucosal spike IgA as a diagnostic tool in the community or in a hospital setting. However, the context in which these measurements were taken was over the course of primary vaccination and in the months soon afterwards. This context will affect how these mucosal markers will perform. Repeating this analysis in a new population as part of a community or hospital survey during a period of stability in transmission and vaccination would give a more accurate measure of how either spike or nucleocapsid mucosal IgA might perform as a diagnostic or public health tool.

5.5.2 Caveats

There are several caveats to the mucosal work presented. Some have already been mentioned alongside the discussion to qualify interpretation of results. Caveats affecting the body of work more generally are discussed below.

Com-COV1 was not set up to focus on mucosal responses, the timepoints are therefore not equally spaced. This has been accounted for by using gradients rather than ratios to reflect changes between timepoints. Seroconversion has been defined by changes in immunological parameters at routine visits. Given the larger amount of time between visits, post-infection / post-seroconversion visits may have been some time after infection and therefore any signal of seroconversion may have been lost through waning. This is potentially of significant importance for the single participant who had mucosal sampling performed: They demonstrated virologically confirmed infection without seroconversion but did not show mucoconversion. Their infection was 2 weeks after their penultimate visit with their final visit occurring months later, which would give time for their mucosal response to wane and therefore any increase to have passed undetected. Analysis of the sample taken at the time of infection is planned.

Comparisons of first and second dose immune responses need to be interpreted with caution as the post-first vaccination timepoint is 56 days later, whereas the post second vaccination timepoint is 28 days later.

This body of work has also not touched upon the airway cellular or cytokine responses, both of which will be of critical and, likely synergistic, importance alongside the humoral response.

The mucosal results presented in this chapter are the ‘raw’ absolute results from the MSD ECLIA assay. Normalisation by total mucosal non-antigen specific IgG and IgA levels has not yet been performed, which would help to account for intra-person variability of antibody production and sampling volume variation.

The secondary antibody used in the IgA work was directed against IgA. This will not differentiate between locally produced dimeric IgA and monomeric IgA which has transudated from the serum. Future work aims to establish the feasibility of using a secondary antibody against the secretory component of sIgA to differentiate between monomeric and dimeric IgA.

Com-COV1 had a very small number of baseline seropositive participants and therefore it has not been possible to pass a more definitive comment on the ability for IM vaccination to induce a local mucosal IgA response in seropositive participants at the time of their second dose.

Further to this, the exploratory subgroup analyses frequently contained small numbers of participants, limiting their power. It is difficult to draw definitive conclusions from these and their role is limited to hypothesis generation.

Although the conclusions of this work regarding mucosal immunological activation in the absence of systemic activation are based on the dynamic titres of mucosal anti-SARS-CoV2 spike IgA, much of this activity occurs below the ascribed pre-pandemic threshold

of positivity. It is possible that the pre-pandemic threshold is falsely high, which may have been caused by inter-laboratory or inter-operator variability using the MSD assay, as the values used to set these thresholds were obtained by a different laboratory who shared their raw data. However, other studies have also reported mucosal IgA responses below the pre-pandemic thresholds that they set independently (183). In this case, their results may represent a pre-pandemic threshold that has been set falsely high, caused by not taking into account the fact there may be significant cross-reactivity between SARS-CoV2 nucleocapsid and the nucleocapsid of the seasonal coronaviruses (244), as there is still significant primary sequence homology between members of the coronavirus family in this protein too (**Table 54**).

Table 54 *– Percentage primary sequence homology between nucleocapsid proteins for the seven members of the coronavirus family which can cause human infection . Alignment comparison performed on https://benchling.com/ using archetypal amino acid sequences for the spike of each coronavirus from the NCBI virus database (https://www.ncbi.nlm.nih.gov/labs/virus/vssi/#/virus?SeqType_s=Nucleotide)*

Nucleocapsid	SARS-CoV2 WT Spike	SARS-CoV1 Spike	MERS-CoV spike	HCoV-OC43 spike	HCoV-HKU1 spike	HCoV-229E spike	HCoV-NL63 spike
SARS-CoV2 WT Spike		91%	50%	32%	32%	27%	31%
SARS-CoV1 Spike	91%		50%	32%	33%	26%	31%
MERS-CoV spike	50%	50%		32%	33%	27%	30%
HCoV-OC43 spike	32%	32%	32%		65%	20%	23%
HCoV-HKU1 spike	32%	33%	33%	65%		21%	24%
HCoV-229E spike	27%	26%	27%	20%	21%		42%
HCoV-NL63 spike	31%	31%	30%	23%	24%	42%	

5.5.3 Final statements & Future work

The importance and relevance of the mucosal immune response is being increasingly recognised with an increase in the number of publications addressing this area. However, there is still great variability in the methodology attempting to delineate mucosal immune responses including sample type, isotypes being investigated and the ability to account for concurrent viral exposure.

Few pieces of research work (235) investigating the locally produced mucosal IgA response have used secondary antibodies that specifically target dimeric IgA rather than total IgA (245). Similarly few studies have dedicated resource to investigating immune measures representative of biologically plausible confounding processes such as mucosal SARS-CoV2 exposure. Finally, none of the few studies which did measure mucosal anti-nucleocapsid IgA have yet managed to incorporate this result into their analyses at an individual participant level.

This body of work addresses most of the issues raised. The main conclusion of this chapter is that IM vaccination is unlikely to produce a locally derived mucosal dimeric IgA response. The evidence that IgA's dimeric valence is what drives its high avidity and neutralisation capability rather than the individual Fab affinity, suggests that locally produced dimeric mucosal IgA is critical in mucosal defence rather than systemically derived monomeric IgA (159). Given the increasingly established importance of mucosally derived dimeric IgA in preventing infection, developing immunisation strategies that can stimulate mucosal dimeric IgA responses is critical (194).

Mucosally delivered vaccines are very attractive given the obviously biologically plausible mechanisms by which mucosal presentation of antigen via immunisation may produce a local mucosal dimeric IgA response. Currently, only the live-attenuated influenza vaccine is licensed in the UK as a mucosally delivered vaccination against a respiratory disease (246), and even then, it is licensed only in children as it did not perform as favourably in older adults when compared against an intramuscular alternative (247). The mucosal immune response of this nasal vaccine is incompletely understood, although trials are planned to further investigate this (248).

There are a few mucosally delivered vaccines against SARS-CoV2 which are in development including a nasally delivered atomised spray containing a replication-deficient H1N1 influenza virus vector carrying the RNA sequence for an ancestral SARS-

CoV-2 receptor-binding domain (RBD) as well as an aerosolised, orally inhaled, replication deficient, adenovirus type-5 vectored vaccine encoding the SARS-CoV-2 spike protein. The former has shown efficacy of around 30% (249) against symptomatic infection, when used as part of a primary course in vaccine-naive and infection-naïve cohorts, as well as when used as a booster in those previously vaccinated. This level of protection was similar to other SARS-CoV2 vaccine trials carried out at a similar point in the pandemic when Omicron subvariants were circulating. It was unable to assess protection against severe disease due to low endpoint numbers in the trial. The Ad5 vaccine has not been tested in an efficacy trial, but shows promise from an immunogenicity point of view in both the systemic and mucosal compartments (250,251).

There is much yet to understand about mucosally-delivered vaccines including the importance of the method of administration – inhaled vaccines may produce a larger systemic response as antigen presentation in the small airways may be more similar to IM vaccination, whereas nasally inhaled vaccines might produce a larger mucosal response and fail to produce a systemic response (252). It is likely that any vaccination schedule in the future will need to stimulate both compartments. The mucosal immune response has not been adequately quantitatively analysed nor is there a sufficient understanding of its functionality.

The work in this chapter adds to this field and will inform how to assess mucosal immune responses to mucosal vaccines. There is much work planned to further elucidate the mucosal immune response:

- Total non-antigen specific mucosal IgG & IgA titres to allow normalisation
- Quantification of antibody-specific mucosal neutralisation capacity
- Evaluation of paired saliva and SAM-strip samples to establish the key differences between these sample types including variability.

- Paired analysis of serum IgG, mucosal IgG & mucosal IgA investigating glycosylation, Fc function, IgG/IgA subclasses and epitope specificity
- Analysis of mucosal samples from COV-Boost (heterologous 3rd dose vaccination study), which would allow a more definitive assessment of whether seropositive participants can produce a mucosal IgA response to IM vaccination

Chapter 6 Final Discussion & Conclusions

6.1 Overview

Flexibility of vaccination schedules is important. It eases the logistical constraints of immunisation programme delivery, especially in situations where vaccine supply is limited and programme roll out needs to be rapid, such as in a pandemic.

Additionally this flexibility might allow national immunisation programmes to reduce reliance on a single vaccine manufacturer. This makes the programme more robust against supply shocks, but also has the potential to allow a fairer market for vaccine purchase and drive down the costs of these life saving medications (253).

This thesis provides robust evidence for the feasibility of flexible vaccination, both through the use of heterologous vaccination as well as through a permissive approach to the priming interval.

The data from these trials have helped inform policy-relevant decisions to the UK COVID-19 immunisation programme as well as WHO-endorsed advice. However, they also presented an opportunity to gain insight into the way different vaccine platforms or their combinations might lay down immunity. The conclusions drawn from these more exploratory analyses must always be looked at with some caution as some subgroup analyses contain smaller numbers of participants and there may be unmeasured confounding factors. Multiple additional analyses will have a greater chance of yielding false positive results. Nevertheless, these data provide a valuable resource from which to draw hypotheses for further investigation, as never before have there been such large numbers of people vaccinated with different vaccine platforms to allow their direct comparison.

These studies are unique in their decision to take mucosal samples so intensively and extensively to be able prospectively to explore more definitively the mucosal immune responses to intramuscular vaccination and are therefore uniquely placed to pass a more definitive comment on this topic – that intramuscular vaccination does not induce a locally produced mucosal IgA response.

6.2 Principal findings, Conclusions & Implications

6.2.1 COVID-19 Heterologous priming schedules

In this study, I have shown the heterologous schedule ChAd/BNT to be non-inferior and superior to its relative homologous schedule, ChAd/ChAd, by total peak serum anti-SARS-CoV2 spike binding IgG. This was true at both short and long intervals. Similarly I have shown ChAd/Mod and ChAd/NVX to be non-inferior and superior to ChAd/ChAd. BNT/Mod was also shown to be superior to BNT/BNT, but BNT/ChAd and BNT/NVX did not meet the non-inferiority margin against BNT/BNT.

In the absence of a universally accepted correlate of protection, serum binding anti-SARS-CoV2 spike IgG was used to infer the likely protective capability of these heterologous schedules.

If the serum binding anti-SARS-CoV2 spike IgG is indeed a definitive correlate of protection, one should not then put too much weight on the failure of BNT/ChAd and BNT/NVX to meet non-inferiority against BNT/BNT, as their absolute serum binding anti-SARS-CoV2 spike IgG titres are still in excess of that produced by ChAd/ChAd, a schedule which has been shown to provide high levels of protection against severe SARS-CoV2 infection and death, and, like all other licensed COVID vaccines, short-lived protection against symptomatic infection. This has been shown both in the setting of a randomised clinical trial (87) and subsequently in real-world test-negative case control studies (254).

Although an immunological measure such as serum binding anti-SARS-CoV2 spike IgG is helpful in giving a clear answer to the comparison of schedules, it is not a nuanced approach. It fails to consider the importance of the cellular response. In fact, it is clear that the most humorally immunogenic schedules, such as those with two doses of mRNA vaccine are not the most immunogenic in terms of T cell response. It was ChAd-primed schedules followed on by a non-ChAd vaccine (BNT, Mod or NVX) that produced the largest T cell response with the longest persistence. The clinical significance of these cellular differences are unclear, but do indicate that there is not a clear 'winner' between all these schedules.

The over-simplified view of regarding only serum anti-SARS-CoV2 spike binding IgG also fails to take into account differences in the functionality of the humoral immune response. This is most clearly demonstrated by the comparison of BNT/BNT to BNT/NVX, where the former produces double the amount of binding anti-SARS-CoV2 spike IgG , but the latter produces a greater neutralisation response against WT SARS-CoV2.

What is clear is that heterologous priming schedules are more than suitable alternatives to homologous priming schedules, where homologous schedules are either clinically contra-indicated or logistically unfeasible. Some of the more commonly used heterologous schedules have been shown to be effective in real world effectiveness studies (255). However, there is insufficient evidence to suggest that heterologous schedules should be preferentially administered over homologous ones. It was suspected by some, that heterologous schedules may offer additional benefits in terms of breadth or longevity of response, however this has not been convincingly borne out by the data thus far.

One should not focus too much on the word 'heterologous.' Defining a schedule by what it is not (i.e. two doses of the same vaccine) is not as important as defining it by exactly

what is there. In other words, the differences observed between homologous and heterologous schedules do not depend on a schedule being labelled as ‘heterologous’ per se, but on exactly which vaccines make up that schedule and the order in which they are received.

6.2.2 COVID-19 vaccine schedule priming Interval

The decision to randomise both by heterologous/homologous schedule and by interval was an important one. This allowed a definitive description of both reactogenicity and immunogenicity. Other studies (121,123) conflated interval and schedule when describing the immune responses and reactogenicity resulting in misleading conclusions.

Prolonging interval resulted in an increase in serum binding anti-SARS-CoV2 spike IgG in ChAd/ChAd (aGMR 1.9), BNT/BNT (aGMR 1.3) and BNT/ChAd (aGMR 1.5), but not for ChAd/BNT. However, neutralising responses were increased for all schedules between 1.5 and 2.8 times. This again suggested that longer schedules may have qualitative differences from shorter schedules.

The cellular response was also affected differently by prolonging interval, with all schedules universally showing a lower T cell response. This drop in measured response is of uncertain significance.

These results support the flexibility of approach to priming intervals when rolling out large scale immunisation programmes. Although these data do not support aiming for a longer interval rather than a shorter one, post-hoc results from the ChAdOx1 nCoV-19 efficacy trial (256) do show improved clinical efficacy in terms of protection against symptomatic infection, with a prolonged priming interval. Ultimately, it is highly likely that there will be more pressing practical constraints other than optimising the humoral response that will dictate the priming interval.

6.2.3 Reactogenicity

Ultimately the reactogenicity of all schedules studied were acceptable and there were no safety concerns. There is therefore little more to say from a policy perspective. However, the differences in reactogenicity rates by schedule, by interval and by serostatus raise interesting hypotheses that tie in well with other research. With further work, these data have the potential to contribute to a more complete understanding of how reactogenicity is mediated and how one might consider strategies to reduce it. This may have the potential to reduce vaccine hesitancy in some spheres, although how great an impact this may have is unclear.

6.2.4 Mucosal response to vaccination

The vast majority of literature on the induction of mucosal antibody responses by intramuscular vaccination support the notion that mucosal IgG was mostly the result of active or passive transport from blood and that mucosal IgA was not induced by intramuscular vaccination. However, a number of studies looking at mucosal IgA against COVID performed during the pandemic concluded that mRNA vaccine schedules could indeed induce the local production of a mucosal IgA response. These papers, however, did not account for mucosal SARS-CoV2 exposure.

The data presented in this thesis do not support local IgA production in seronegative participants. There was no relationship between serum anti-SARS-CoV2 spike IgG induction and mucosal anti-SARS-CoV2 spike IgA induction. The one exception to this is in a single subgroup analysis, where seronegative ChAd/BNT participants demonstrate a statistically non-significant association between serum IgG induction and mucosal IgA induction at the time of the second dose. Given the pre-existing literature, the lack of biological plausibility and the fact that this result occurs only for this specific combination (when it does not for the first BNT dose in either seronegative or

seropositive participants, nor at the time of the second dose for the BNT/BNT schedule) suggests that this may not be a true result, but does require further investigation.

There was additionally no convincing evidence that first doses of vaccine in seropositive participants induced a local mucosal IgA response. There were insufficient seropositive participants in this study to pass definitive comment on the effect of intramuscular vaccination at the time of the second dose, however, there was no clear signal of induction of local mucosal IgA here either.

It is important not to confound ‘time spent in pandemic’ with response to intramuscular vaccination. The work presented here highlights the importance of accounting for confounding mucosal exposure in assigning the origins of a mucosal IgA response. Critically, it also highlights the judicious use of participant-level data and multivariate analysis in order to deconvolute complex biological systems.

6.2.5 Immune statuses including mucostatus

It has been well described that not all SARS-CoV2 infection results in a systemic antibody response, but that some individuals may develop a T cell response in the absence of seroconversion (187–189). This highlights that the model of seronegative vs seropositive based on serum antibody response alone may be inadequate to describe the whole spectrum of immune statuses following pathogen or antigen exposure. In the above described case alone, there are four groups based on the combinations of the presence and absence of both T cell and antibody responses. The data presented here gives further support to this concept but also expands up on it. There is likely a syndrome of nasal mucoconversion that is the result of low level SARS-CoV2 mucosal exposure. The biological mechanism underlying this may well be that the immune response at the mucosa is sufficient to mitigate the infection prior to any systemic activation.

Given the separation between mucosal and systemic systems as well as the further compartmentalisation within the mucosal system itself, it is important to identify those who have been mucosally 'primed' and characterise their immune responses. This may well inform how the mucosal immune response might be induced through mucosal immunisation to reduce infection and transmission. Monitoring the mucostatus of the population also has the potential to provide insight into identifying patterns of sub-clinical transmission (257).

6.3 Limitations

The work presented in this thesis has several caveats, discussed specifically in each chapter. There are also some over-arching caveats that are worthy of mention:

The external validity of this work is limited by the age range of the participants enrolled (over 50). Additionally, given the relatively narrow range of ages studied, any analysis of the effect of age on immunogenicity is limited.

The period of follow up was relatively short, which was in part due to the rapidly changing vaccination policies of the time including the addition of a national third dose programme. It is therefore impossible to know how protection against severe disease may have waned, if at all. Long term data for homologous schedules, too, are lacking as the rate of uptake for third doses was so high (258).

An important factor beyond the scope of this trial was the effect of vaccine dose on immunogenicity. Each licensed vaccine was administered at the manufacturer's recommended dose without modification. The doses chosen in these trials were based on the original Phase I and II trials. An additional complication is that for mRNA and adenovirus vectored vaccines, even though there may be a specified dose of mRNA or adenovirus respectively, what that dose translates into in terms of antigenic dose, and how variable that is between people is unknown. Within these trials, it is impossible to

tease out whether the observed differences are the result of different vaccine platform modalities or the result of different relative antigenic doses.

In order to deliver these large studies so rapidly, there had to be sufficient laboratory processing capacity to perform the immunological assays in a timely manner. These studies therefore made use of contracted clinical research organisations to deliver these endpoints. This not only increased efficiency, but also allowed direct comparison of results across many trials, as the same laboratories processed samples for many of these studies through coordination by bodies such as CEPI.

Although assays were validated, verified and appropriately quality controlled, the nature of having to ship lithium heparin tubes from many sites to one centralised laboratory, meant that PBMCs could not be processed within 4 hours – a normal requirement in order to guarantee the quality of cells and an accurate ELISpot response. This was mitigated through the addition of the proprietary reagent, T-cell Xtend, which the manufacturer claimed made ELISpot results valid, even up to 32 hours post venepuncture. Although the T-cell results are likely qualitatively correct and are in agreement with results from other trials, other work has shown that these assays perform poorly at lower T cell concentrations, likely resulting in some degree of lower bound censoring (259).

Regarding the mucosal work, the lack of significant numbers of seropositive participants makes it difficult confidently to assert whether IM vaccination does or does not induce a local mucosal IgA response.

6.4 Future work

There are several areas of interest that spring from the work conducted in this thesis. To further the mucosal investigations, a deeper characterisation of the response is warranted, which includes measuring the neutralising and functional responses of the

mucosal antibodies and how this differs from the serum response. Given the established importance of neutralisation for mucosal IgA, investigation of the glycosylation pattern of mucosal IgA would be critical in understanding how dimeric IgA is able to confer protection.

To answer the question of whether intramuscular vaccination in a seropositive cohort might affect anti-SARS-CoV2 spike mucosal IgA, it is planned to expand this body of work to include samples from COV-Boost – the heterologous third dose study which guided the UK booster programme policy.

Further analyses regarding reactogenicity are ongoing, including assessing whether levels of anti-adenovirus neutralising antibody (anti-vector immunity) might explain the reduced reactogenicity of a second dose of homologous ChAd/ChAd. Additionally, whether waning levels of anti-vector immunity might be associated with increasing reactogenicity seen with second doses of homologous ChAd when administered at a prolonged interval.

Finally, it is clear from the kinetics data that the waning of antibody titres is not simple logarithmic decay down to a baseline of zero. Mathematical modelling in collaboration with statistical colleagues is planned to assess whether there is indeed a non-zero baseline to which antibodies might be decaying or whether the pattern of decay is due to different populations of antibody producing cells, such as short-lived and long-lived plasma cells waning at different rates. The demonstration of a plateau of both humoral and cellular immunity may give insight into long term protection against severe disease and whether further doses are required.

6.5 Final comment

The primary outcomes of these trials have been policy influencing. Bodies such as the JCVI frequently have to make decisions based on inference and related areas of

immunology and vaccinology – There is rarely evidence to hand precisely to answer policy questions in a relevant timeframe. The work here, however, will contribute to a more robust assessment of heterologous schedules, priming interval and mucosal responses that will help inform future decision making. The comparison of schedules and the vaccine platforms that make them up will inform future vaccine and vaccine schedule design against future pathogens.

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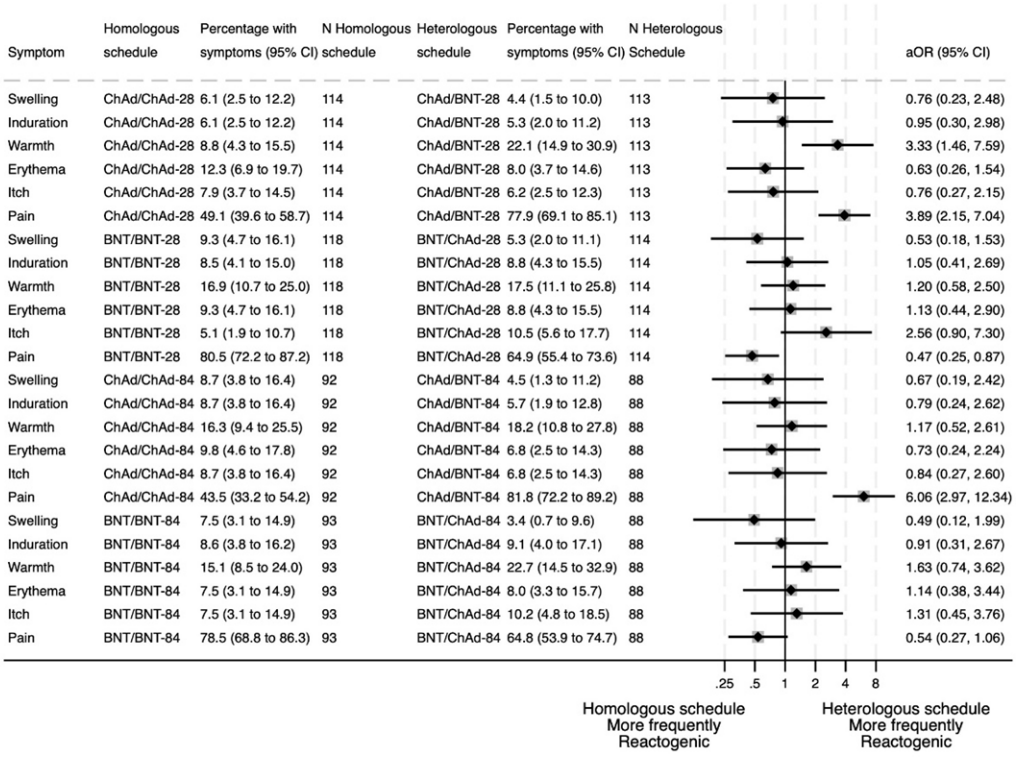
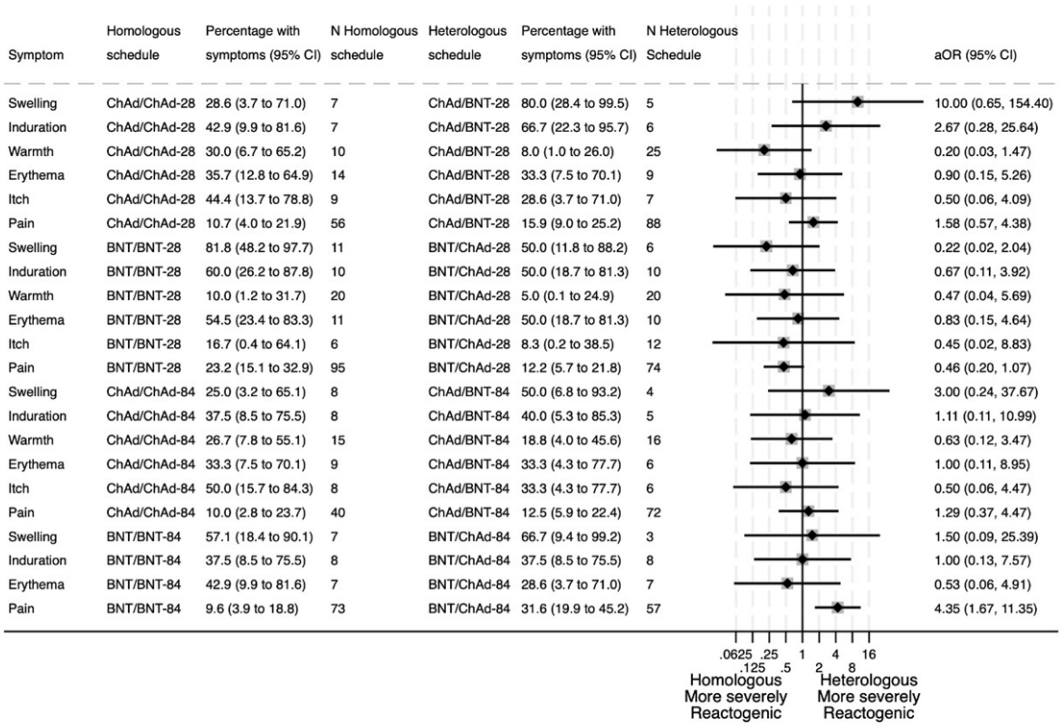
Supplementary Appendix

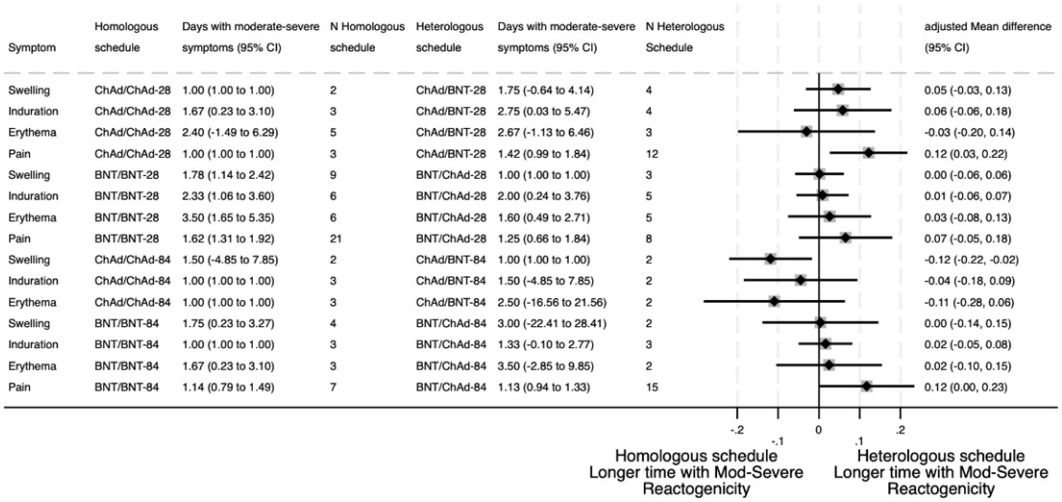


Supplementary Figure 1 *– Forest plot comparing proportions of participants (seronegative and seropositive combined) suffering local reactogenicity symptoms in the first 7 days after the second COVID-19 vaccination between homologous & heterologous schedules "Were you more*

likely to suffer a symptom of any grade after the second dose if the schedule was homologous or heterologous?"

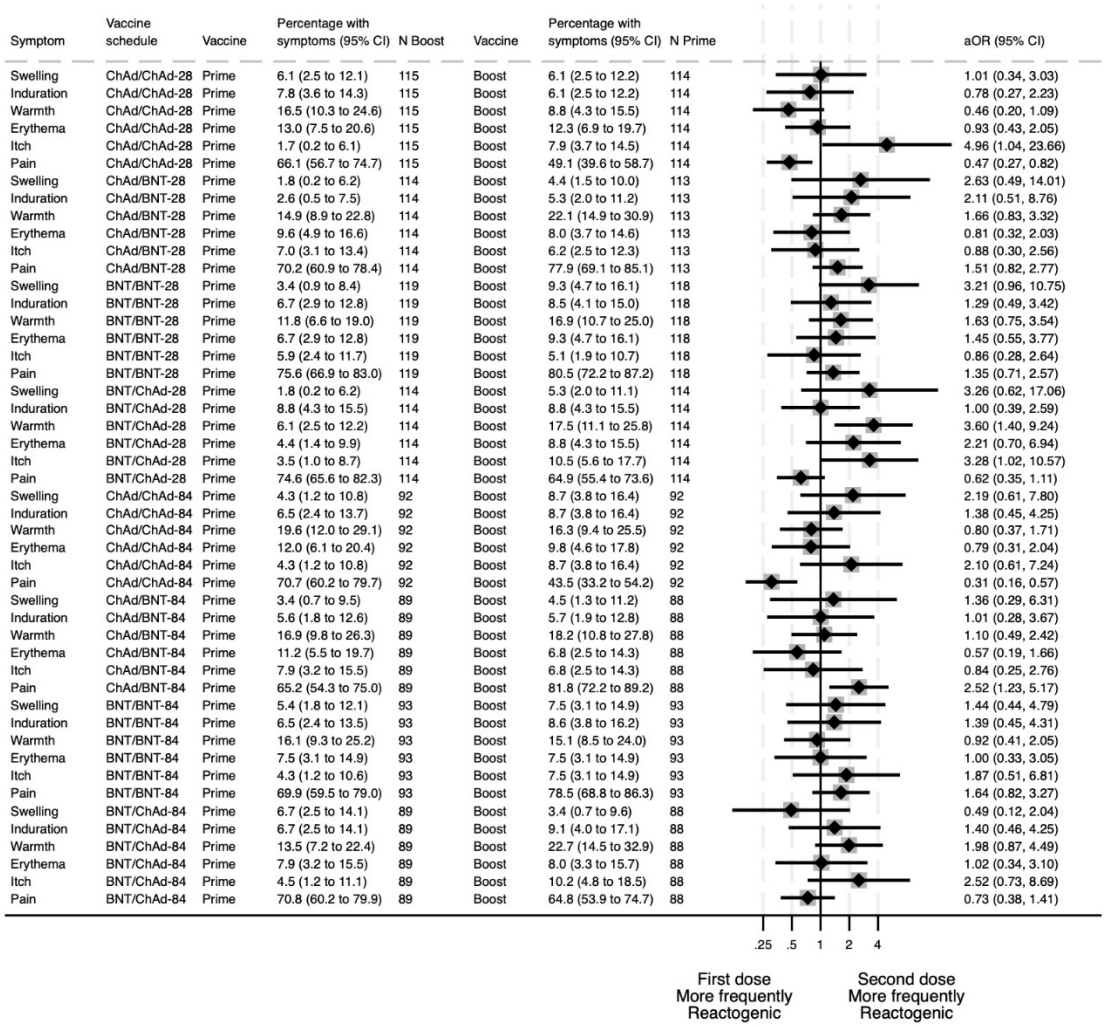


Supplementary Figure 2 *– Forest plot comparing proportions of participants (seronegative and seropositive combined) suffering moderate-severe (Grade 2 or above) local reactogenicity symptoms out of the total number of participants suffering any grade symptoms, in the first 7 days after a second COVID-19 vaccination between homologous and heterologous schedules . "If you did suffer a symptom of any grade after the second dose, was it more likely to be moderate/severe (Grade 2 or above) if the schedule was homologous or heterolgous?"*



Supplementary Figure 3 *– Forest plot comparing durations of time spent with grade 2 or higher local reactogenicity symptoms after the second COVID-19 vaccination between participants (seronegative and seropositive combined) receiving homologous or heterologous schedules . "If*

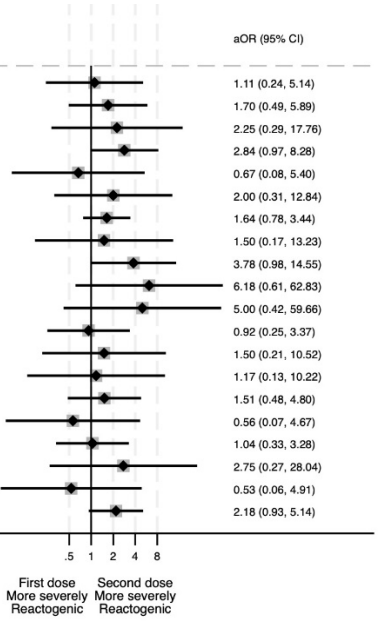
you did suffer with moderate/severe symptoms (Grade 2 or above) after the second dose, were the symptoms likely to last longer after a homologous or heterologous schedule?"



Symptom	Vaccine schedule	Vaccine	Percentage with symptoms (95% CI)	N Boost	Vaccine	Percentage with symptoms (95% CI)	N Prime	aOR (95% CI)
Swelling	ChAd/ChAd-28	Prime	6.1 (2.5 to 12.1)	115	Boost	6.1 (2.5 to 12.2)	114	1.01 (0.34, 3.03)
Induration	ChAd/ChAd-28	Prime	7.8 (3.6 to 14.3)	115	Boost	6.1 (2.5 to 12.2)	114	0.78 (0.27, 2.23)
Warmth	ChAd/ChAd-28	Prime	16.5 (10.3 to 24.6)	115	Boost	8.8 (4.3 to 15.5)	114	0.46 (0.20, 1.09)
Erythema	ChAd/ChAd-28	Prime	13.0 (7.5 to 20.6)	115	Boost	12.3 (6.9 to 19.7)	114	0.93 (0.43, 2.05)
Itch	ChAd/ChAd-28	Prime	1.7 (0.2 to 6.1)	115	Boost	7.9 (3.7 to 14.5)	114	4.96 (1.04, 23.66)
Pain	ChAd/ChAd-28	Prime	66.1 (56.7 to 74.7)	115	Boost	49.1 (39.6 to 58.7)	114	0.47 (0.27, 0.82)
Swelling	ChAd/BNT-28	Prime	1.8 (0.2 to 6.2)	114	Boost	4.4 (1.5 to 10.0)	113	2.63 (0.49, 14.01)
Induration	ChAd/BNT-28	Prime	2.6 (0.5 to 7.5)	114	Boost	5.3 (2.0 to 11.2)	113	2.11 (0.51, 8.76)
Warmth	ChAd/BNT-28	Prime	14.9 (8.9 to 22.8)	114	Boost	22.1 (14.9 to 30.9)	113	1.66 (0.83, 3.32)
Erythema	ChAd/BNT-28	Prime	9.6 (4.9 to 16.6)	114	Boost	8.0 (3.7 to 14.6)	113	0.81 (0.32, 2.03)
Itch	ChAd/BNT-28	Prime	7.0 (3.1 to 13.4)	114	Boost	6.2 (2.5 to 12.3)	113	0.88 (0.30, 2.56)
Pain	ChAd/BNT-28	Prime	70.2 (60.9 to 78.4)	114	Boost	77.9 (69.1 to 85.1)	113	1.51 (0.82, 2.77)
Swelling	BNT/BNT-28	Prime	3.4 (0.9 to 8.4)	119	Boost	9.3 (4.7 to 16.1)	118	3.21 (0.96, 10.75)
Induration	BNT/BNT-28	Prime	6.7 (2.9 to 12.8)	119	Boost	8.5 (4.1 to 15.0)	118	1.29 (0.49, 3.42)
Warmth	BNT/BNT-28	Prime	11.8 (6.6 to 19.0)	119	Boost	16.9 (10.7 to 25.0)	118	1.63 (0.75, 3.54)
Erythema	BNT/BNT-28	Prime	6.7 (2.9 to 12.8)	119	Boost	9.3 (4.7 to 16.1)	118	1.45 (0.55, 3.77)
Itch	BNT/BNT-28	Prime	5.9 (2.4 to 11.7)	119	Boost	5.1 (1.9 to 10.7)	118	0.86 (0.28, 2.64)
Pain	BNT/BNT-28	Prime	75.6 (66.9 to 83.0)	119	Boost	80.5 (72.2 to 87.2)	118	1.35 (0.71, 2.57)
Swelling	BNT/ChAd-28	Prime	1.8 (0.2 to 6.2)	114	Boost	5.3 (2.0 to 11.1)	114	3.26 (0.62, 17.06)
Induration	BNT/ChAd-28	Prime	8.8 (4.3 to 15.5)	114	Boost	8.8 (4.3 to 15.5)	114	1.00 (0.39, 2.59)
Warmth	BNT/ChAd-28	Prime	6.1 (2.5 to 12.2)	114	Boost	17.5 (11.1 to 25.8)	114	3.60 (1.40, 9.24)
Erythema	BNT/ChAd-28	Prime	4.4 (1.4 to 9.9)	114	Boost	8.8 (4.3 to 15.5)	114	2.21 (0.70, 6.94)
Itch	BNT/ChAd-28	Prime	3.5 (1.0 to 8.7)	114	Boost	10.5 (5.6 to 17.7)	114	3.28 (1.02, 10.57)
Pain	BNT/ChAd-28	Prime	74.6 (65.6 to 82.3)	114	Boost	64.9 (55.4 to 73.6)	114	0.62 (0.35, 1.11)
Swelling	ChAd/ChAd-84	Prime	4.3 (1.2 to 10.8)	92	Boost	8.7 (3.8 to 16.4)	92	2.19 (0.61, 7.80)
Induration	ChAd/ChAd-84	Prime	6.5 (2.4 to 13.7)	92	Boost	8.7 (3.8 to 16.4)	92	1.38 (0.45, 4.25)
Warmth	ChAd/ChAd-84	Prime	19.6 (12.0 to 29.1)	92	Boost	16.3 (9.4 to 25.5)	92	0.80 (0.37, 1.71)
Erythema	ChAd/ChAd-84	Prime	12.0 (6.1 to 20.4)	92	Boost	9.8 (4.6 to 17.8)	92	0.79 (0.31, 2.04)
Itch	ChAd/ChAd-84	Prime	4.3 (1.2 to 10.8)	92	Boost	8.7 (3.8 to 16.4)	92	2.10 (0.61, 7.24)
Pain	ChAd/ChAd-84	Prime	70.7 (60.2 to 79.7)	92	Boost	43.5 (33.2 to 54.2)	92	0.31 (0.16, 0.57)
Swelling	ChAd/BNT-84	Prime	3.4 (0.7 to 9.5)	89	Boost	4.5 (1.3 to 11.2)	88	1.36 (0.29, 6.31)
Induration	ChAd/BNT-84	Prime	5.6 (1.8 to 12.6)	89	Boost	5.7 (1.9 to 12.8)	88	1.01 (0.28, 3.67)
Warmth	ChAd/BNT-84	Prime	16.9 (9.8 to 26.3)	89	Boost	18.2 (10.8 to 27.8)	88	1.10 (0.49, 2.42)
Erythema	ChAd/BNT-84	Prime	11.2 (5.5 to 19.7)	89	Boost	6.8 (2.5 to 14.3)	88	0.57 (0.19, 1.66)
Itch	ChAd/BNT-84	Prime	7.9 (3.2 to 15.5)	89	Boost	6.8 (2.5 to 14.3)	88	0.84 (0.25, 2.76)
Pain	ChAd/BNT-84	Prime	65.2 (54.3 to 75.0)	89	Boost	81.8 (72.2 to 89.2)	88	2.52 (1.23, 5.17)
Swelling	BNT/BNT-84	Prime	5.4 (1.8 to 12.1)	93	Boost	7.5 (3.1 to 14.9)	93	1.44 (0.44, 4.79)
Induration	BNT/BNT-84	Prime	6.5 (2.4 to 13.5)	93	Boost	8.6 (3.8 to 16.2)	93	1.39 (0.45, 4.31)
Warmth	BNT/BNT-84	Prime	16.1 (9.3 to 25.2)	93	Boost	15.1 (8.5 to 24.0)	93	0.92 (0.41, 2.05)
Erythema	BNT/BNT-84	Prime	7.5 (3.1 to 14.9)	93	Boost	7.5 (3.1 to 14.9)	93	1.00 (0.33, 3.05)
Itch	BNT/BNT-84	Prime	4.3 (1.2 to 10.6)	93	Boost	7.5 (3.1 to 14.9)	93	1.87 (0.51, 6.81)
Pain	BNT/BNT-84	Prime	69.9 (59.5 to 79.0)	93	Boost	78.5 (68.8 to 86.3)	93	1.64 (0.82, 3.27)
Swelling	BNT/ChAd-84	Prime	6.7 (2.5 to 14.1)	89	Boost	3.4 (0.7 to 9.6)	88	0.49 (0.12, 2.04)
Induration	BNT/ChAd-84	Prime	6.7 (2.5 to 14.1)	89	Boost	9.1 (4.0 to 17.1)	88	1.40 (0.46, 4.25)
Warmth	BNT/ChAd-84	Prime	13.5 (7.2 to 22.4)	89	Boost	22.7 (14.5 to 32.9)	88	1.98 (0.87, 4.49)
Erythema	BNT/ChAd-84	Prime	7.9 (3.2 to 15.5)	89	Boost	8.0 (3.3 to 15.7)	88	1.02 (0.34, 3.10)
Itch	BNT/ChAd-84	Prime	4.5 (1.2 to 11.1)	89	Boost	10.2 (4.8 to 18.5)	88	2.52 (0.73, 8.69)
Pain	BNT/ChAd-84	Prime	70.8 (60.2 to 79.9)	89	Boost	64.8 (53.9 to 74.7)	88	0.73 (0.38, 1.41)

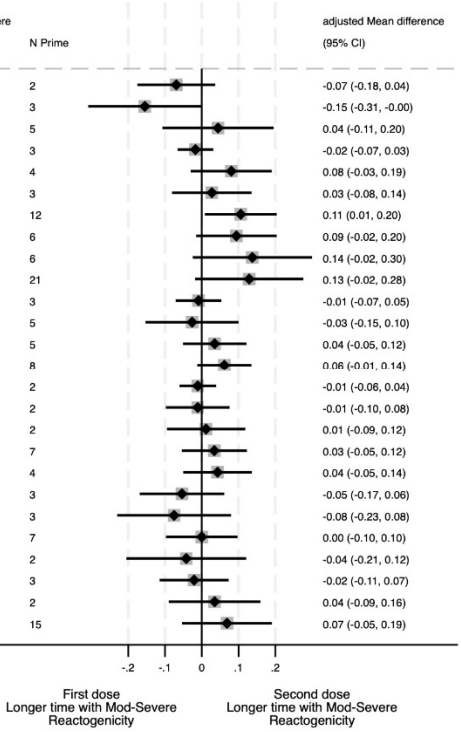
Supplementary Figure 4 *– Forest plot comparing presence/absence of local reactogenicity symptoms in the first 7 days after the first vs second COVID-19 vaccination . "Were you more likely to be symptomatic for your first or second dose?"*

Symptom	Vaccine schedule	Vaccine	Percentage with symptoms (95% CI)	N Boost	Vaccine	Percentage with symptoms (95% CI)	N Prime	aOR (95% CI)
Erythema	ChAd/ChAd-28	Prime	33.3 (11.8 to 61.6)	15	Boost	35.7 (12.8 to 64.9)	14	1.11 (0.24, 5.14)
Pain	ChAd/ChAd-28	Prime	6.6 (2.2 to 14.7)	76	Boost	10.7 (4.0 to 21.9)	56	1.70 (0.49, 5.89)
Erythema	ChAd/BNT-28	Prime	18.2 (2.3 to 51.8)	11	Boost	33.3 (7.5 to 70.1)	9	2.25 (0.29, 17.76)
Pain	ChAd/BNT-28	Prime	6.2 (2.1 to 14.0)	80	Boost	15.9 (9.0 to 25.2)	88	2.84 (0.97, 8.28)
Warmth	BNT/BNT-28	Prime	14.3 (1.8 to 42.8)	14	Boost	10.0 (1.2 to 31.7)	20	0.67 (0.08, 5.40)
Erythema	BNT/BNT-28	Prime	37.5 (8.5 to 75.5)	8	Boost	54.5 (23.4 to 83.3)	11	2.00 (0.31, 12.84)
Pain	BNT/BNT-28	Prime	15.6 (8.8 to 24.7)	90	Boost	23.2 (15.1 to 32.9)	95	1.64 (0.78, 3.44)
Erythema	BNT/ChAd-28	Prime	40.0 (5.3 to 85.3)	5	Boost	50.0 (18.7 to 81.3)	10	1.50 (0.17, 13.23)
Pain	BNT/ChAd-28	Prime	3.5 (0.7 to 10.0)	85	Boost	12.2 (5.7 to 21.8)	74	3.78 (0.98, 14.55)
Warmth	ChAd/ChAd-84	Prime	5.6 (0.1 to 27.3)	18	Boost	26.7 (7.8 to 55.1)	15	6.18 (0.61, 62.83)
Erythema	ChAd/ChAd-84	Prime	9.1 (0.2 to 41.3)	11	Boost	33.3 (7.5 to 70.1)	9	5.00 (0.42, 59.66)
Pain	ChAd/ChAd-84	Prime	10.8 (4.4 to 20.9)	65	Boost	10.0 (2.8 to 23.7)	40	0.92 (0.25, 3.37)
Warmth	ChAd/BNT-84	Prime	13.3 (1.7 to 40.5)	15	Boost	18.8 (4.0 to 45.6)	16	1.50 (0.21, 10.52)
Erythema	ChAd/BNT-84	Prime	30.0 (6.7 to 65.2)	10	Boost	33.3 (4.3 to 77.7)	6	1.17 (0.13, 10.22)
Pain	ChAd/BNT-84	Prime	8.6 (2.9 to 19.0)	58	Boost	12.5 (5.9 to 22.4)	72	1.51 (0.48, 4.80)
Erythema	BNT/BNT-84	Prime	57.1 (18.4 to 90.1)	7	Boost	42.9 (9.9 to 81.6)	7	0.56 (0.07, 4.67)
Pain	BNT/BNT-84	Prime	9.2 (3.5 to 19.0)	65	Boost	9.6 (3.9 to 18.8)	73	1.04 (0.33, 3.28)
Warmth	BNT/ChAd-84	Prime	8.3 (0.2 to 38.5)	12	Boost	20.0 (5.7 to 43.7)	20	2.75 (0.27, 28.04)
Erythema	BNT/ChAd-84	Prime	42.9 (9.9 to 81.6)	7	Boost	28.6 (3.7 to 71.0)	7	0.53 (0.06, 4.91)
Pain	BNT/ChAd-84	Prime	17.5 (9.1 to 29.1)	63	Boost	31.6 (19.9 to 45.2)	57	2.18 (0.93, 5.14)

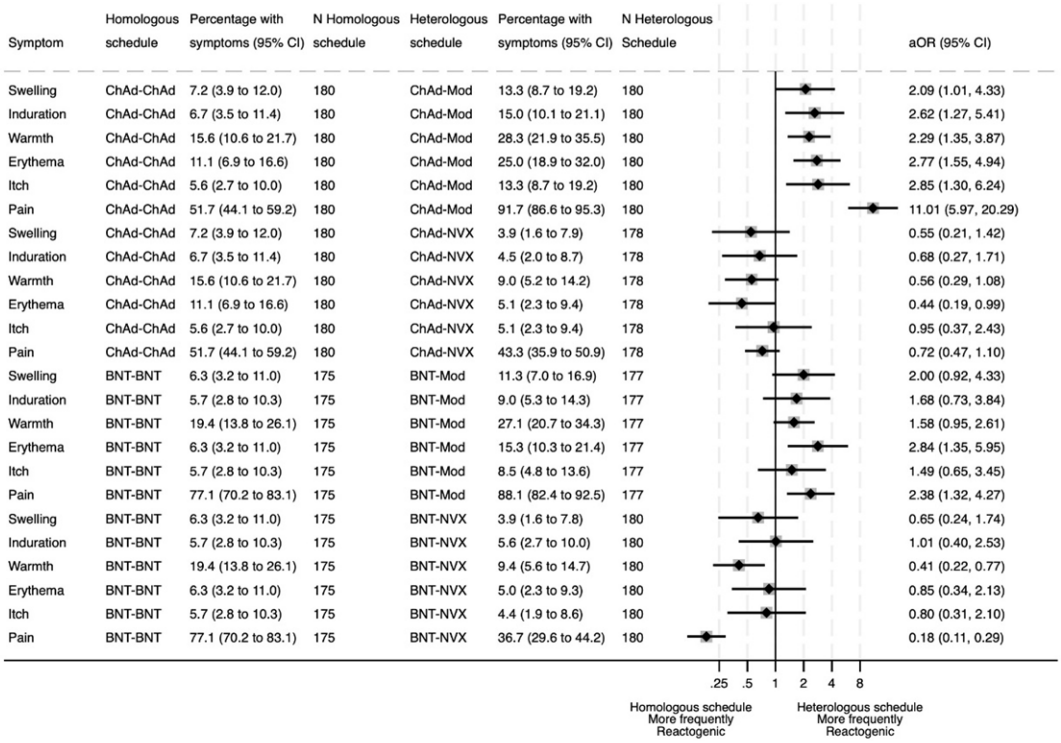


Supplementary Figure 5 *– Forest plot comparing the proportions of seronegative vs seropositive participants suffering moderate-severe (Grade 2 or above) local reactogenicity symptoms out of the total number of participants suffering any grade symptoms, in the first 7 days after a COVID-19 vaccination for first vs second doses. "If you did suffer a symptom of any grade after a vaccine dose, was it more likely to be moderate/severe (Grade 2 or above) if you it was you first or second dose?"*

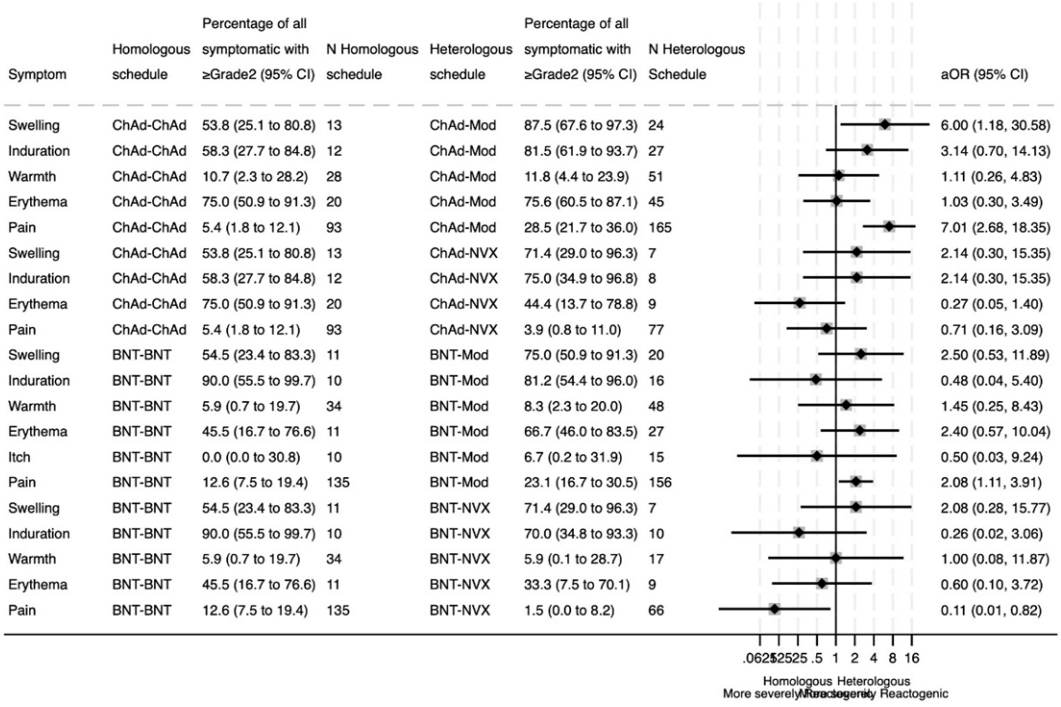
Symptom	Vaccine schedule	Vaccine	Days with moderate-severe symptoms (95% CI)	N Boost	Vaccine	Days with moderate-severe symptoms (95% CI)	N Prime	adjusted Mean difference (95% CI)
Swelling	ChAd/ChAd-28	Prime	2.50 (-0.55 to 5.55)	4	Boost	1.00 (1.00 to 1.00)	2	-0.07 (-0.18, 0.04)
Induration	ChAd/ChAd-28	Prime	2.88 (1.93 to 3.82)	8	Boost	1.67 (0.23 to 3.10)	3	-0.15 (-0.31, -0.00)
Erythema	ChAd/ChAd-28	Prime	1.40 (0.72 to 2.08)	5	Boost	2.40 (-1.49 to 6.29)	5	0.04 (-0.11, 0.20)
Pain	ChAd/ChAd-28	Prime	1.00 (1.00 to 1.00)	5	Boost	1.00 (1.00 to 1.00)	3	-0.02 (-0.07, 0.03)
Induration	ChAd/BNT-28	Prime	1.00 (1.00 to 1.00)	2	Boost	2.75 (0.03 to 5.47)	4	0.08 (-0.03, 0.19)
Erythema	ChAd/BNT-28	Prime	2.50 (-3.85 to 8.85)	2	Boost	2.67 (-1.13 to 6.46)	3	0.03 (-0.08, 0.14)
Pain	ChAd/BNT-28	Prime	1.00 (1.00 to 1.00)	5	Boost	1.42 (0.99 to 1.84)	12	0.11 (0.01, 0.20)
Induration	BNT/BNT-28	Prime	1.50 (-4.85 to 7.85)	2	Boost	2.33 (1.06 to 3.60)	6	0.09 (-0.02, 0.20)
Erythema	BNT/BNT-28	Prime	1.67 (-1.20 to 4.54)	3	Boost	3.50 (1.65 to 5.35)	6	0.14 (-0.02, 0.30)
Pain	BNT/BNT-28	Prime	1.46 (1.06 to 1.86)	13	Boost	1.62 (1.31 to 1.92)	21	0.13 (-0.02, 0.28)
Swelling	BNT/ChAd-28	Prime	2.00 (-10.71 to 14.71)	2	Boost	1.00 (1.00 to 1.00)	3	-0.01 (-0.07, 0.05)
Induration	BNT/ChAd-28	Prime	1.86 (1.22 to 2.50)	7	Boost	2.00 (0.24 to 3.76)	5	-0.03 (-0.15, 0.10)
Erythema	BNT/ChAd-28	Prime	2.00 (-10.71 to 14.71)	2	Boost	1.60 (0.49 to 2.71)	5	0.04 (-0.05, 0.12)
Pain	BNT/ChAd-28	Prime	1.00 (1.00 to 1.00)	3	Boost	1.25 (0.66 to 1.84)	8	0.06 (-0.01, 0.14)
Swelling	ChAd/BNT-84	Prime	1.00 (1.00 to 1.00)	3	Boost	1.00 (1.00 to 1.00)	2	-0.01 (-0.06, 0.04)
Induration	ChAd/BNT-84	Prime	2.00 (-10.71 to 14.71)	2	Boost	1.50 (-4.85 to 7.85)	2	-0.01 (-0.10, 0.08)
Erythema	ChAd/BNT-84	Prime	1.33 (-0.10 to 2.77)	3	Boost	2.50 (-16.56 to 21.56)	2	0.01 (-0.09, 0.12)
Pain	ChAd/BNT-84	Prime	1.25 (0.45 to 2.05)	4	Boost	1.14 (0.79 to 1.49)	7	0.03 (-0.05, 0.12)
Swelling	BNT/BNT-84	Prime	1.50 (-4.85 to 7.85)	2	Boost	1.75 (0.23 to 3.27)	4	0.04 (-0.05, 0.14)
Induration	BNT/BNT-84	Prime	2.67 (-2.50 to 7.84)	3	Boost	1.00 (1.00 to 1.00)	3	-0.05 (-0.17, 0.06)
Erythema	BNT/BNT-84	Prime	3.00 (-1.50 to 7.50)	4	Boost	1.67 (0.23 to 3.10)	3	-0.08 (-0.23, 0.08)
Pain	BNT/BNT-84	Prime	1.33 (0.79 to 1.88)	6	Boost	1.14 (0.79 to 1.49)	7	0.00 (-0.10, 0.10)
Swelling	BNT/ChAd-84	Prime	2.50 (0.91 to 4.09)	4	Boost	3.00 (-22.41 to 28.41)	2	-0.04 (-0.21, 0.12)
Induration	BNT/ChAd-84	Prime	1.50 (-0.09 to 3.09)	4	Boost	1.33 (-0.10 to 2.77)	3	-0.02 (-0.11, 0.07)
Erythema	BNT/ChAd-84	Prime	1.33 (-0.10 to 2.77)	3	Boost	3.50 (-2.85 to 9.85)	2	0.04 (-0.09, 0.16)
Pain	BNT/ChAd-84	Prime	1.10 (0.87 to 1.33)	10	Boost	1.13 (0.94 to 1.33)	15	0.07 (-0.05, 0.19)



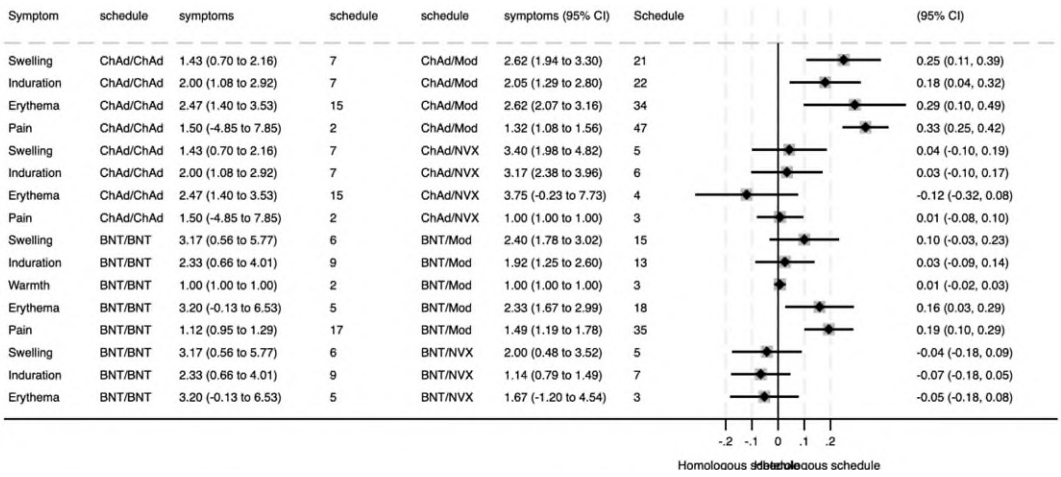
Supplementary Figure 6 *– Forest plot comparing durations of time spent with grade 2 or higher local reactogenicity symptoms after a COVID-19 vaccination between first and second dose . "If you did suffer with moderate/severe symptoms (Grade 2 or above), were the symptoms likely to last longer after a single dose of either ChAd or BNT"*



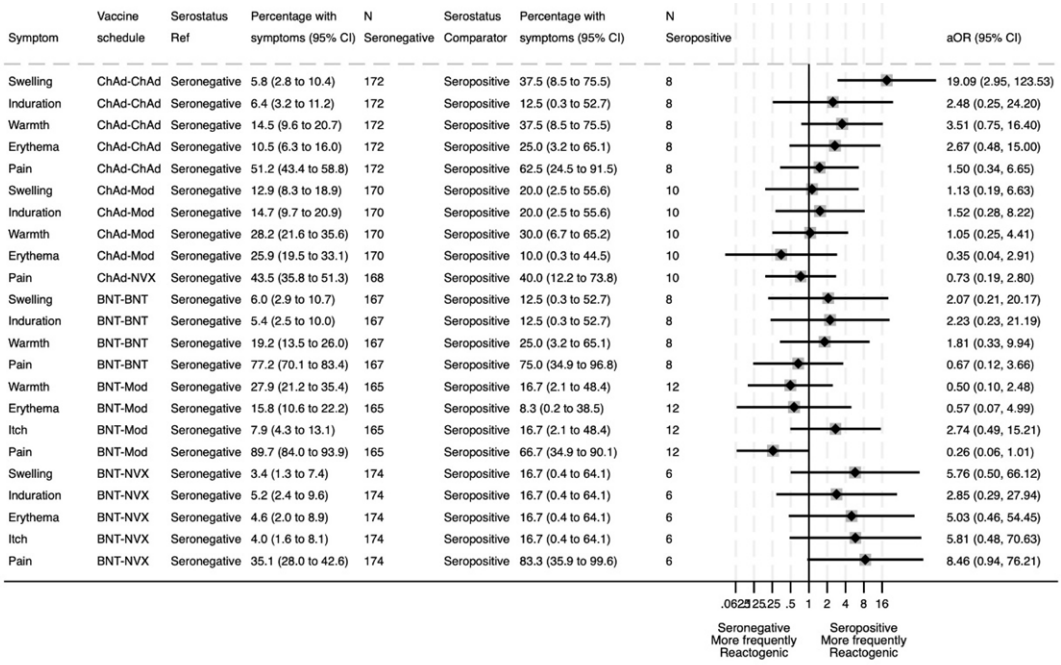
Supplementary Figure 7 *– Forest plot comparing the proportions of participants (seronegative and seropositive combined) suffering local reactogenicity symptoms in the first 7 days after the second COVID-19 vaccination between homologous and heterologous schedules "Were you more likely to suffer a local symptom of any grade after the second dose if the schedule was homologous or heterologous?"*



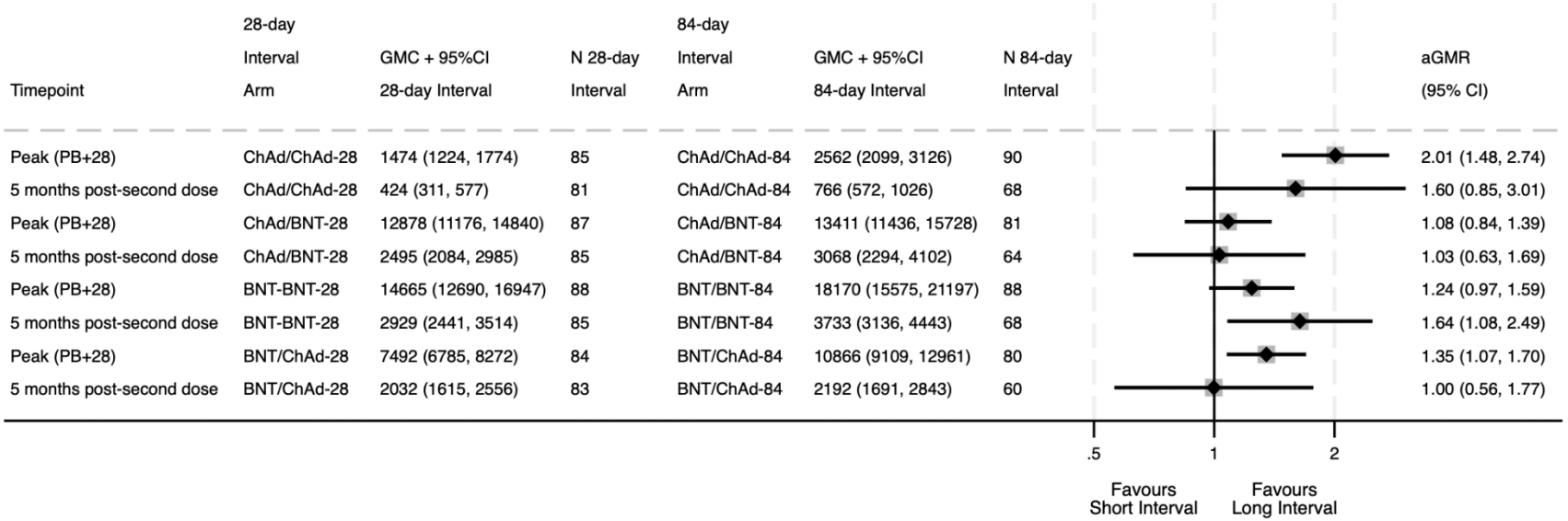
Supplementary Figure 8 *– Forest plot comparing proportions of participants (seronegative and seropositive combined) suffering moderate-severe (Grade 2 or above) local reactogenicity symptoms out of the total number of participants suffering any grade symptoms, in the first 7 days after a second COVID-19 vaccination between a particular homologous and relative heterologous schedule. "If you did suffer a symptom of any grade after the second dose, was it more likely to be moderate/severe (Grade 2 or above) if the schedule was homologous or heterolgous?"*



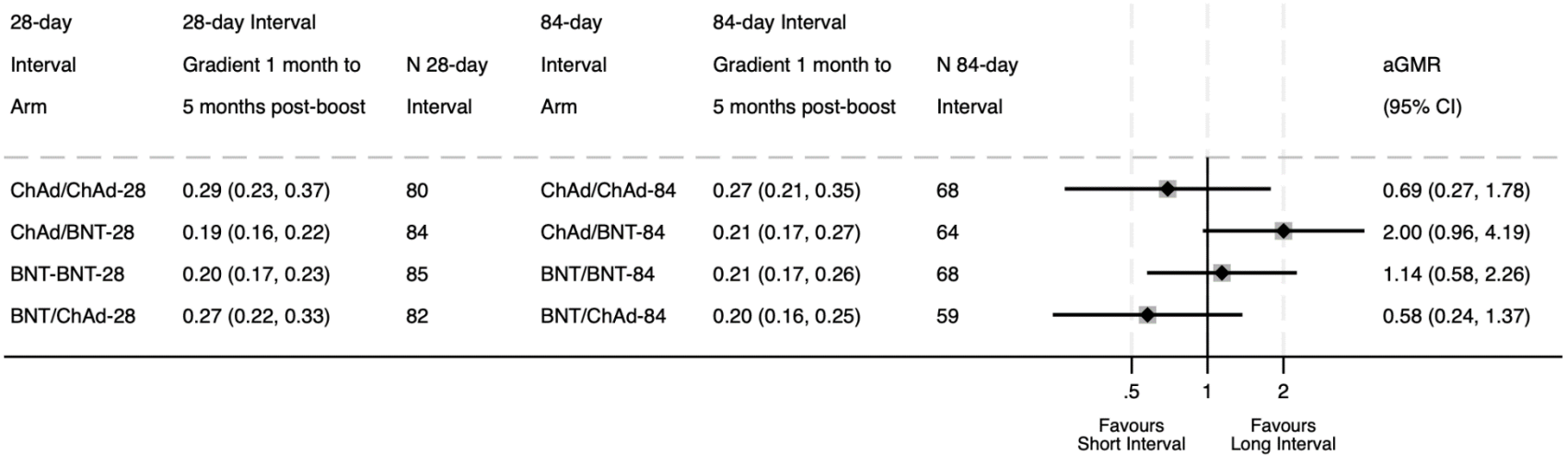
Supplementary Figure 9 *– Forest plot comparing durations of time spent with grade 2 or higher local reactogenicity symptoms after the second COVID-19 vaccination between participants (seronegative and seropositive combined) receiving homologous or heterologous schedules . "If you did suffer with moderate/severe symptoms (Grade 2 or above) after the second dose, were the symptoms likely to last longer after a homologous or heterologous schedule?"*



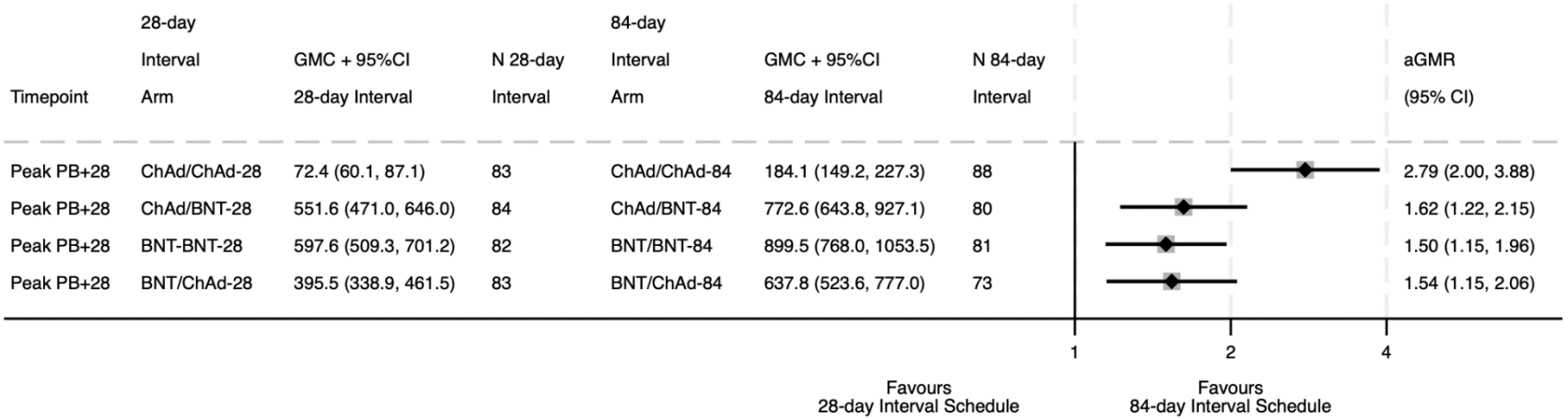
Supplementary Figure 10 *– Forest plot comparing the proportions of seronegative vs seropositive participants for each schedule suffering local reactogenicity symptoms in the first 7 days after the second COVID-19 vaccination "Were you more likely to suffer a symptom of any grade after the second dose if you were seropositive or seronegative at the point of second dose administration?"*



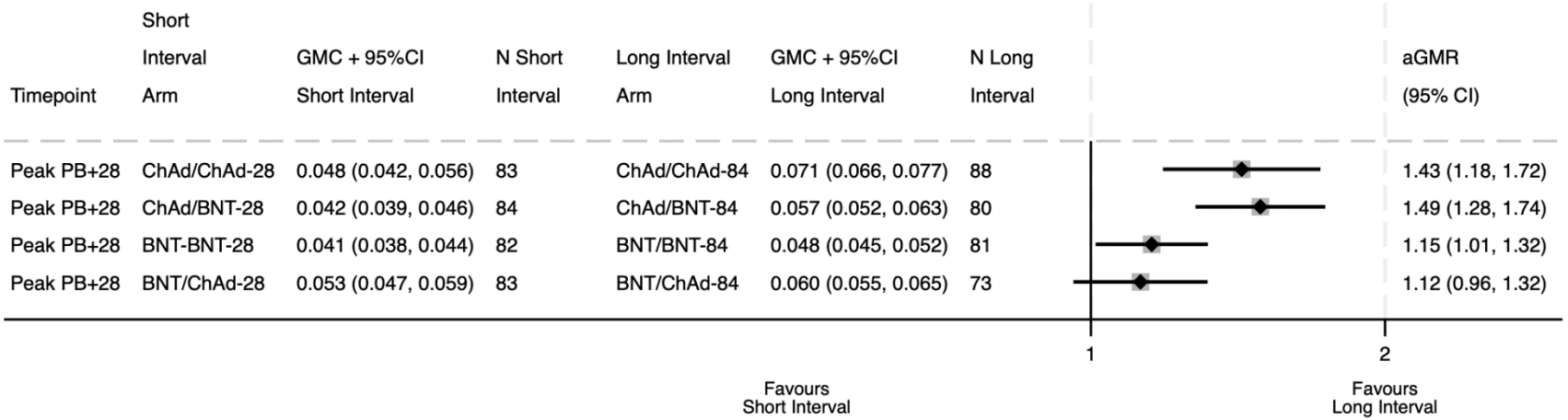
Supplementary Figure 11 *– Forest plot paracetamol sensitivity analysis comparing peak (28 days post second dose) and 5-months post second dose antibody responses between 4-week and 12-week interval schedules . Peak antibody response for the 4 week schedules was at 56 days, whilst for 12-week this was at 112 days. The 5-month timepoint was at day 182 for 4-week schedules, and at day 255 for the 12-week schedules. Left sided columns represent the raw, unadjusted GMC ±95% CI. The forest plot represents the right sided aGMR derived from a multivariate linear regression.*



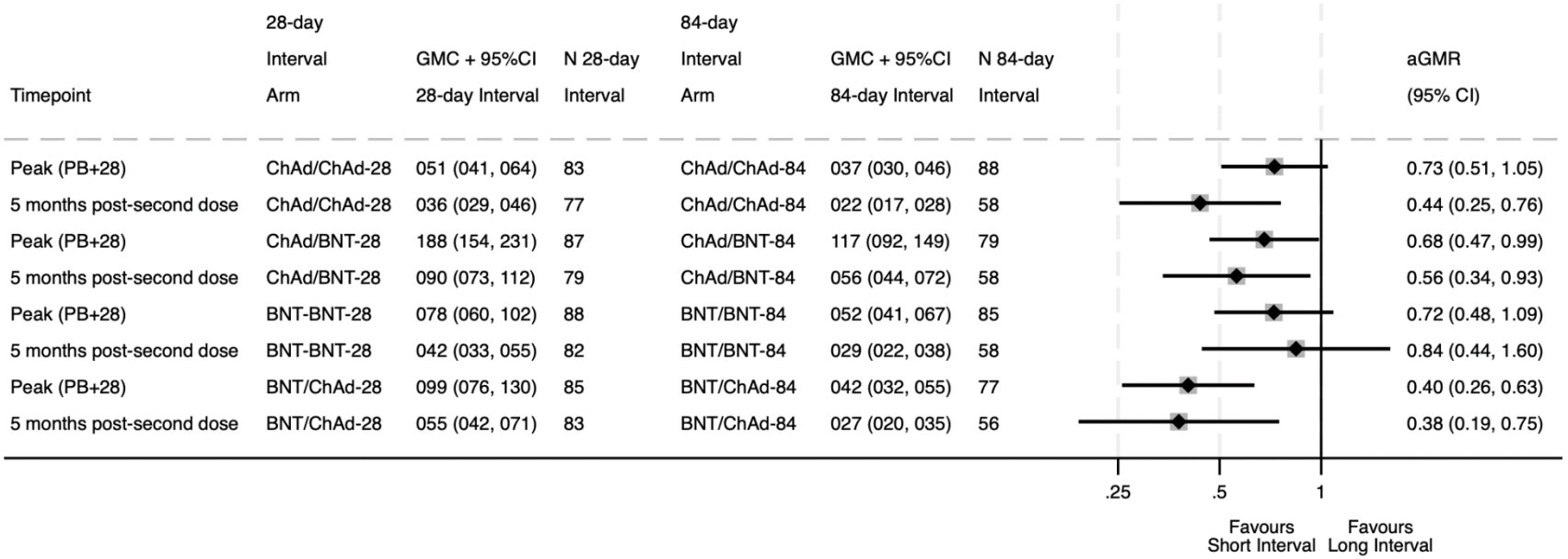
Supplementary Figure 12 *– Forest plot paracetamol sensitivity analysis comparing rates of antibody response wane between 4-week and 12-week interval schedules . Left sided columns represent the raw, unadjusted GMC ± 95% CI of the ratio of the 5-month antibody level over the peak antibody level. The forest plot represents the right sided aGMR derived from a multivariate linear regression.*



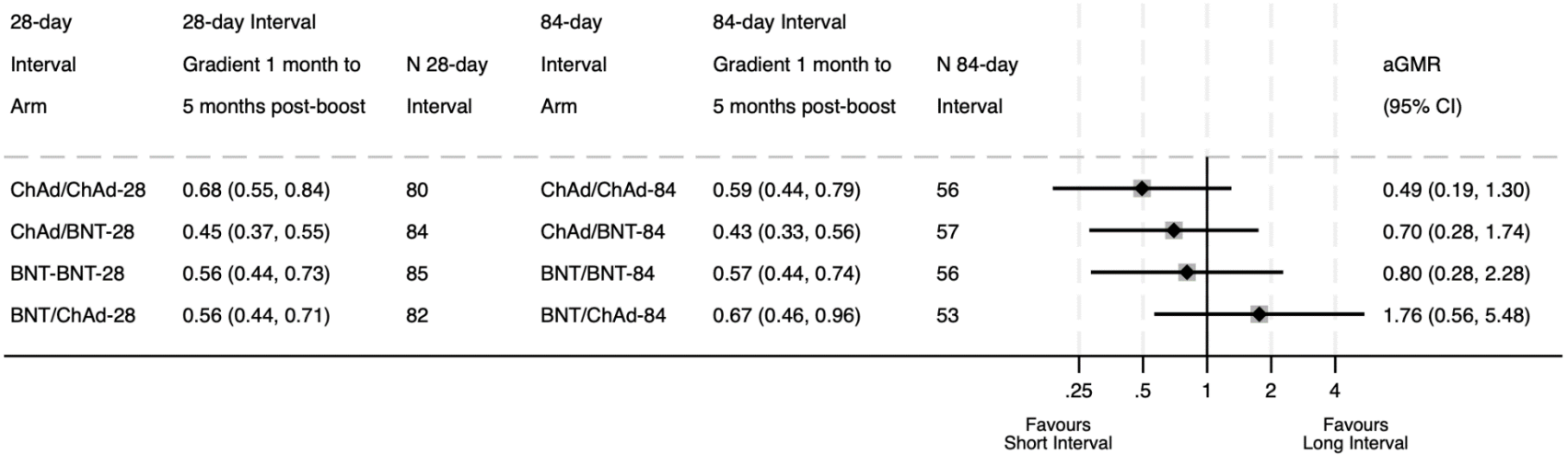
Supplementary Figure 13 *– Forest plot paracetamol sensitivity analysis comparing peak (28 days post second dose) pseudotype virus neutralisation 50 titres between 4-week and 12-week interval schedules . Left sided columns represent the raw, unadjusted GMC ± 95% CI. The forest plot represents the right sided aGMR derived from a multivariate linear regression.*



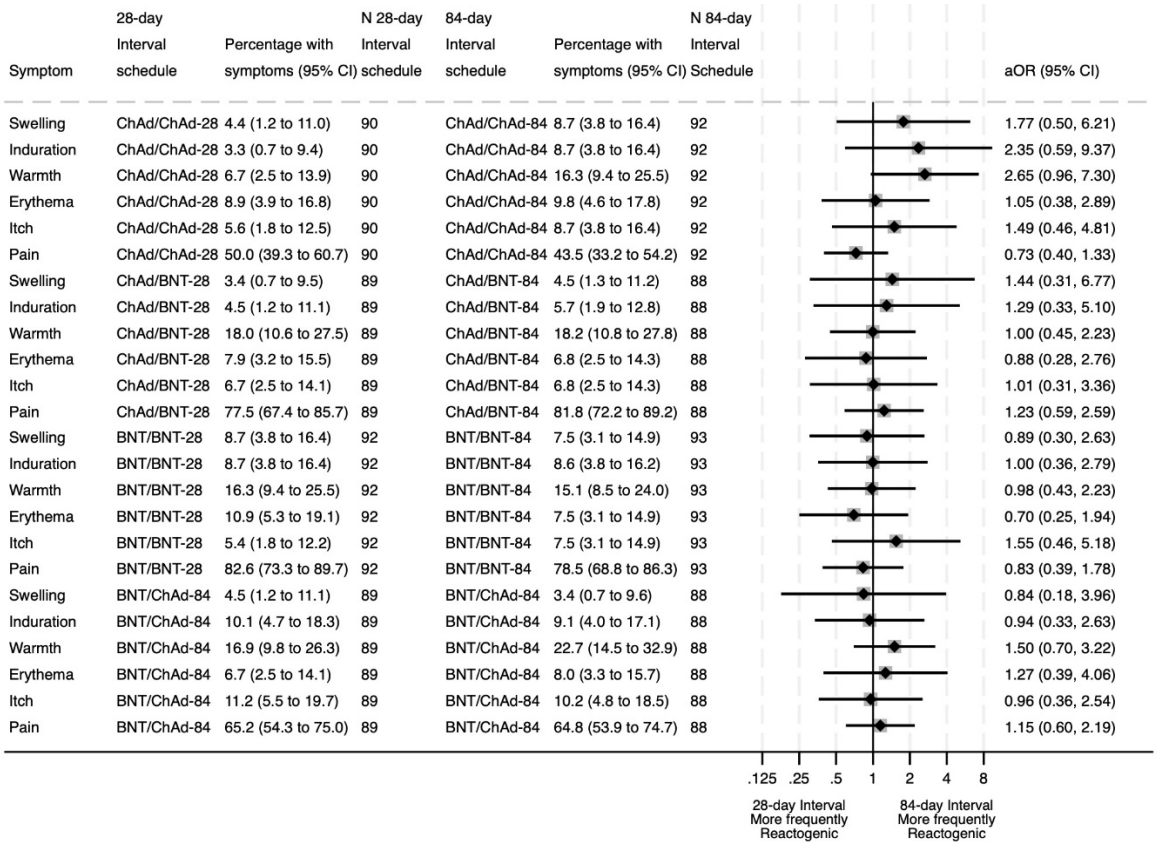
Supplementary Figure 14 *– Forest plot paracetamol sensitivity analysis comparing the ratio between pseudotype virus neutralisation 50 titre and anti-SARS-CoV2 spike IgG at peak timepoint (28 days post second dose) between 4-week and 12-week interval schedules . Left sided columns represent the raw, unadjusted GMC ± 95% CI. The forest plot represents the right sided aGMR derived from a multivariate linear regression.*



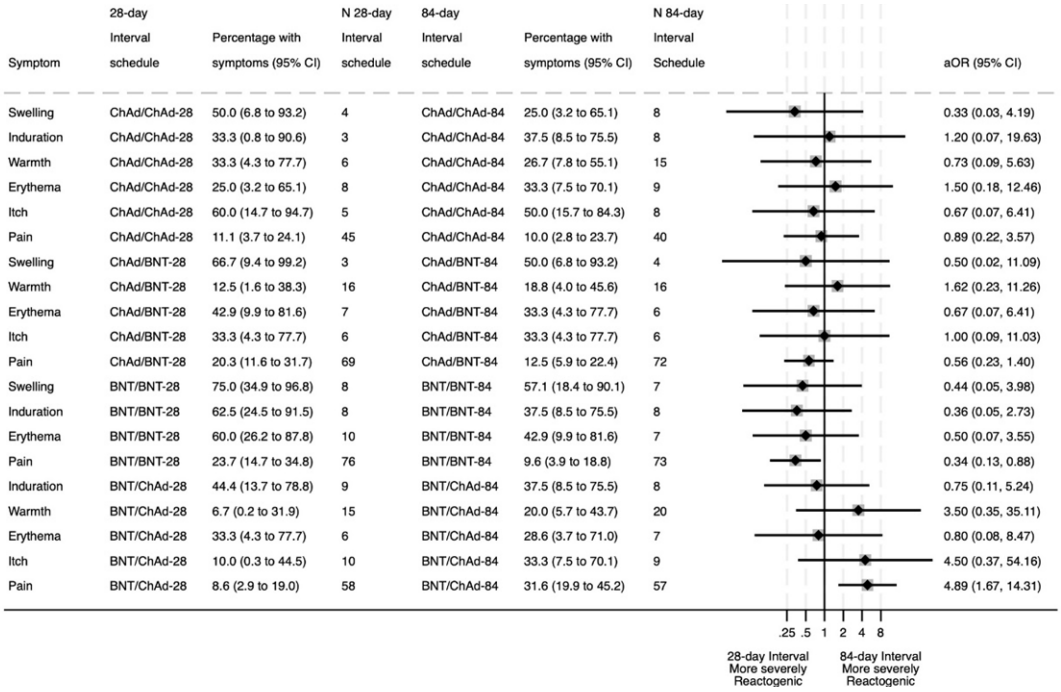
Supplementary Figure 15 *– Forest plot paracetamol sensitivity analysis comparing peak (28 days post second dose) and 5-months post second dose IFNγ-SFC between 4-week and 12-week interval schedules . Peak response for the 4 week schedules was at 56 days, whilst for 12-week this was at 112 days. The 5-month timepoint was at day 182 for 4-week schedules, and at day 255 for the 12-week schedules. Left sided columns represent the raw, unadjusted GMC with 95% confidence intervals. The forest plot represents the right sided aGMR derived from a multivariate linear regression.*



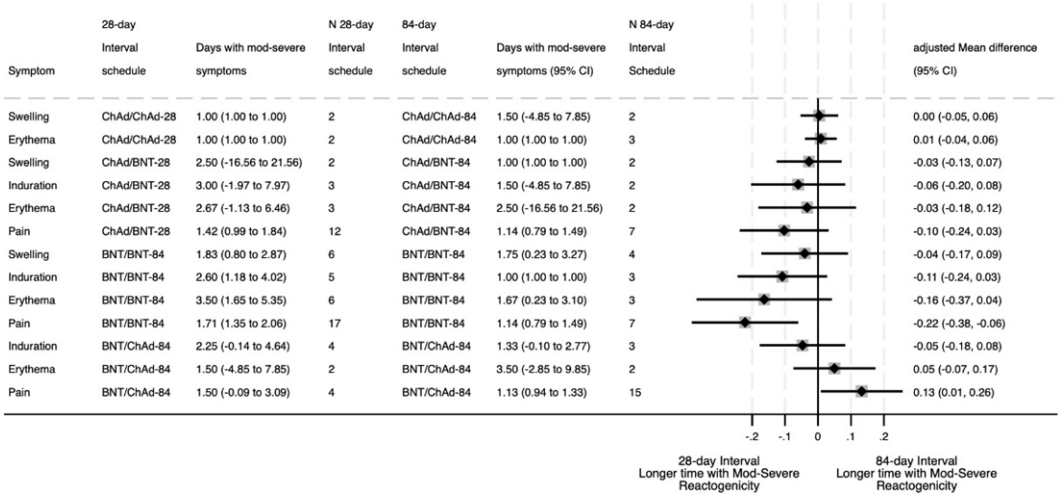
Supplementary Figure 16 *– Forest plot paracetamol sensitivity analysis comparing rates of IFNγ-SFC wane between short and relative long interval schedules. Left sided columns represent the raw, unadjusted GMC ± 95% CI of the ratio of the 5-month antibody level over the peak T-cell response. The forest plot represents the right sided aGMR derived from a multivariate linear regression*



Supplementary Figure 17 *– Forest plot comparing the proportions of participants (seronegative and seropositive combined) suffering local reactogenicity symptoms in the first 7 days after the second COVID-19 vaccination between 4-week and 12-week interval schedules "Were you more likely to suffer a symptom of any grade after the second dose if the schedule had a short or long interval?"*

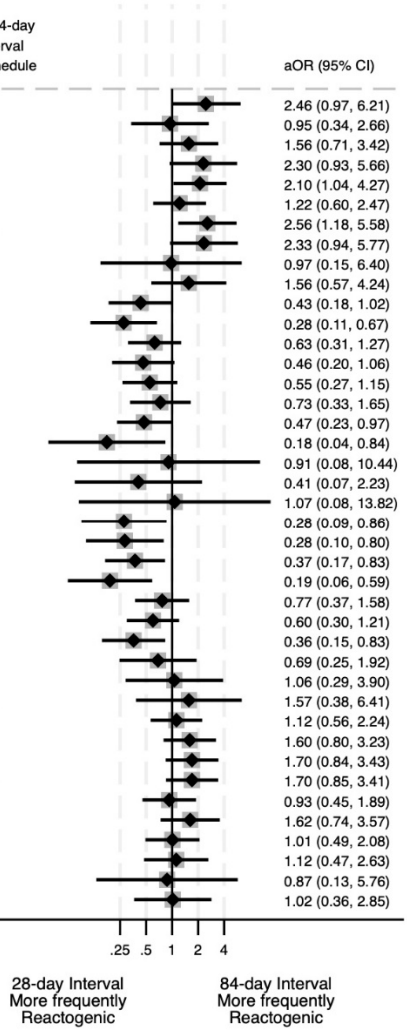


Supplementary Figure 18 *– Forest plot comparing proportions of participants (seronegative and seropositive combined) suffering moderate-severe (Grade 2 or above) local reactogenicity symptoms out of the total number of participants suffering any grade symptoms, in the first 7 days after a second COVID-19 vaccination between a particular short and relative long interval schedule. "If you did suffer a symptom of any grade after the second dose, was it more likely to be moderate/severe if the schedule had a short or long interval?"*

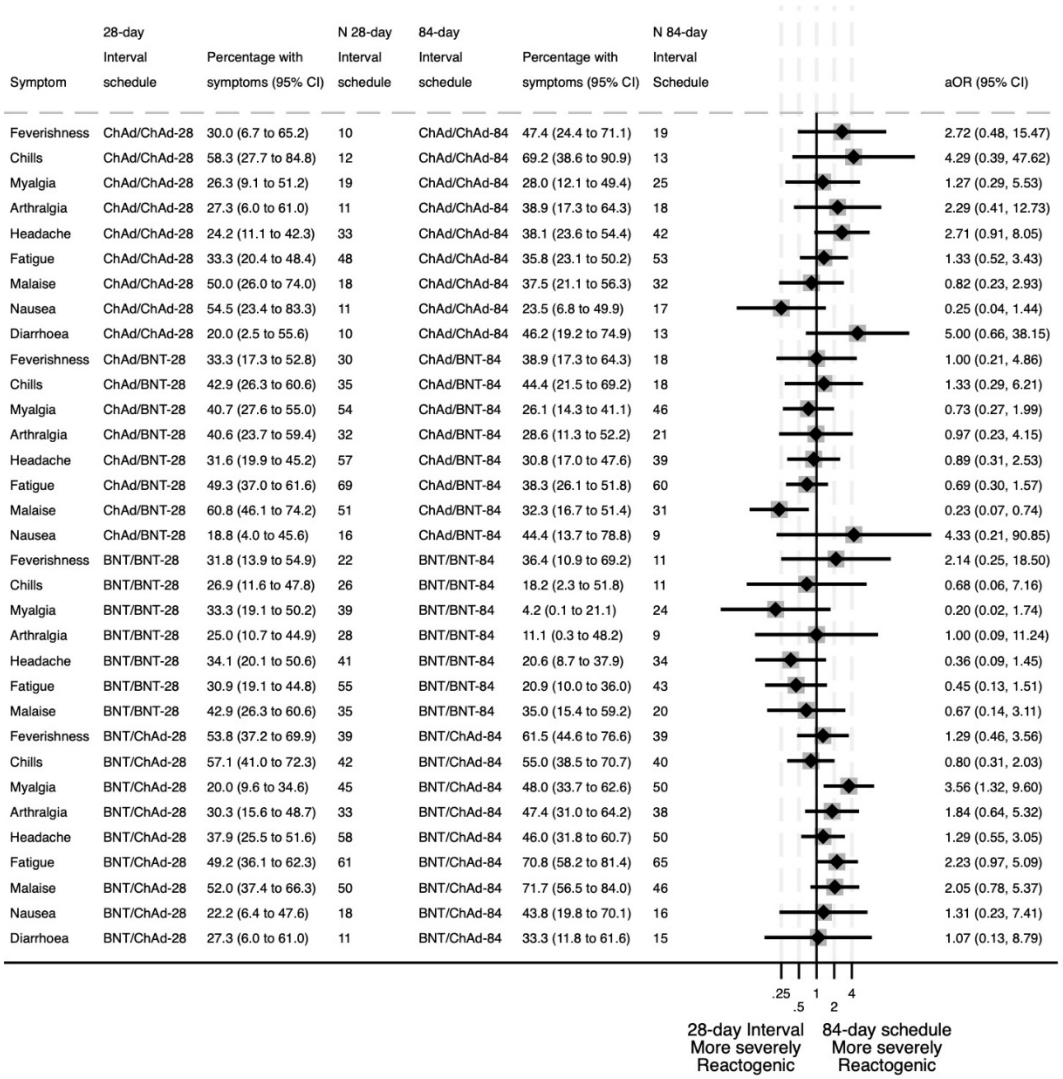


Supplementary Figure 19 *– Forest plot comparing durations of time spent with grade 2 or higher local reactogenicity symptoms after the second COVID-19 vaccination between participants (seronegative and seropositive combined) receiving schedules with either 4-week or 12-week intervals . "If you did suffer with moderate/severe symptoms after the second dose, were the symptoms likely to last longer after a short or long interval schedule?"*

Symptom	28-day Interval schedule	Percentage with symptoms (95% CI)	N 28-day Interval schedule	84-day Interval schedule	Percentage with symptoms (95% CI)	N 84-day Interval Schedule	aOR (95% CI)
Feverishness	ChAd/ChAd-28	11.1 (5.5 to 19.5)	90	ChAd/ChAd-84	20.7 (12.9 to 30.4)	92	2.46 (0.97, 6.21)
Chills	ChAd/ChAd-28	13.3 (7.1 to 22.1)	90	ChAd/ChAd-84	14.1 (7.7 to 23.0)	92	0.95 (0.34, 2.66)
Myalgia	ChAd/ChAd-28	21.1 (13.2 to 31.0)	90	ChAd/ChAd-84	27.2 (18.4 to 37.4)	92	1.56 (0.71, 3.42)
Arthralgia	ChAd/ChAd-28	12.2 (6.3 to 20.8)	90	ChAd/ChAd-84	19.6 (12.0 to 29.1)	92	2.30 (0.93, 5.66)
Headache	ChAd/ChAd-28	36.7 (26.8 to 47.5)	90	ChAd/ChAd-84	45.7 (35.2 to 56.4)	92	2.10 (1.04, 4.27)
Fatigue	ChAd/ChAd-28	53.3 (42.5 to 63.9)	90	ChAd/ChAd-84	57.6 (46.9 to 67.9)	92	1.22 (0.60, 2.47)
Malaise	ChAd/ChAd-28	20.0 (12.3 to 29.8)	90	ChAd/ChAd-84	34.8 (25.1 to 45.4)	92	2.56 (1.18, 5.58)
Nausea	ChAd/ChAd-28	12.2 (6.3 to 20.8)	90	ChAd/ChAd-84	18.5 (11.1 to 27.9)	92	2.33 (0.94, 5.77)
Vomiting	ChAd/ChAd-28	3.3 (0.7 to 9.4)	90	ChAd/ChAd-84	3.3 (0.7 to 9.2)	92	0.97 (0.15, 6.40)
Diarrhoea	ChAd/ChAd-28	11.1 (5.5 to 19.5)	90	ChAd/ChAd-84	14.1 (7.7 to 23.0)	92	1.56 (0.57, 4.24)
Feverishness	ChAd/BNT-28	33.7 (24.0 to 44.5)	89	ChAd/BNT-84	20.5 (12.6 to 30.4)	88	0.43 (0.18, 1.02)
Chills	ChAd/BNT-28	39.3 (29.1 to 50.3)	89	ChAd/BNT-84	20.5 (12.6 to 30.4)	88	0.28 (0.11, 0.67)
Myalgia	ChAd/BNT-28	60.7 (49.7 to 70.9)	89	ChAd/BNT-84	52.3 (41.4 to 63.0)	88	0.63 (0.31, 1.27)
Arthralgia	ChAd/BNT-28	36.0 (26.1 to 46.8)	89	ChAd/BNT-84	23.9 (15.4 to 34.1)	88	0.46 (0.20, 1.06)
Headache	ChAd/BNT-28	64.0 (53.2 to 73.9)	89	ChAd/BNT-84	44.3 (33.7 to 55.3)	88	0.55 (0.27, 1.15)
Fatigue	ChAd/BNT-28	77.5 (67.4 to 85.7)	89	ChAd/BNT-84	68.2 (57.4 to 77.7)	88	0.73 (0.33, 1.65)
Malaise	ChAd/BNT-28	57.3 (46.4 to 67.7)	89	ChAd/BNT-84	35.2 (25.3 to 46.1)	88	0.47 (0.23, 0.97)
Nausea	ChAd/BNT-28	18.0 (10.6 to 27.5)	89	ChAd/BNT-84	10.2 (4.8 to 18.5)	88	0.18 (0.04, 0.84)
Vomiting	ChAd/BNT-28	2.2 (0.3 to 7.9)	89	ChAd/BNT-84	3.4 (0.7 to 9.6)	88	0.91 (0.08, 10.44)
Diarrhoea	ChAd/BNT-28	7.9 (3.2 to 15.5)	89	ChAd/BNT-84	8.0 (3.3 to 15.7)	88	0.41 (0.07, 2.23)
Fever	BNT/BNT-28	2.2 (0.3 to 7.6)	92	BNT/BNT-84	2.2 (0.3 to 7.6)	93	1.07 (0.08, 13.82)
Feverishness	BNT/BNT-28	23.9 (15.6 to 33.9)	92	BNT/BNT-84	11.8 (6.1 to 20.2)	93	0.28 (0.09, 0.86)
Chills	BNT/BNT-28	28.3 (19.4 to 38.6)	92	BNT/BNT-84	11.8 (6.1 to 20.2)	93	0.28 (0.10, 0.80)
Myalgia	BNT/BNT-28	42.4 (32.1 to 53.1)	92	BNT/BNT-84	25.8 (17.3 to 35.9)	93	0.37 (0.17, 0.83)
Arthralgia	BNT/BNT-28	30.4 (21.3 to 40.9)	92	BNT/BNT-84	9.7 (4.5 to 17.6)	93	0.19 (0.06, 0.59)
Headache	BNT/BNT-28	44.6 (34.2 to 55.3)	92	BNT/BNT-84	36.6 (26.8 to 47.2)	93	0.77 (0.37, 1.58)
Fatigue	BNT/BNT-28	59.8 (49.0 to 69.9)	92	BNT/BNT-84	46.2 (35.8 to 56.9)	93	0.60 (0.30, 1.21)
Malaise	BNT/BNT-28	38.0 (28.1 to 48.8)	92	BNT/BNT-84	21.5 (13.7 to 31.2)	93	0.36 (0.15, 0.83)
Nausea	BNT/BNT-28	17.4 (10.3 to 26.7)	92	BNT/BNT-84	11.8 (6.1 to 20.2)	93	0.69 (0.25, 1.92)
Diarrhoea	BNT/BNT-28	7.6 (3.1 to 15.1)	92	BNT/BNT-84	7.5 (3.1 to 14.9)	93	1.06 (0.29, 3.90)
Fever	BNT/ChAd-84	5.6 (1.8 to 12.6)	89	BNT/ChAd-84	8.0 (3.3 to 15.7)	88	1.57 (0.38, 6.41)
Feverishness	BNT/ChAd-84	43.8 (33.3 to 54.7)	89	BNT/ChAd-84	44.3 (33.7 to 55.3)	88	1.12 (0.56, 2.24)
Chills	BNT/ChAd-84	47.2 (36.5 to 58.1)	89	BNT/ChAd-84	45.5 (34.8 to 56.4)	88	1.60 (0.80, 3.23)
Myalgia	BNT/ChAd-84	50.6 (39.8 to 61.3)	89	BNT/ChAd-84	56.8 (45.8 to 67.3)	88	1.70 (0.84, 3.43)
Arthralgia	BNT/ChAd-84	37.1 (27.1 to 48.0)	89	BNT/ChAd-84	43.2 (32.7 to 54.2)	88	1.70 (0.85, 3.41)
Headache	BNT/ChAd-84	65.2 (54.3 to 75.0)	89	BNT/ChAd-84	56.8 (45.8 to 67.3)	88	0.93 (0.45, 1.89)
Fatigue	BNT/ChAd-84	68.5 (57.8 to 78.0)	89	BNT/ChAd-84	73.9 (63.4 to 82.7)	88	1.62 (0.74, 3.57)
Malaise	BNT/ChAd-84	56.2 (45.3 to 66.7)	89	BNT/ChAd-84	52.3 (41.4 to 63.0)	88	1.01 (0.49, 2.08)
Nausea	BNT/ChAd-84	20.2 (12.4 to 30.1)	89	BNT/ChAd-84	18.2 (10.8 to 27.8)	88	1.12 (0.47, 2.63)
Vomiting	BNT/ChAd-84	3.4 (0.7 to 9.5)	89	BNT/ChAd-84	4.5 (1.3 to 11.2)	88	0.87 (0.13, 5.76)
Diarrhoea	BNT/ChAd-84	12.4 (6.3 to 21.0)	89	BNT/ChAd-84	17.0 (9.9 to 26.6)	88	1.02 (0.36, 2.85)

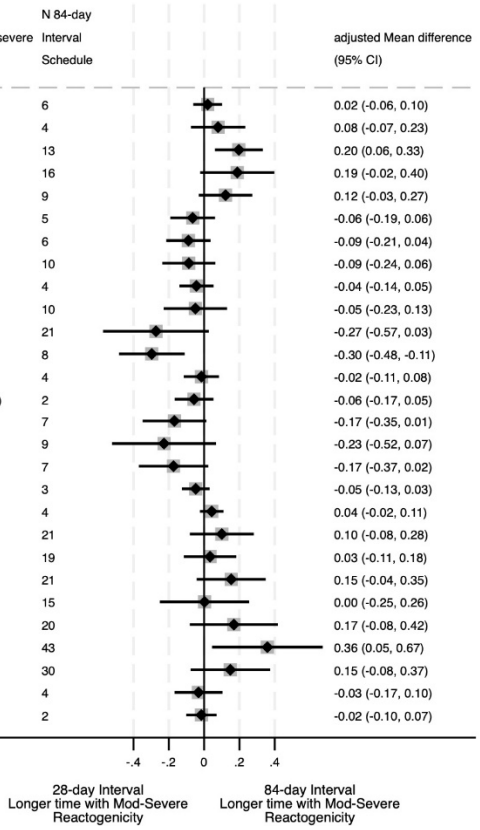


Supplementary Figure 20 *– Forest plot paracetamol sensitivity analysis comparing the proportions of participants (seronegative and seropositive combined) suffering systemic reactogenicity symptoms in the first 7 days after the second COVID-19 vaccination between 4-week and 12-week interval schedules "Were you more likely to suffer a symptom of any grade after the second dose if the schedule had a short or long interval?"*

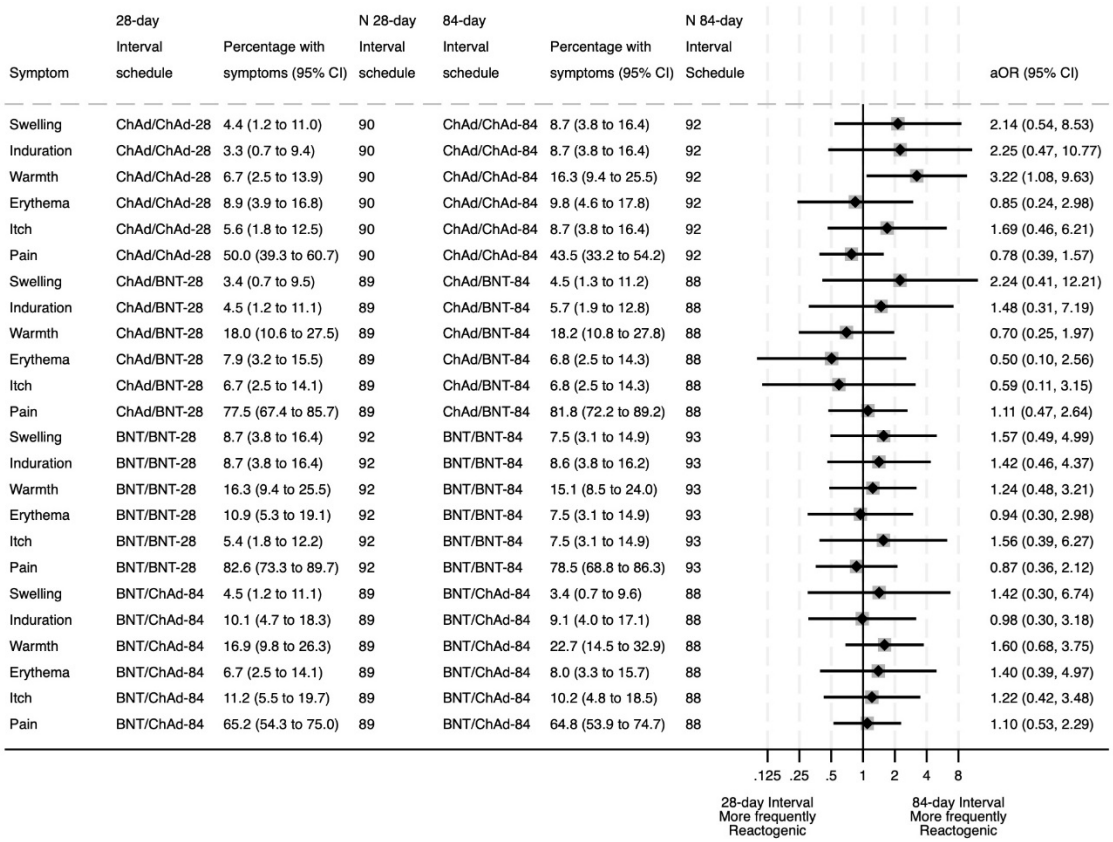


Supplementary Figure 21 *– Forest plot paracetamol sensitivity analysis comparing proportions of participants (both seronegative and seropositive combined) suffering moderate-severe (Grade 2 or above) systemic reactogenicity symptoms out of the total number of participants suffering any grade symptoms, in the first 7 days after a second COVID-19 vaccination between a 4-week and 12-week interval schedules. "If you did suffer a symptom of any grade after the second dose, was it more likely to be moderate/severe if the schedule had a short or long interval?"*

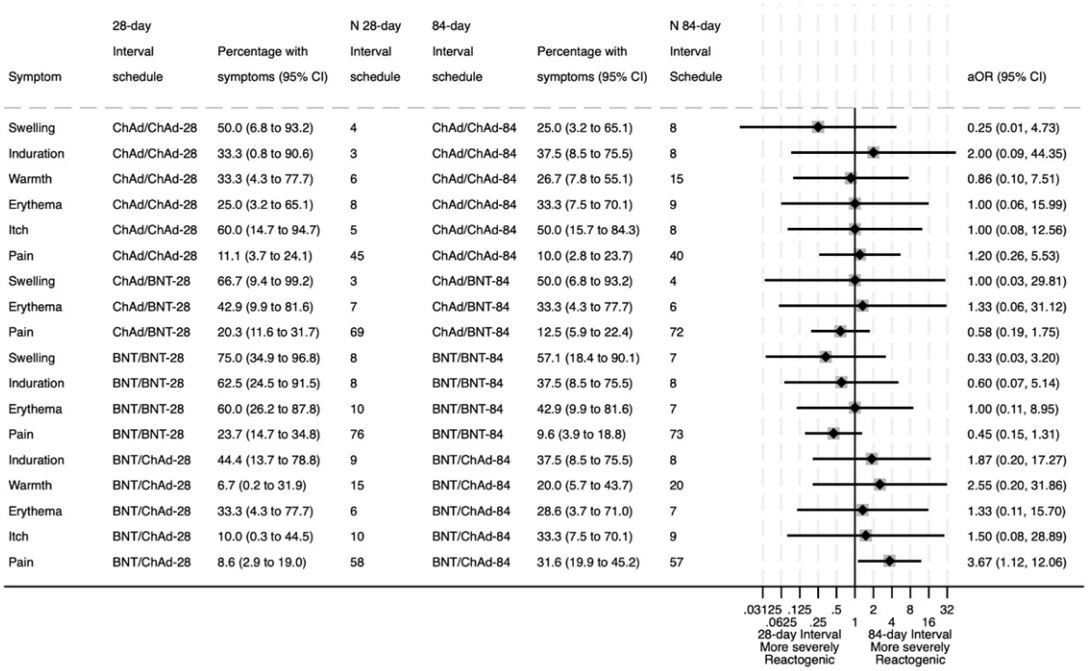
Symptom	28-day Interval schedule	Days with moderate-severe symptoms	N 28-day Interval schedule	84-day Interval schedule	Days with moderate-severe symptoms (95% CI)	N 84-day Interval Schedule	adjusted Mean difference (95% CI)
Chills	ChAd/ChAd-28	1.00 (1.00 to 1.00)	5	ChAd/ChAd-84	1.00 (1.00 to 1.00)	6	0.02 (-0.06, 0.10)
Myalgia	ChAd/ChAd-28	1.33 (-0.10 to 2.77)	3	ChAd/ChAd-84	2.25 (-0.76 to 5.26)	4	0.08 (-0.07, 0.23)
Headache	ChAd/ChAd-28	1.00 (1.00 to 1.00)	6	ChAd/ChAd-84	1.31 (0.93 to 1.69)	13	0.20 (0.06, 0.33)
Fatigue	ChAd/ChAd-28	1.14 (0.93 to 1.35)	14	ChAd/ChAd-84	1.69 (1.18 to 2.19)	16	0.19 (-0.02, 0.40)
Malaise	ChAd/ChAd-28	1.14 (0.79 to 1.49)	7	ChAd/ChAd-84	1.56 (0.88 to 2.23)	9	0.12 (-0.03, 0.27)
Feverishness	ChAd/BNT-28	1.38 (0.75 to 2.00)	8	ChAd/BNT-84	1.00 (1.00 to 1.00)	5	-0.06 (-0.19, 0.06)
Chills	ChAd/BNT-28	1.15 (0.93 to 1.38)	13	ChAd/BNT-84	1.00 (1.00 to 1.00)	6	-0.09 (-0.21, 0.04)
Myalgia	ChAd/BNT-28	1.10 (0.96 to 1.24)	20	ChAd/BNT-84	1.10 (0.87 to 1.33)	10	-0.09 (-0.24, 0.06)
Arthralgia	ChAd/BNT-28	1.00 (1.00 to 1.00)	11	ChAd/BNT-84	1.00 (1.00 to 1.00)	4	-0.04 (-0.14, 0.05)
Headache	ChAd/BNT-28	1.19 (0.90 to 1.48)	16	ChAd/BNT-84	1.40 (0.80 to 2.00)	10	-0.05 (-0.23, 0.13)
Fatigue	ChAd/BNT-28	1.59 (1.24 to 1.95)	32	ChAd/BNT-84	1.29 (0.87 to 1.70)	21	-0.27 (-0.57, 0.03)
Malaise	ChAd/BNT-28	1.24 (1.05 to 1.44)	29	ChAd/BNT-84	1.12 (0.83 to 1.42)	8	-0.30 (-0.48, -0.11)
Feverishness	BNT/BNT-84	1.17 (0.74 to 1.60)	6	BNT/BNT-84	1.25 (0.45 to 2.05)	4	-0.02 (-0.11, 0.08)
Chills	BNT/BNT-84	1.17 (0.74 to 1.60)	6	BNT/BNT-84	2.00 (-10.71 to 14.71)	2	-0.06 (-0.17, 0.05)
Headache	BNT/BNT-84	1.62 (1.15 to 2.08)	13	BNT/BNT-84	1.29 (0.59 to 1.98)	7	-0.17 (-0.35, 0.01)
Fatigue	BNT/BNT-84	2.19 (1.36 to 3.02)	16	BNT/BNT-84	1.89 (1.18 to 2.60)	9	-0.23 (-0.52, 0.07)
Malaise	BNT/BNT-84	1.64 (0.91 to 2.38)	14	BNT/BNT-84	1.00 (1.00 to 1.00)	7	-0.17 (-0.37, 0.02)
Nausea	BNT/BNT-84	1.25 (0.45 to 2.05)	4	BNT/BNT-84	1.00 (1.00 to 1.00)	3	-0.05 (-0.13, 0.03)
Fever	BNT/ChAd-84	1.00 (1.00 to 1.00)	3	BNT/ChAd-84	1.00 (1.00 to 1.00)	4	0.04 (-0.02, 0.11)
Feverishness	BNT/ChAd-84	1.10 (0.96 to 1.24)	20	BNT/ChAd-84	1.19 (0.96 to 1.42)	21	0.10 (-0.08, 0.28)
Chills	BNT/ChAd-84	1.04 (0.95 to 1.13)	23	BNT/ChAd-84	1.00 (1.00 to 1.00)	19	0.03 (-0.11, 0.18)
Myalgia	BNT/ChAd-84	1.75 (0.78 to 2.72)	8	BNT/ChAd-84	1.14 (0.98 to 1.31)	21	0.15 (-0.04, 0.35)
Arthralgia	BNT/ChAd-84	2.22 (0.80 to 3.65)	9	BNT/ChAd-84	1.27 (0.94 to 1.60)	15	0.00 (-0.25, 0.26)
Headache	BNT/ChAd-84	1.29 (1.03 to 1.54)	21	BNT/ChAd-84	1.50 (0.94 to 2.06)	20	0.17 (-0.08, 0.42)
Fatigue	BNT/ChAd-84	1.45 (1.17 to 1.73)	29	BNT/ChAd-84	1.56 (1.28 to 1.84)	43	0.36 (0.05, 0.67)
Malaise	BNT/ChAd-84	1.28 (1.03 to 1.53)	25	BNT/ChAd-84	1.23 (1.05 to 1.42)	30	0.15 (-0.08, 0.37)
Nausea	BNT/ChAd-84	2.33 (-1.46 to 6.13)	3	BNT/ChAd-84	1.25 (0.45 to 2.05)	4	-0.03 (-0.17, 0.10)
Diarrhoea	BNT/ChAd-84	2.00 (-10.71 to 14.71)	2	BNT/ChAd-84	1.00 (1.00 to 1.00)	2	-0.02 (-0.10, 0.07)



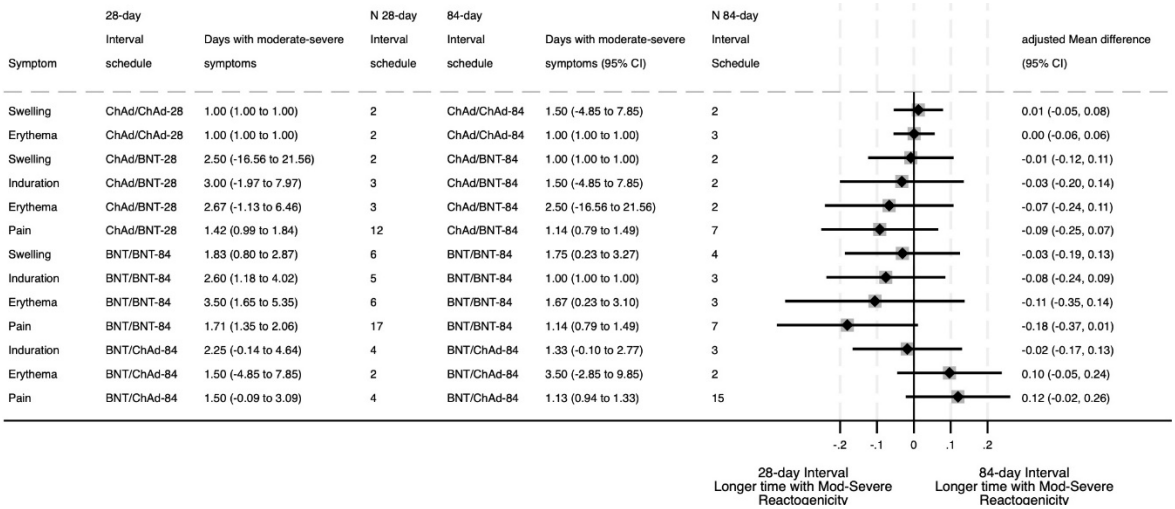
Supplementary Figure 22 *– Forest plot paracetamol sensitivity analysis comparing durations of time spent with grade 2 or higher systemic reactogenicity symptoms after the second COVID-19 vaccination between participants (seronegative and seropositive combined) receiving schedules with either 4-week or 12-week intervals . "If you did suffer with moderate/severe symptoms (Grade 2 or above) after the second dose, were the symptoms likely to last longer after a short or long interval schedule?"*



Supplementary Figure 23 *– Forest plot paracetamol sensitivity analysis comparing the proportions of participants (seronegative and seropositive combined) suffering local reactogenicity symptoms in the first 7 days after the second COVID-19 vaccination between 4-week and 12-week interval schedules "Were you more likely to suffer a symptom of any grade after the second dose if the schedule had a short or long interval?"*



Supplementary Figure 24 *– Forest plot paracetamol sensitivity analysis comparing proportions of participants (seronegative and seropositive combined) suffering moderate-severe (Grade 2 or above) local reactogenicity symptoms out of the total number of participants suffering any grade symptoms, in the first 7 days after a second COVID-19 vaccination between 4-week and 12-week interval schedules. "If you did suffer a symptom of any grade after the second dose, was it more likely to be moderate/severe if the schedule had a short or long interval?"*



Supplementary Figure 25 *– Forest plot paracetamol sensitivity analysis comparing durations of time spent with grade 2 or higher local reactogenicity symptoms after the second COVID-19 vaccination between participants (seronegative and seropositive combined) receiving schedules with either 4-week or 12-week intervals . "If you did suffer with moderate/severe symptoms (Grade 2 or above) after the second dose, were the symptoms likely to last longer after a short or long interval schedule?"*

Supplementary Table 1 *– Multivariate linear regressions exploring different baseline immunological statuses on peak immunological outcomes*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.003	.008	0.37	.711	-.013	.019	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.155	.081	1.91	.059	-.006	.316	*
BMI	.004	.008	0.54	.593	-.011	.02	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.116	.089	-1.30	.198	-.293	.061	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.2	.086	2.33	.022	.029	.37	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.661	.113	5.86	0	.437	.885	***
BNT/BNT-84	.786	.11	7.13	0	.567	1.005	***
BNT/ChAd-84	.599	.105	5.68	0	.389	.808	***
Baseline serum anti-SARS-CoV2 spike IgG	.079	.076	1.05	.299	-.071	.23	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.251	.08	3.13	.002	.092	.41	***
Constant	3.775	.588	6.42	0	2.606	4.944	***

Mean dependent var	4.959	SD dependent var	0.520
R-squared	0.543	Number of obs	98
F-test	10.347	Prob > F	0.000
Akaike crit. (AIC)	94.107	Bayesian crit. (BIC)	122.542

*** *p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.002	.008	0.24	.809	-.014	.018	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.153	.081	1.88	.064	-.009	.315	*
BMI	.003	.008	0.33	.742	-.013	.018	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.109	.09	-1.21	.228	-.288	.07	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.207	.086	2.42	.018	.037	.378	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.655	.114	5.75	0	.429	.881	***
BNT/BNT-84	.78	.112	6.93	0	.556	1.003	***
BNT/ChAd-84	.597	.108	5.53	0	.382	.811	***
Baseline serum anti-SARS-CoV2 spike IgA	.033	.073	0.45	.654	-.113	.178	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.268	.079	3.41	.001	.112	.424	***
Constant	3.963	.556	7.12	0	2.857	5.069	***

Mean dependent var	4.959	SD dependent var	0.520
R-squared	0.539	Number of obs	98
F-test	10.155	Prob > F	0.000
Akaike crit. (AIC)	95.103	Bayesian crit. (BIC)	123.538

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.003	.009	0.35	.73	-.014	.02	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.162	.084	1.94	.056	-.004	.328	*
BMI	.004	.008	0.46	.65	-.012	.02	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.117	.093	-1.26	.21	-.301	.067	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.207	.087	2.39	.019	.035	.379	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.672	.116	5.78	0	.44	.903	***
BNT/BNT-84	.791	.114	6.93	0	.564	1.017	***
BNT/ChAd-84	.617	.108	5.72	0	.402	.831	***
Baseline mucosal anti-SARS-CoV2 spike IgG	.021	.067	0.32	.753	-.111	.153	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.267	.08	3.32	.001	.107	.427	***
Constant	3.907	.592	6.60	0	2.73	5.084	***

Mean dependent var	4.962	SD dependent var	0.525
R-squared	0.539	Number of obs	96
F-test	9.940	Prob > F	0.000
Akaike crit. (AIC)	95.224	Bayesian crit. (BIC)	123.431

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.002	.009	0.23	.822	-.015	.019	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.156	.083	1.88	.063	-.009	.321	*
BMI	.005	.008	0.61	.543	-.011	.021	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.122	.092	-1.33	.188	-.305	.061	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.21	.086	2.43	.017	.038	.381	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.675	.116	5.84	0	.446	.905	***
BNT/BNT-84	.808	.113	7.17	0	.584	1.033	***
BNT/ChAd-84	.621	.107	5.79	0	.408	.835	***
Baseline mucosal anti-SARS-CoV2 spike IgA	-.09	.088	-1.02	.308	-.265	.085	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.271	.08	3.40	.001	.113	.43	***
Constant	4.167	.615	6.77	0	2.944	5.39	***

Mean dependent var	4.962	SD dependent var	0.525
R-squared	0.544	Number of obs	96
F-test	10.146	Prob > F	0.000
Akaike crit. (AIC)	94.158	Bayesian crit. (BIC)	122.366

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.003	.008	0.32	.749	-.013	.018	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.169	.08	2.11	.038	.009	.328	**
BMI	.005	.008	0.60	.551	-.011	.02	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.098	.088	-1.12	.268	-.273	.077	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.195	.084	2.32	.023	.028	.362	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.65	.111	5.85	0	.429	.871	***
BNT/BNT-84	.784	.109	7.23	0	.569	1	***
BNT/ChAd-84	.576	.105	5.51	0	.368	.784	***
Baseline serum anti-nucleocapsid IgG	.117	.059	1.99	.05	0	.234	*
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.234	.079	2.99	.004	.078	.39	***
Constant	3.671	.561	6.55	0	2.556	4.785	***

Mean dependent var	4.959	SD dependent var	0.520
R-squared	0.558	Number of obs	98
F-test	10.964	Prob > F	0.000
Akaike crit. (AIC)	90.983	Bayesian crit. (BIC)	119.418

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.002	.008	0.29	.773	-.014	.018	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.161	.081	1.98	.051	-.001	.322	*
BMI	.005	.008	0.59	.558	-.011	.02	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.113	.089	-1.27	.207	-.29	.064	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.202	.085	2.37	.02	.033	.371	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.675	.113	5.95	0	.45	.901	***
BNT/BNT-84	.791	.11	7.18	0	.572	1.01	***
BNT/ChAd-84	.603	.105	5.74	0	.394	.811	***
Baseline serum anti-nucleocapsid IgA	.094	.081	1.16	.247	-.067	.255	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.251	.08	3.15	.002	.093	.409	***
Constant	3.731	.593	6.29	0	2.553	4.91	***

Mean dependent var	4.959	SD dependent var	0.520
R-squared	0.545	Number of obs	98
F-test	10.404	Prob > F	0.000
Akaike crit. (AIC)	93.816	Bayesian crit. (BIC)	122.250

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.003	.009	0.36	.72	-.014	.02	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.165	.083	1.98	.051	-.001	.331	*
BMI	.004	.008	0.49	.626	-.012	.02	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.114	.092	-1.24	.219	-.298	.069	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.208	.086	2.41	.018	.037	.38	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.663	.116	5.70	0	.432	.895	***
BNT/BNT-84	.782	.114	6.89	0	.556	1.008	***
BNT/ChAd-84	.607	.108	5.61	0	.391	.822	***
Baseline mucosal anti-nucleocapsid IgG	.044	.053	0.84	.406	-.061	.148	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.267	.08	3.35	.001	.109	.426	***
Constant	3.856	.587	6.57	0	2.689	5.023	***

Mean dependent var	4.962	SD dependent var	0.525
R-squared	0.542	Number of obs	96
F-test	10.070	Prob > F	0.000
Akaike crit. (AIC)	94.552	Bayesian crit. (BIC)	122.760

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.003	.009	0.34	.738	-.014	.02	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.161	.084	1.93	.057	-.005	.328	*
BMI	.004	.008	0.47	.637	-.012	.02	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.117	.093	-1.26	.211	-.301	.067	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.209	.087	2.41	.018	.036	.381	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.673	.116	5.78	0	.442	.905	***
BNT/BNT-84	.796	.113	7.05	0	.571	1.02	***
BNT/ChAd-84	.617	.108	5.72	0	.402	.831	***
Baseline mucosal anti-nucleocapsid IgA	.017	.093	0.18	.855	-.167	.201	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.263	.08	3.27	.002	.103	.422	***
Constant	3.882	.675	5.75	0	2.54	5.225	***

Mean dependent var	4.962	SD dependent var	0.525
R-squared	0.539	Number of obs	96
F-test	9.926	Prob > F	0.000
Akaike crit. (AIC)	95.299	Bayesian crit. (BIC)	123.506

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.001	.008	0.09	.927	-.015	.017	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.16	.081	1.97	.052	-.001	.322	*
BMI	.004	.008	0.46	.648	-.012	.019	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.094	.091	-1.03	.305	-.274	.087	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.186	.088	2.12	.037	.011	.36	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.653	.113	5.78	0	.428	.877	***
BNT/BNT-84	.8	.111	7.23	0	.58	1.021	***
BNT/ChAd-84	.593	.106	5.60	0	.382	.803	***
Baseline serum anti-OC43 spike IgG	.115	.105	1.09	.279	-.094	.324	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.267	.077	3.45	.001	.113	.421	***
Constant	3.555	.686	5.18	0	2.192	4.918	***

Mean dependent var	4.959	SD dependent var	0.520
R-squared	0.544	Number of obs	98
F-test	10.368	Prob > F	0.000
Akaike crit. (AIC)	94.001	Bayesian crit. (BIC)	122.436

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.002	.008	0.20	.84	-.015	.018	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.152	.082	1.87	.065	-.01	.315	*
BMI	.003	.008	0.44	.663	-.012	.019	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.114	.09	-1.27	.209	-.292	.065	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.209	.086	2.44	.017	.038	.379	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.657	.114	5.78	0	.431	.883	***
BNT/BNT-84	.785	.111	7.05	0	.564	1.006	***
BNT/ChAd-84	.6	.108	5.56	0	.386	.815	***
Baseline serum anti-OC43 spike IgA	.025	.082	0.30	.761	-.138	.188	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.269	.079	3.41	.001	.112	.426	***
Constant	3.939	.597	6.60	0	2.752	5.127	***

Mean dependent var	4.959	SD dependent var	0.520
R-squared	0.538	Number of obs	98
F-test	10.131	Prob > F	0.000
Akaike crit. (AIC)	95.226	Bayesian crit. (BIC)	123.661

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.003	.009	0.34	.736	-.014	.02	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.161	.083	1.93	.057	-.005	.327	*
BMI	.003	.008	0.41	.68	-.013	.02	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.117	.092	-1.27	.208	-.301	.067	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.205	.087	2.37	.02	.033	.378	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.674	.116	5.80	0	.443	.905	***
BNT/BNT-84	.788	.114	6.91	0	.561	1.015	***
BNT/ChAd-84	.617	.108	5.73	0	.403	.831	***
Baseline mucosal anti-OC43 spike IgG	.031	.07	0.45	.657	-.108	.17	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.269	.081	3.33	.001	.108	.429	***
Constant	3.837	.628	6.11	0	2.589	5.086	***

Mean dependent var	4.962	SD dependent var	0.525
R-squared	0.540	Number of obs	96
F-test	9.962	Prob > F	0.000
Akaike crit. (AIC)	95.112	Bayesian crit. (BIC)	123.320

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.003	.009	0.32	.748	-.014	.02	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.162	.083	1.95	.055	-.004	.328	*
BMI	.003	.008	0.36	.723	-.014	.019	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.111	.093	-1.19	.236	-.296	.074	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.208	.087	2.41	.018	.036	.38	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.667	.117	5.71	0	.434	.899	***
BNT/BNT-84	.797	.113	7.08	0	.573	1.021	***
BNT/ChAd-84	.613	.108	5.67	0	.398	.828	***
Baseline mucosal anti-OC43 spike IgA	-.035	.07	-0.50	.62	-.173	.104	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.266	.08	3.33	.001	.107	.426	***
Constant	4.099	.655	6.25	0	2.796	5.402	***

Mean dependent var	4.962	SD dependent var	0.525
R-squared	0.540	Number of obs	96
F-test	9.973	Prob > F	0.000
Akaike crit. (AIC)	95.056	Bayesian crit. (BIC)	123.264

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.001	.008	0.08	.937	-.016	.017	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.161	.081	1.98	.051	-.001	.323	*
BMI	.005	.008	0.62	.536	-.011	.021	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.124	.09	-1.38	.171	-.302	.054	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.19	.087	2.18	.032	.017	.363	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.647	.113	5.70	0	.421	.872	***
BNT/BNT-84	.795	.11	7.20	0	.575	1.014	***
BNT/ChAd-84	.593	.106	5.60	0	.383	.804	***
Baseline serum anti-HKU1 spike IgG	.096	.091	1.05	.295	-.085	.277	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.254	.079	3.21	.002	.097	.412	***
Constant	3.67	.632	5.80	0	2.413	4.927	***

Mean dependent var	4.959	SD dependent var	0.520
R-squared	0.543	Number of obs	98
F-test	10.351	Prob > F	0.000
Akaike crit. (AIC)	94.088	Bayesian crit. (BIC)	122.523

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.001	.008	0.18	.856	-.015	.018	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.154	.082	1.89	.062	-.008	.316	*
BMI	.003	.008	0.44	.662	-.012	.019	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.113	.09	-1.27	.209	-.292	.065	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.209	.086	2.44	.017	.039	.38	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.657	.114	5.78	0	.431	.883	***
BNT/BNT-84	.784	.111	7.05	0	.563	1.005	***
BNT/ChAd-84	.601	.107	5.65	0	.39	.813	***
Baseline serum anti-HKU1 spike IgA	.033	.083	0.39	.696	-.132	.197	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.266	.08	3.34	.001	.108	.425	***
Constant	3.936	.58	6.79	0	2.783	5.089	***

Mean dependent var	4.959	SD dependent var	0.520
R-squared	0.538	Number of obs	98
F-test	10.144	Prob > F	0.000
Akaike crit. (AIC)	95.157	Bayesian crit. (BIC)	123.592

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.003	.009	0.33	.742	-.014	.02	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.163	.084	1.95	.055	-.003	.329	*
BMI	.004	.008	0.46	.646	-.012	.02	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.127	.094	-1.34	.184	-.315	.061	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.206	.087	2.37	.02	.033	.378	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.671	.116	5.77	0	.44	.902	***
BNT/BNT-84	.784	.115	6.81	0	.555	1.013	***
BNT/ChAd-84	.618	.108	5.73	0	.403	.832	***
Baseline mucosal anti-HKU1 spike IgG	.031	.062	0.49	.622	-.092	.154	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.266	.08	3.32	.001	.107	.425	***
Constant	3.851	.61	6.32	0	2.639	5.063	***

Mean dependent var	4.962	SD dependent var	0.525
R-squared	0.540	Number of obs	96
F-test	9.972	Prob > F	0.000
Akaike crit. (AIC)	95.061	Bayesian crit. (BIC)	123.269

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.003	.009	0.32	.753	-.014	.02	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.159	.083	1.90	.061	-.007	.325	*
BMI	.004	.008	0.52	.606	-.012	.021	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.118	.093	-1.28	.205	-.302	.066	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.208	.087	2.40	.018	.036	.38	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.674	.116	5.79	0	.443	.905	***
BNT/BNT-84	.796	.113	7.05	0	.571	1.02	***
BNT/ChAd-84	.62	.108	5.73	0	.405	.835	***
Baseline mucosal anti-HKU1 spike IgA	.021	.067	0.31	.758	-.113	.155	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.261	.081	3.23	.002	.1	.421	***
Constant	3.857	.646	5.97	0	2.573	5.142	***

Mean dependent var	4.962	SD dependent var	0.525
R-squared	0.539	Number of obs	96
F-test	9.939	Prob > F	0.000
Akaike crit. (AIC)	95.228	Bayesian crit. (BIC)	123.436

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.002	.008	0.23	.816	-.014	.018	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.15	.082	1.83	.071	-.013	.313	*
BMI	.002	.008	0.27	.789	-.014	.019	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.111	.09	-1.24	.217	-.29	.067	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.213	.087	2.44	.017	.04	.386	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.66	.113	5.82	0	.435	.886	***
BNT/BNT-84	.783	.112	6.98	0	.56	1.006	***
BNT/ChAd-84	.61	.106	5.74	0	.399	.821	***
Baseline serum anti-NL63 spike IgG	-.029	.097	-0.30	.766	-.222	.164	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.274	.078	3.52	.001	.119	.428	***
Constant	4.141	.691	5.99	0	2.766	5.515	***

Mean dependent var	4.959	SD dependent var	0.520
R-squared	0.538	Number of obs	98
F-test	10.130	Prob > F	0.000
Akaike crit. (AIC)	95.231	Bayesian crit. (BIC)	123.665

**** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.002	.008	0.28	.778	-.014	.018	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.157	.081	1.93	.057	-.005	.319	*
BMI	.004	.008	0.56	.577	-.011	.02	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.125	.091	-1.38	.17	-.306	.055	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.215	.086	2.51	.014	.044	.385	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.661	.113	5.85	0	.437	.886	***
BNT/BNT-84	.789	.111	7.14	0	.569	1.009	***
BNT/ChAd-84	.592	.107	5.55	0	.38	.805	***
Baseline serum anti-NL63 spike IgA	.066	.08	0.82	.414	-.093	.224	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.256	.08	3.19	.002	.097	.415	***
Constant	3.776	.616	6.13	0	2.552	5	***

Mean dependent var	4.959	SD dependent var	0.520
R-squared	0.541	Number of obs	98
F-test	10.257	Prob > F	0.000
Akaike crit. (AIC)	94.573	Bayesian crit. (BIC)	123.008

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.002	.009	0.27	.786	-.015	.02	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.159	.083	1.91	.06	-.007	.325	*
BMI	.004	.008	0.45	.651	-.012	.02	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.113	.094	-1.21	.23	-.3	.073	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.207	.087	2.39	.019	.035	.38	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.672	.116	5.78	0	.44	.903	***
BNT/BNT-84	.799	.113	7.04	0	.573	1.024	***
BNT/ChAd-84	.617	.108	5.71	0	.402	.831	***
Baseline mucosal anti-NL63 spike IgG	-.016	.065	-0.25	.803	-.144	.112	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.261	.081	3.23	.002	.1	.422	***
Constant	4.006	.628	6.38	0	2.757	5.256	***

Mean dependent var	4.962	SD dependent var	0.525
R-squared	0.539	Number of obs	96
F-test	9.932	Prob > F	0.000
Akaike crit. (AIC)	95.265	Bayesian crit. (BIC)	123.473

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.003	.009	0.31	.76	-.015	.02	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.16	.083	1.92	.059	-.006	.326	*
BMI	.004	.008	0.45	.653	-.013	.02	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.116	.094	-1.24	.219	-.303	.071	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.208	.087	2.38	.02	.034	.381	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.672	.116	5.78	0	.441	.904	***
BNT/BNT-84	.796	.113	7.05	0	.572	1.02	***
BNT/ChAd-84	.617	.108	5.72	0	.402	.832	***
Baseline mucosal anti-NL63 spike IgA	-.002	.086	-0.03	.978	-.172	.168	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.264	.08	3.30	.001	.105	.423	***
Constant	3.957	.701	5.64	0	2.562	5.351	***

Mean dependent var	4.962	SD dependent var	0.525
R-squared	0.539	Number of obs	96
F-test	9.919	Prob > F	0.000
Akaike crit. (AIC)	95.335	Bayesian crit. (BIC)	123.543

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.002	.008	0.26	.797	-.014	.018	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.148	.081	1.82	.073	-.014	.31	*
BMI	.002	.008	0.25	.806	-.014	.018	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.108	.089	-1.20	.232	-.285	.07	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.217	.086	2.52	.013	.046	.387	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.657	.113	5.81	0	.432	.882	***
BNT/BNT-84	.772	.112	6.90	0	.55	.994	***
BNT/ChAd-84	.607	.105	5.76	0	.397	.816	***
Baseline serum anti-229E spike IgG	-.08	.09	-0.89	.377	-.258	.099	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.275	.077	3.56	.001	.121	.429	***
Constant	4.391	.689	6.37	0	3.021	5.76	***

Mean dependent var	4.959	SD dependent var	0.520
R-squared	0.542	Number of obs	98
F-test	10.281	Prob > F	0.000
Akaike crit. (AIC)	94.448	Bayesian crit. (BIC)	122.882

**** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.002	.008	0.21	.837	-.014	.018	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.143	.082	1.74	.086	-.021	.306	*
BMI	.002	.008	0.25	.802	-.014	.018	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.099	.091	-1.09	.28	-.28	.082	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.202	.086	2.35	.021	.031	.372	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.663	.113	5.85	0	.438	.887	***
BNT/BNT-84	.79	.111	7.14	0	.57	1.01	***
BNT/ChAd-84	.619	.107	5.81	0	.407	.831	***
Baseline serum anti-229E spike IgA	-.063	.082	-0.77	.446	-.225	.1	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.281	.078	3.60	.001	.126	.437	***
Constant	4.261	.632	6.74	0	3.005	5.517	***

Mean dependent var	4.959	SD dependent var	0.520
R-squared	0.541	Number of obs	98
F-test	10.238	Prob > F	0.000
Akaike crit. (AIC)	94.672	Bayesian crit. (BIC)	123.107

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.002	.009	0.18	.855	-.015	.019	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.158	.083	1.92	.059	-.006	.323	*
BMI	.004	.008	0.50	.619	-.012	.02	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.1	.093	-1.08	.284	-.285	.084	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.206	.086	2.40	.019	.035	.377	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.657	.116	5.66	0	.426	.887	***
BNT/BNT-84	.809	.112	7.20	0	.586	1.032	***
BNT/ChAd-84	.602	.108	5.58	0	.387	.816	***
Baseline mucosal anti-229E spike IgG	-.079	.064	-1.23	.222	-.207	.049	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.253	.08	3.16	.002	.094	.411	***
Constant	4.282	.636	6.73	0	3.016	5.547	***

Mean dependent var	4.962	SD dependent var	0.525
R-squared	0.547	Number of obs	96
F-test	10.246	Prob > F	0.000
Akaike crit. (AIC)	93.645	Bayesian crit. (BIC)	121.853

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.003	.009	0.29	.769	-.014	.019	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.155	.083	1.87	.065	-.01	.32	*
BMI	.003	.008	0.37	.716	-.013	.019	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.109	.092	-1.19	.237	-.292	.073	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.199	.086	2.31	.023	.028	.371	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.671	.115	5.82	0	.441	.9	***
BNT/BNT-84	.806	.112	7.18	0	.583	1.029	***
BNT/ChAd-84	.611	.107	5.71	0	.398	.824	***
Baseline mucosal anti-229E spike IgA	-.098	.081	-1.21	.231	-.26	.064	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.265	.079	3.34	.001	.107	.423	***
Constant	4.386	.681	6.44	0	3.032	5.74	***

Mean dependent var	4.962	SD dependent var	0.525
R-squared	0.546	Number of obs	96
F-test	10.233	Prob > F	0.000
Akaike crit. (AIC)	93.710	Bayesian crit. (BIC)	121.918

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.002	.008	0.24	.81	-.015	.019	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.179	.082	2.19	.031	.017	.342	**
BMI	.004	.008	0.56	.577	-.011	.02	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.093	.092	-1.01	.317	-.275	.09	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.185	.086	2.14	.035	.013	.356	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.597	.113	5.27	0	.372	.822	***
BNT/BNT-84	.778	.113	6.87	0	.553	1.003	***
BNT/ChAd-84	.58	.11	5.25	0	.36	.799	***
Baseline Seromucostatus (Reference: Seronegative/Muconegative)	0	.	.	.	.	.	
Seronegative/Mucopositive	-.074	.131	-0.57	.572	-.335	.186	
Seropositive	.108	.113	0.95	.342	-.117	.332	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.228	.079	2.87	.005	.07	.386	***
Constant	3.987	.555	7.18	0	2.884	5.091	***

Mean dependent var	4.954	SD dependent var	0.517
R-squared	0.521	Number of obs	100
F-test	8.688	Prob > F	0.000
Akaike crit. (AIC)	101.145	Bayesian crit. (BIC)	132.407

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.003	.008	0.37	.712	-.013	.019	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.175	.082	2.13	.036	.012	.337	**
BMI	.004	.008	0.54	.589	-.012	.02	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.096	.091	-1.05	.298	-.277	.086	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.188	.086	2.19	.031	.017	.36	**
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.602	.114	5.29	0	.376	.829	***
BNT/BNT-84	.786	.113	6.97	0	.562	1.011	***
BNT/ChAd-84	.609	.108	5.65	0	.395	.822	***
Baseline Mucostatus (Reference: Muconegative)	0	.	.	.	.	.	
Mucopositive	.036	.099	0.36	.718	-.161	.233	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.248	.078	3.16	.002	.092	.403	***
Constant	3.911	.554	7.06	0	2.811	5.012	***

Mean dependent var	4.954	SD dependent var	0.517
R-squared	0.514	Number of obs	100
F-test	9.405	Prob > F	0.000
Akaike crit. (AIC)	100.555	Bayesian crit. (BIC)	129.212

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.013	.011	1.18	.24	-.009	.036	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.118	.112	1.06	.293	-.104	.34	
BMI	.012	.011	1.08	.284	-.01	.033	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.101	.123	-0.82	.413	-.345	.143	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.052	.118	-0.44	.66	-.286	.182	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.496	.155	3.19	.002	.187	.805	***
BNT/BNT-84	.824	.152	5.42	0	.522	1.126	***
BNT/ChAd-84	.769	.145	5.30	0	.48	1.057	***
Baseline serum anti-SARS-CoV2 spike IgG	.189	.104	1.81	.074	-.019	.396	*
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.213	.11	1.93	.057	-.006	.433	*
Constant	1.285	.81	1.59	.116	-.326	2.896	

Mean dependent var	3.402	SD dependent var	0.621
R-squared	0.392	Number of obs	98
F-test	5.613	Prob > F	0.000
Akaike crit. (AIC)	156.935	Bayesian crit. (BIC)	185.369

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.011	.01	1.09	.278	-.009	.032	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.121	.104	1.16	.247	-.085	.327	
BMI	.003	.01	0.31	.76	-.017	.023	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.053	.114	-0.47	.643	-.281	.174	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.042	.109	-0.38	.702	-.259	.175	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.437	.145	3.01	.003	.149	.725	***
BNT/BNT-84	.727	.143	5.08	0	.443	1.012	***
BNT/ChAd-84	.67	.137	4.87	0	.397	.943	***
Baseline serum anti-SARS-CoV2 spike IgA	.39	.093	4.18	0	.205	.575	***
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.202	.1	2.01	.047	.003	.401	**
Constant	1.249	.709	1.76	.082	-.16	2.658	*

Mean dependent var	3.402	SD dependent var	0.621
R-squared	0.475	Number of obs	98
F-test	7.870	Prob > F	0.000
Akaike crit. (AIC)	142.588	Bayesian crit. (BIC)	171.023

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.018	.011	1.57	.121	-.005	.04	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.125	.11	1.15	.255	-.092	.343	
BMI	.011	.011	0.99	.323	-.011	.032	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.14	.121	-1.16	.251	-.381	.101	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.055	.113	-0.48	.632	-.28	.171	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.47	.152	3.09	.003	.167	.773	***
BNT/BNT-84	.742	.149	4.97	0	.445	1.039	***
BNT/ChAd-84	.741	.141	5.24	0	.46	1.022	***
Baseline mucosal anti-SARS-CoV2 spike IgG	.144	.087	1.65	.102	-.029	.317	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.293	.105	2.78	.007	.083	.502	***
Constant	1.31	.775	1.69	.095	-.231	2.852	*

Mean dependent var	3.426	SD dependent var	0.600
R-squared	0.396	Number of obs	96
F-test	5.564	Prob > F	0.000
Akaike crit. (AIC)	146.994	Bayesian crit. (BIC)	175.201

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.016	.011	1.40	.164	-.007	.039	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.114	.111	1.02	.309	-.107	.334	
BMI	.01	.011	0.94	.348	-.011	.032	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.138	.123	-1.12	.266	-.382	.107	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.048	.115	-0.42	.677	-.277	.181	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.473	.155	3.06	.003	.166	.78	***
BNT/BNT-84	.771	.151	5.12	0	.471	1.071	***
BNT/ChAd-84	.742	.143	5.18	0	.457	1.028	***
Baseline mucosal anti-SARS-CoV2 spike IgA	.056	.118	0.48	.634	-.178	.29	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.269	.107	2.52	.014	.057	.481	**
Constant	1.437	.822	1.75	.084	-.197	3.071	*

Mean dependent var	3.426	SD dependent var	0.600
R-squared	0.378	Number of obs	96
F-test	5.163	Prob > F	0.000
Akaike crit. (AIC)	149.770	Bayesian crit. (BIC)	177.978

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.011	.011	0.99	.325	-.011	.034	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.125	.114	1.10	.272	-.1	.351	
BMI	.01	.011	0.94	.348	-.011	.032	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.082	.124	-0.66	.514	-.329	.166	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.043	.119	-0.36	.721	-.279	.194	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.486	.157	3.09	.003	.173	.798	***
BNT/BNT-84	.825	.154	5.37	0	.52	1.13	***
BNT/ChAd-84	.762	.148	5.15	0	.468	1.056	***
Baseline serum anti-nucleocapsid IgG	.097	.083	1.16	.249	-.069	.262	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.234	.111	2.11	.038	.013	.455	**
Constant	1.571	.793	1.98	.051	-.006	3.148	*

Mean dependent var	3.402	SD dependent var	0.621
R-squared	0.379	Number of obs	98
F-test	5.308	Prob > F	0.000
Akaike crit. (AIC)	159.045	Bayesian crit. (BIC)	187.480

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.012	.011	1.11	.271	-.01	.034	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.14	.109	1.28	.204	-.077	.357	
BMI	.014	.011	1.33	.186	-.007	.035	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.095	.119	-0.80	.428	-.333	.142	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.052	.114	-0.46	.65	-.28	.175	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.545	.152	3.58	.001	.242	.847	***
BNT/BNT-84	.838	.148	5.67	0	.544	1.132	***
BNT/ChAd-84	.775	.141	5.50	0	.495	1.055	***
Baseline serum anti-nucleocapsid IgA	.313	.109	2.88	.005	.097	.529	***
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.191	.107	1.79	.077	-.021	.404	*
Constant	.916	.797	1.15	.253	-.667	2.499	

Mean dependent var	3.402	SD dependent var	0.621
R-squared	0.424	Number of obs	98
F-test	6.409	Prob > F	0.000
Akaike crit. (AIC)	151.631	Bayesian crit. (BIC)	180.065

*** p<.01, ** p<.05, * p<.1

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.016	.011	1.43	.156	-.006	.039	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.12	.111	1.08	.282	-.1	.34	
BMI	.011	.011	1.06	.294	-.01	.033	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.137	.122	-1.12	.268	-.38	.107	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.046	.114	-0.40	.687	-.274	.181	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.46	.154	2.98	.004	.153	.767	***
BNT/BNT-84	.756	.151	5.02	0	.457	1.056	***
BNT/ChAd-84	.728	.144	5.07	0	.442	1.013	***
Baseline mucosal anti-nucleocapsid IgG	.072	.07	1.03	.308	-.067	.21	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.279	.106	2.63	.01	.068	.489	**
Constant	1.429	.779	1.83	.07	-.12	2.977	*

Mean dependent var	3.426	SD dependent var	0.600
R-squared	0.384	Number of obs	96
F-test	5.295	Prob > F	0.000
Akaike crit. (AIC)	148.847	Bayesian crit. (BIC)	177.054

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.016	.012	1.40	.166	-.007	.039	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.114	.111	1.02	.309	-.107	.335	
BMI	.011	.011	1.04	.302	-.01	.033	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.14	.123	-1.14	.257	-.385	.104	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.045	.115	-0.39	.696	-.274	.184	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.477	.155	3.08	.003	.17	.785	***
BNT/BNT-84	.778	.15	5.19	0	.48	1.076	***
BNT/ChAd-84	.744	.143	5.19	0	.459	1.029	***
Baseline mucosal anti-nucleocapsid IgA	.036	.123	0.29	.77	-.209	.281	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.27	.107	2.53	.013	.058	.483	**
Constant	1.44	.897	1.60	.112	-.344	3.224	

Mean dependent var	3.426	SD dependent var	0.600
R-squared	0.377	Number of obs	96
F-test	5.140	Prob > F	0.000
Akaike crit. (AIC)	149.931	Bayesian crit. (BIC)	178.139

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.01	.011	0.84	.402	-.013	.032	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.119	.114	1.04	.3	-.108	.345	
BMI	.01	.011	0.86	.389	-.012	.031	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.077	.127	-0.61	.545	-.33	.175	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.051	.123	-0.42	.678	-.295	.193	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.488	.158	3.08	.003	.173	.802	***
BNT/BNT-84	.839	.155	5.41	0	.53	1.147	***
BNT/ChAd-84	.776	.148	5.23	0	.481	1.07	***
Baseline serum anti-OC43 spike IgG	.1	.147	0.68	.501	-.194	.393	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.261	.108	2.41	.018	.045	.476	**
Constant	1.457	.961	1.52	.133	-.452	3.366	

Mean dependent var	3.402	SD dependent var	0.621
R-squared	0.373	Number of obs	98
F-test	5.167	Prob > F	0.000
Akaike crit. (AIC)	160.040	Bayesian crit. (BIC)	188.475

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.009	.011	0.82	.416	-.013	.032	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.112	.113	1.00	.322	-.112	.337	
BMI	.011	.011	0.99	.323	-.011	.033	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.099	.124	-0.80	.428	-.346	.148	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.029	.119	-0.24	.81	-.265	.207	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.48	.158	3.05	.003	.167	.793	***
BNT/BNT-84	.812	.154	5.26	0	.505	1.119	***
BNT/ChAd-84	.753	.149	5.04	0	.456	1.051	***
Baseline serum anti-OC43 spike IgA	.13	.114	1.14	.256	-.096	.356	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.244	.109	2.23	.028	.027	.461	**
Constant	1.468	.827	1.77	.08	-.176	3.112	*

Mean dependent var	3.402	SD dependent var	0.621
R-squared	0.379	Number of obs	98
F-test	5.302	Prob > F	0.000
Akaike crit. (AIC)	159.091	Bayesian crit. (BIC)	187.525

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.016	.011	1.38	.172	-.007	.039	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.112	.111	1.01	.317	-.109	.332	
BMI	.011	.011	1.00	.322	-.011	.032	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.141	.123	-1.15	.254	-.386	.103	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.048	.115	-0.42	.676	-.278	.181	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.476	.155	3.08	.003	.168	.783	***
BNT/BNT-84	.774	.152	5.10	0	.473	1.076	***
BNT/ChAd-84	.745	.143	5.19	0	.46	1.03	***
Baseline mucosal anti-OC43 spike IgG	.018	.093	0.19	.848	-.167	.202	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.276	.107	2.57	.012	.063	.489	**
Constant	1.512	.836	1.81	.074	-.149	3.174	*

Mean dependent var	3.426	SD dependent var	0.600
R-squared	0.376	Number of obs	96
F-test	5.133	Prob > F	0.000
Akaike crit. (AIC)	149.986	Bayesian crit. (BIC)	178.194

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.016	.011	1.42	.161	-.006	.038	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.121	.11	1.10	.272	-.097	.339	
BMI	.008	.011	0.71	.477	-.014	.029	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.118	.122	-0.96	.338	-.361	.125	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.045	.114	-0.40	.693	-.271	.181	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.452	.153	2.95	.004	.147	.757	***
BNT/BNT-84	.785	.148	5.30	0	.49	1.079	***
BNT/ChAd-84	.728	.142	5.12	0	.445	1.01	***
Baseline mucosal anti-OC43 spike IgA	-.138	.092	-1.50	.136	-.32	.044	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.283	.105	2.69	.009	.074	.492	***
Constant	2.181	.861	2.53	.013	.469	3.894	**

Mean dependent var	3.426	SD dependent var	0.600
R-squared	0.392	Number of obs	96
F-test	5.489	Prob > F	0.000
Akaike crit. (AIC)	147.509	Bayesian crit. (BIC)	175.716

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.01	.011	0.88	.381	-.013	.033	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.115	.114	1.01	.315	-.112	.343	
BMI	.01	.011	0.88	.384	-.013	.032	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.098	.126	-0.78	.437	-.348	.152	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.039	.122	-0.32	.749	-.282	.203	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.488	.159	3.07	.003	.172	.805	***
BNT/BNT-84	.831	.155	5.36	0	.523	1.139	***
BNT/ChAd-84	.782	.148	5.27	0	.487	1.078	***
Baseline serum anti-HKU1 spike IgG	.039	.128	0.31	.759	-.214	.293	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.258	.111	2.32	.023	.037	.479	**
Constant	1.713	.887	1.93	.057	-.05	3.477	*

Mean dependent var	3.402	SD dependent var	0.621
R-squared	0.370	Number of obs	98
F-test	5.110	Prob > F	0.000
Akaike crit. (AIC)	160.445	Bayesian crit. (BIC)	188.880

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.008	.011	0.71	.48	-.014	.03	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.123	.112	1.10	.275	-.099	.345	
BMI	.011	.011	1.04	.302	-.01	.033	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.1	.123	-0.81	.419	-.344	.144	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.024	.117	-0.21	.836	-.258	.209	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.476	.156	3.05	.003	.166	.785	***
BNT/BNT-84	.804	.152	5.27	0	.501	1.107	***
BNT/ChAd-84	.753	.146	5.16	0	.463	1.044	***
Baseline serum anti-HKU1 spike IgA	.207	.114	1.82	.072	-.019	.433	*
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.222	.109	2.03	.046	.005	.439	**
Constant	1.359	.795	1.71	.091	-.221	2.938	*

Mean dependent var	3.402	SD dependent var	0.621
R-squared	0.392	Number of obs	98
F-test	5.619	Prob > F	0.000
Akaike crit. (AIC)	156.892	Bayesian crit. (BIC)	185.327

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.016	.011	1.37	.173	-.007	.038	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.113	.111	1.01	.314	-.108	.334	
BMI	.011	.011	1.02	.31	-.01	.032	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.146	.126	-1.16	.249	-.396	.104	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.048	.115	-0.42	.677	-.278	.181	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.474	.155	3.06	.003	.166	.782	***
BNT/BNT-84	.773	.153	5.04	0	.468	1.077	***
BNT/ChAd-84	.745	.143	5.20	0	.46	1.03	***
Baseline mucosal anti-HKU1 spike IgG	.016	.082	0.19	.851	-.148	.179	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.274	.106	2.58	.012	.063	.486	**
Constant	1.526	.812	1.88	.063	-.087	3.14	*

Mean dependent var	3.426	SD dependent var	0.600
R-squared	0.376	Number of obs	96
F-test	5.132	Prob > F	0.000
Akaike crit. (AIC)	149.988	Bayesian crit. (BIC)	178.196

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.016	.011	1.37	.174	-.007	.038	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.11	.111	0.99	.325	-.111	.331	
BMI	.012	.011	1.05	.296	-.01	.033	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.142	.123	-1.16	.251	-.387	.102	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.047	.115	-0.41	.685	-.276	.182	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.477	.155	3.08	.003	.169	.784	***
BNT/BNT-84	.778	.15	5.19	0	.48	1.076	***
BNT/ChAd-84	.748	.144	5.20	0	.462	1.034	***
Baseline mucosal anti-HKU1 spike IgA	.022	.09	0.24	.808	-.156	.2	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.27	.107	2.51	.014	.056	.483	**
Constant	1.481	.859	1.72	.088	-.227	3.19	*

Mean dependent var	3.426	SD dependent var	0.600
R-squared	0.377	Number of obs	96
F-test	5.136	Prob > F	0.000
Akaike crit. (AIC)	149.960	Bayesian crit. (BIC)	178.168

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.011	.011	0.93	.354	-.012	.033	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.104	.114	0.92	.362	-.122	.331	
BMI	.006	.012	0.55	.581	-.017	.03	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.09	.125	-0.72	.472	-.338	.158	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.017	.121	-0.14	.887	-.258	.224	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.495	.158	3.14	.002	.181	.809	***
BNT/BNT-84	.813	.156	5.20	0	.502	1.123	***
BNT/ChAd-84	.798	.148	5.39	0	.504	1.092	***
Baseline serum anti-NL63 spike IgG	-.087	.135	-0.64	.522	-.356	.182	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.267	.108	2.47	.016	.052	.482	**
Constant	2.235	.963	2.32	.023	.322	4.148	**

Mean dependent var	3.402	SD dependent var	0.621
R-squared	0.372	Number of obs	98
F-test	5.160	Prob > F	0.000
Akaike crit. (AIC)	160.089	Bayesian crit. (BIC)	188.524

*** *p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.012	.011	1.10	.274	-.01	.034	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.132	.111	1.19	.236	-.088	.352	
BMI	.015	.011	1.33	.187	-.007	.036	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.145	.123	-1.18	.243	-.389	.1	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.005	.116	-0.04	.967	-.236	.226	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.499	.153	3.26	.002	.195	.804	***
BNT/BNT-84	.832	.15	5.55	0	.534	1.13	***
BNT/ChAd-84	.731	.145	5.05	0	.443	1.019	***
Baseline serum anti-NL63 spike IgA	.26	.108	2.40	.019	.045	.475	**
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.198	.109	1.82	.073	-.019	.414	*
Constant	.912	.835	1.09	.278	-.747	2.571	

Mean dependent var	3.402	SD dependent var	0.621
R-squared	0.408	Number of obs	98
F-test	6.008	Prob > F	0.000
Akaike crit. (AIC)	154.269	Bayesian crit. (BIC)	182.704

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.015	.012	1.27	.206	-.008	.038	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.109	.111	0.98	.329	-.111	.329	
BMI	.011	.011	1.00	.32	-.011	.032	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.13	.124	-1.05	.298	-.378	.117	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.049	.115	-0.43	.671	-.278	.18	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.473	.154	3.06	.003	.166	.78	***
BNT/BNT-84	.787	.151	5.23	0	.488	1.087	***
BNT/ChAd-84	.743	.143	5.19	0	.458	1.028	***
Baseline mucosal anti-NL63 spike IgG	-.047	.086	-0.55	.584	-.217	.123	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.265	.107	2.47	.016	.052	.478	**
Constant	1.752	.834	2.10	.039	.093	3.41	**

Mean dependent var	3.426	SD dependent var	0.600
R-squared	0.378	Number of obs	96
F-test	5.175	Prob > F	0.000
Akaike crit. (AIC)	149.687	Bayesian crit. (BIC)	177.895

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.015	.012	1.34	.183	-.007	.038	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.112	.111	1.01	.317	-.109	.332	
BMI	.011	.011	0.98	.329	-.011	.033	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.138	.125	-1.11	.272	-.387	.11	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.049	.116	-0.42	.675	-.28	.182	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.476	.155	3.07	.003	.168	.783	***
BNT/BNT-84	.779	.15	5.19	0	.481	1.077	***
BNT/ChAd-84	.745	.144	5.19	0	.459	1.03	***
Baseline mucosal anti-NL63 spike IgA	-.014	.114	-0.13	.9	-.24	.212	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.274	.106	2.57	.012	.062	.486	**
Constant	1.64	.932	1.76	.082	-.213	3.494	*

Mean dependent var	3.426	SD dependent var	0.600
R-squared	0.376	Number of obs	96
F-test	5.129	Prob > F	0.000
Akaike crit. (AIC)	150.010	Bayesian crit. (BIC)	178.218

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.011	.011	0.94	.349	-.012	.033	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.11	.114	0.97	.337	-.117	.337	
BMI	.009	.011	0.77	.441	-.014	.031	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.092	.125	-0.73	.466	-.341	.157	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.028	.12	-0.24	.814	-.267	.211	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.493	.158	3.11	.003	.178	.807	***
BNT/BNT-84	.822	.157	5.24	0	.51	1.133	***
BNT/ChAd-84	.788	.147	5.35	0	.495	1.081	***
Baseline serum anti-229E spike IgG	-.031	.126	-0.25	.804	-.282	.219	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.267	.108	2.46	.016	.051	.482	**
Constant	2.002	.965	2.07	.041	.084	3.921	**

Mean dependent var	3.402	SD dependent var	0.621
R-squared	0.370	Number of obs	98
F-test	5.104	Prob > F	0.000
Akaike crit. (AIC)	160.483	Bayesian crit. (BIC)	188.918

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.011	.011	1.02	.312	-.011	.034	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.14	.113	1.24	.218	-.085	.366	
BMI	.012	.011	1.13	.26	-.009	.034	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.134	.125	-1.07	.287	-.384	.115	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.012	.118	-0.10	.92	-.247	.223	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.486	.156	3.12	.002	.176	.796	***
BNT/BNT-84	.822	.152	5.39	0	.519	1.124	***
BNT/ChAd-84	.75	.147	5.11	0	.459	1.042	***
Baseline serum anti-229E spike IgA	.189	.113	1.68	.097	-.035	.413	*
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.242	.108	2.24	.027	.028	.456	**
Constant	1.108	.871	1.27	.206	-.622	2.839	

Mean dependent var	3.402	SD dependent var	0.621
R-squared	0.389	Number of obs	98
F-test	5.542	Prob > F	0.000
Akaike crit. (AIC)	157.426	Bayesian crit. (BIC)	185.860

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.015	.011	1.32	.189	-.008	.038	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.111	.111	1.00	.321	-.11	.331	
BMI	.011	.011	1.03	.305	-.01	.033	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.135	.124	-1.08	.282	-.382	.113	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.048	.115	-0.42	.679	-.277	.181	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.469	.155	3.02	.003	.16	.778	***
BNT/BNT-84	.784	.151	5.20	0	.484	1.083	***
BNT/ChAd-84	.739	.144	5.12	0	.452	1.026	***
Baseline mucosal anti-229E spike IgG	-.03	.086	-0.35	.726	-.202	.141	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.269	.107	2.51	.014	.056	.482	**
Constant	1.703	.853	2.00	.049	.007	3.399	**

Mean dependent var	3.426	SD dependent var	0.600
R-squared	0.377	Number of obs	96
F-test	5.147	Prob > F	0.000
Akaike crit. (AIC)	149.888	Bayesian crit. (BIC)	178.096

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.015	.011	1.36	.178	-.007	.038	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.108	.111	0.97	.334	-.113	.328	
BMI	.01	.011	0.97	.335	-.011	.032	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.136	.123	-1.10	.273	-.38	.109	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.053	.115	-0.46	.647	-.282	.176	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.474	.154	3.07	.003	.167	.781	***
BNT/BNT-84	.785	.15	5.23	0	.487	1.084	***
BNT/ChAd-84	.741	.143	5.17	0	.456	1.026	***
Baseline mucosal anti-229E spike IgA	-.069	.109	-0.63	.531	-.285	.148	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.274	.106	2.58	.012	.063	.485	**
Constant	1.882	.911	2.07	.042	.07	3.693	**

Mean dependent var	3.426	SD dependent var	0.600
R-squared	0.379	Number of obs	96
F-test	5.190	Prob > F	0.000
Akaike crit. (AIC)	149.582	Bayesian crit. (BIC)	177.790

*** p<.01, ** p<.05, * p<.1

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.006	.012	0.51	.613	-.017	.029	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.09	.113	0.80	.426	-.134	.315	
BMI	.008	.011	0.71	.482	-.014	.03	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.092	.127	-0.73	.469	-.344	.16	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.002	.119	-0.02	.986	-.239	.234	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.544	.156	3.48	.001	.233	.855	***
BNT/BNT-84	.817	.156	5.23	0	.507	1.128	***
BNT/ChAd-84	.705	.153	4.62	0	.402	1.008	***
Baseline Seromucostatus (Reference: Seronegative/Muconegative)	0	.	.	.	.	.	
Seronegative/Mucopositive	-.152	.181	-0.84	.403	-.511	.208	
Seropositive	.293	.156	1.88	.063	-.017	.603	*
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.266	.11	2.42	.017	.048	.484	**
Constant	2.175	.766	2.84	.006	.652	3.698	***

Mean dependent var	3.422	SD dependent var	0.633
R-squared	0.391	Number of obs	100
F-test	5.140	Prob > F	0.000
Akaike crit. (AIC)	165.631	Bayesian crit. (BIC)	196.893

**** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Serum anti-SARS-CoV2 spike IgA	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	.009	.012	0.76	.451	-.014	.032	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.081	.115	0.71	.482	-.147	.309	
BMI	.008	.011	0.67	.503	-.015	.03	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.101	.128	-0.79	.434	-.355	.154	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	.006	.121	0.05	.961	-.233	.245	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	.553	.16	3.47	.001	.236	.87	***
BNT/BNT-84	.837	.158	5.30	0	.523	1.15	***
BNT/ChAd-84	.777	.151	5.16	0	.478	1.076	***
Baseline Mucostatus (Reference: Muconegative)	0	.	.	.	.	.	
Mucopositive	.131	.139	0.94	.35	-.145	.406	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.317	.11	2.89	.005	.099	.535	***
Constant	1.967	.775	2.54	.013	.427	3.508	**

Mean dependent var	3.422	SD dependent var	0.633
R-squared	0.365	Number of obs	100
F-test	5.118	Prob > F	0.000
Akaike crit. (AIC)	167.823	Bayesian crit. (BIC)	196.480

*** p<.01, ** p<.05, * p<.1

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.014	.013	-1.06	.293	-.041	.012	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.266	.133	1.99	.05	0	.531	**
BMI	-.004	.013	-0.31	.759	-.03	.022	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.118	.147	-0.80	.425	-.409	.174	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.047	.141	-0.34	.738	-.327	.233	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.067	.186	5.75	0	.698	1.436	***
BNT/BNT-84	1.201	.181	6.62	0	.841	1.562	***
BNT/ChAd-84	.771	.173	4.45	0	.427	1.116	***
Baseline serum anti-SARS-CoV2 spike IgG	-.113	.125	-0.91	.366	-.361	.134	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.475	.132	3.60	.001	.213	.737	***
Constant	3.873	.967	4.00	0	1.95	5.796	***

Mean dependent var	3.759	SD dependent var	0.820
R-squared	0.504	Number of obs	98
F-test	8.826	Prob > F	0.000
Akaike crit. (AIC)	191.665	Bayesian crit. (BIC)	220.100

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.013	.013	-0.96	.339	-.039	.014	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.267	.133	2.00	.049	.002	.532	**
BMI	-.001	.013	-0.05	.962	-.027	.025	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.134	.147	-0.91	.364	-.426	.158	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.056	.14	-0.40	.688	-.335	.222	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.085	.186	5.83	0	.715	1.455	***
BNT/BNT-84	1.229	.184	6.68	0	.863	1.594	***
BNT/ChAd-84	.795	.176	4.50	0	.444	1.146	***
Baseline serum anti-SARS-CoV2 spike IgA	-.116	.12	-0.97	.336	-.354	.122	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.462	.129	3.60	.001	.207	.718	***
Constant	3.712	.91	4.08	0	1.903	5.521	***

Mean dependent var	3.759	SD dependent var	0.820
R-squared	0.504	Number of obs	98
F-test	8.848	Prob > F	0.000
Akaike crit. (AIC)	191.541	Bayesian crit. (BIC)	219.976

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.011	.014	-0.77	.446	-.039	.017	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.289	.137	2.11	.038	.016	.561	**
BMI	-.001	.013	-0.11	.914	-.028	.025	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.127	.152	-0.84	.404	-.428	.174	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.061	.142	-0.43	.668	-.343	.221	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.093	.19	5.74	0	.714	1.472	***
BNT/BNT-84	1.207	.187	6.46	0	.835	1.578	***
BNT/ChAd-84	.782	.177	4.43	0	.431	1.134	***
Baseline mucosal anti-SARS-CoV2 spike IgG	.043	.109	0.40	.692	-.173	.26	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.43	.132	3.26	.002	.168	.692	***
Constant	3.339	.97	3.44	.001	1.411	5.267	***

Mean dependent var	3.760	SD dependent var	0.829
R-squared	0.504	Number of obs	96
F-test	8.653	Prob > F	0.000
Akaike crit. (AIC)	190.002	Bayesian crit. (BIC)	218.210

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.012	.014	-0.89	.377	-.04	.015	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.279	.136	2.05	.044	.008	.55	**
BMI	0	.013	0.03	.98	-.026	.027	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.135	.151	-0.89	.376	-.435	.166	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.057	.141	-0.40	.69	-.338	.225	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.099	.19	5.79	0	.721	1.476	***
BNT/BNT-84	1.235	.185	6.67	0	.866	1.603	***
BNT/ChAd-84	.789	.176	4.48	0	.439	1.14	***
Baseline mucosal anti-SARS-CoV2 spike IgA	-.122	.144	-0.85	.399	-.41	.165	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.435	.131	3.31	.001	.174	.695	***
Constant	3.719	1.01	3.68	0	1.71	5.727	***

Mean dependent var	3.760	SD dependent var	0.829
R-squared	0.508	Number of obs	96
F-test	8.765	Prob > F	0.000
Akaike crit. (AIC)	189.372	Bayesian crit. (BIC)	217.580

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.013	.013	-0.95	.346	-.039	.014	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.267	.135	1.98	.05	0	.535	*
BMI	-.003	.013	-0.20	.841	-.029	.023	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.124	.148	-0.84	.403	-.418	.169	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.058	.141	-0.41	.684	-.338	.223	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.07	.187	5.73	0	.699	1.44	***
BNT/BNT-84	1.199	.182	6.58	0	.837	1.561	***
BNT/ChAd-84	.764	.176	4.35	0	.415	1.113	***
Baseline serum anti-nucleocapsid IgG	-.016	.099	-0.16	.874	-.212	.18	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.449	.132	3.40	.001	.187	.711	***
Constant	3.578	.941	3.80	0	1.707	5.449	***

Mean dependent var	3.759	SD dependent var	0.820
R-squared	0.499	Number of obs	98
F-test	8.667	Prob > F	0.000
Akaike crit. (AIC)	192.562	Bayesian crit. (BIC)	220.996

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.013	.013	-0.96	.338	-.039	.014	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.264	.134	1.96	.053	-.003	.531	*
BMI	-.003	.013	-0.26	.795	-.03	.023	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.122	.147	-0.83	.41	-.414	.17	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.055	.141	-0.39	.695	-.335	.225	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.058	.187	5.64	0	.686	1.431	***
BNT/BNT-84	1.197	.182	6.57	0	.835	1.558	***
BNT/ChAd-84	.763	.174	4.39	0	.418	1.107	***
Baseline serum anti-nucleocapsid IgA	-.062	.134	-0.46	.644	-.328	.204	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.458	.131	3.49	.001	.197	.719	***
Constant	3.718	.98	3.79	0	1.77	5.667	***

Mean dependent var	3.759	SD dependent var	0.820
R-squared	0.500	Number of obs	98
F-test	8.705	Prob > F	0.000
Akaike crit. (AIC)	192.348	Bayesian crit. (BIC)	220.782

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.011	.014	-0.80	.425	-.039	.017	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.287	.137	2.09	.04	.014	.559	**
BMI	-.001	.013	-0.09	.927	-.028	.025	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.126	.152	-0.83	.408	-.428	.176	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.059	.142	-0.41	.681	-.341	.224	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.091	.191	5.70	0	.71	1.471	***
BNT/BNT-84	1.212	.187	6.49	0	.841	1.584	***
BNT/ChAd-84	.779	.178	4.38	0	.425	1.133	***
Baseline mucosal anti-nucleocapsid IgG	.018	.086	0.21	.833	-.154	.19	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.426	.131	3.24	.002	.165	.687	***
Constant	3.381	.966	3.50	.001	1.461	5.302	***

Mean dependent var	3.760	SD dependent var	0.829
R-squared	0.504	Number of obs	96
F-test	8.630	Prob > F	0.000
Akaike crit. (AIC)	190.130	Bayesian crit. (BIC)	218.337

*** *p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.012	.014	-0.86	.39	-.041	.016	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.28	.137	2.04	.044	.007	.553	**
BMI	-.002	.013	-0.12	.905	-.028	.025	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.129	.152	-0.85	.399	-.43	.173	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.062	.142	-0.43	.665	-.344	.221	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.09	.191	5.72	0	.711	1.47	***
BNT/BNT-84	1.219	.185	6.60	0	.852	1.586	***
BNT/ChAd-84	.785	.177	4.44	0	.434	1.136	***
Baseline mucosal anti-nucleocapsid IgA	-.058	.152	-0.38	.704	-.36	.244	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.429	.132	3.26	.002	.168	.691	***
Constant	3.635	1.106	3.29	.001	1.436	5.833	***

Mean dependent var	3.760	SD dependent var	0.829
R-squared	0.504	Number of obs	96
F-test	8.650	Prob > F	0.000
Akaike crit. (AIC)	190.016	Bayesian crit. (BIC)	218.224

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.013	.013	-0.98	.33	-.04	.014	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.274	.134	2.04	.045	.006	.541	**
BMI	-.002	.013	-0.17	.868	-.028	.024	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.112	.15	-0.75	.456	-.41	.186	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.071	.145	-0.49	.623	-.359	.216	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.065	.187	5.70	0	.694	1.436	***
BNT/BNT-84	1.205	.183	6.58	0	.841	1.569	***
BNT/ChAd-84	.752	.175	4.30	0	.405	1.1	***
Baseline serum anti-OC43 spike IgG	.061	.174	0.35	.728	-.285	.407	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.44	.128	3.44	.001	.186	.694	***
Constant	3.29	1.133	2.90	.005	1.037	5.542	***

Mean dependent var	3.759	SD dependent var	0.820
R-squared	0.500	Number of obs	98
F-test	8.686	Prob > F	0.000
Akaike crit. (AIC)	192.453	Bayesian crit. (BIC)	220.888

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.011	.013	-0.83	.411	-.038	.016	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.269	.133	2.02	.047	.004	.533	**
BMI	-.005	.013	-0.35	.729	-.03	.021	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.116	.146	-0.79	.429	-.407	.175	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.063	.14	-0.45	.655	-.341	.215	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.083	.186	5.83	0	.714	1.452	***
BNT/BNT-84	1.217	.182	6.70	0	.856	1.578	***
BNT/ChAd-84	.799	.176	4.54	0	.449	1.149	***
Baseline serum anti-OC43 spike IgA	-.146	.134	-1.09	.277	-.412	.12	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.468	.129	3.63	0	.212	.724	***
Constant	3.968	.975	4.07	0	2.03	5.905	***

Mean dependent var	3.759	SD dependent var	0.820
R-squared	0.506	Number of obs	98
F-test	8.900	Prob > F	0.000
Akaike crit. (AIC)	191.253	Bayesian crit. (BIC)	219.688

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.01	.014	-0.72	.475	-.037	.018	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.291	.134	2.17	.033	.025	.557	**
BMI	-.004	.013	-0.31	.756	-.03	.022	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.13	.148	-0.87	.385	-.425	.165	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.076	.139	-0.55	.586	-.353	.201	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.105	.187	5.92	0	.734	1.476	***
BNT/BNT-84	1.164	.183	6.36	0	.8	1.528	***
BNT/ChAd-84	.783	.173	4.53	0	.439	1.127	***
Baseline mucosal anti-OC43 spike IgG	.218	.112	1.94	.055	-.005	.44	*
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.456	.129	3.53	.001	.199	.713	***
Constant	2.66	1.008	2.64	.01	.655	4.664	***

Mean dependent var	3.760	SD dependent var	0.829
R-squared	0.525	Number of obs	96
F-test	9.381	Prob > F	0.000
Akaike crit. (AIC)	186.008	Bayesian crit. (BIC)	214.216

*** *p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.011	.014	-0.81	.423	-.039	.017	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.289	.137	2.11	.038	.017	.561	**
BMI	-.003	.014	-0.20	.84	-.03	.024	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.117	.153	-0.77	.445	-.421	.186	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.058	.142	-0.41	.684	-.34	.224	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.084	.191	5.67	0	.704	1.465	***
BNT/BNT-84	1.22	.185	6.61	0	.853	1.588	***
BNT/ChAd-84	.776	.177	4.38	0	.424	1.128	***
Baseline mucosal anti-OC43 spike IgA	-.061	.114	-0.53	.597	-.288	.166	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.429	.131	3.27	.002	.168	.689	***
Constant	3.686	1.074	3.43	.001	1.551	5.821	***

Mean dependent var	3.760	SD dependent var	0.829
R-squared	0.505	Number of obs	96
F-test	8.678	Prob > F	0.000
Akaike crit. (AIC)	189.862	Bayesian crit. (BIC)	218.070

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.015	.013	-1.11	.272	-.042	.012	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.285	.134	2.13	.036	.019	.551	**
BMI	.001	.013	0.07	.947	-.025	.027	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.142	.147	-0.97	.337	-.435	.15	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.092	.143	-0.64	.521	-.375	.192	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.045	.186	5.61	0	.675	1.415	***
BNT/BNT-84	1.21	.181	6.68	0	.85	1.57	***
BNT/ChAd-84	.736	.174	4.24	0	.391	1.081	***
Baseline serum anti-HKU1 spike IgG	.169	.149	1.13	.261	-.128	.466	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.41	.13	3.15	.002	.151	.669	***
Constant	2.927	1.038	2.82	.006	.864	4.99	***

Mean dependent var	3.759	SD dependent var	0.820
R-squared	0.506	Number of obs	98
F-test	8.916	Prob > F	0.000
Akaike crit. (AIC)	191.162	Bayesian crit. (BIC)	219.597

*** *p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.011	.013	-0.84	.402	-.038	.015	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.264	.134	1.97	.052	-.002	.53	*
BMI	-.003	.013	-0.27	.791	-.029	.022	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.119	.147	-0.81	.419	-.411	.173	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.063	.14	-0.45	.655	-.342	.216	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.077	.186	5.78	0	.707	1.447	***
BNT/BNT-84	1.21	.182	6.64	0	.848	1.573	***
BNT/ChAd-84	.776	.175	4.45	0	.429	1.123	***
Baseline serum anti-HKU1 spike IgA	-.098	.136	-0.72	.471	-.369	.172	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.464	.131	3.56	.001	.205	.724	***
Constant	3.768	.951	3.96	0	1.878	5.658	***

Mean dependent var	3.759	SD dependent var	0.820
R-squared	0.502	Number of obs	98
F-test	8.766	Prob > F	0.000
Akaike crit. (AIC)	192.002	Bayesian crit. (BIC)	220.436

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.01	.014	-0.75	.453	-.037	.017	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.306	.133	2.30	.024	.041	.57	**
BMI	-.002	.013	-0.12	.903	-.027	.024	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.2	.15	-1.33	.187	-.499	.099	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.076	.138	-0.55	.582	-.35	.198	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.081	.185	5.85	0	.714	1.449	***
BNT/BNT-84	1.131	.183	6.17	0	.766	1.495	***
BNT/ChAd-84	.787	.171	4.59	0	.446	1.128	***
Baseline mucosal anti-HKU1 spike IgG	.229	.098	2.32	.023	.033	.424	**
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.438	.127	3.44	.001	.185	.691	***
Constant	2.711	.97	2.79	.006	.782	4.64	***

Mean dependent var	3.760	SD dependent var	0.829
R-squared	0.533	Number of obs	96
F-test	9.706	Prob > F	0.000
Akaike crit. (AIC)	184.281	Bayesian crit. (BIC)	212.489

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.011	.014	-0.81	.42	-.039	.017	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.28	.137	2.05	.043	.009	.552	**
BMI	0	.014	0.02	.985	-.027	.027	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.131	.152	-0.87	.388	-.433	.17	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.058	.142	-0.41	.682	-.34	.224	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.099	.19	5.77	0	.721	1.478	***
BNT/BNT-84	1.217	.185	6.59	0	.85	1.584	***
BNT/ChAd-84	.792	.177	4.47	0	.44	1.144	***
Baseline mucosal anti-HKU1 spike IgA	.062	.11	0.56	.575	-.157	.281	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.414	.132	3.13	.002	.152	.677	***
Constant	3.155	1.057	2.98	.004	1.053	5.257	***

Mean dependent var	3.760	SD dependent var	0.829
R-squared	0.505	Number of obs	96
F-test	8.685	Prob > F	0.000
Akaike crit. (AIC)	189.823	Bayesian crit. (BIC)	218.031

*** *p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.013	.013	-0.94	.349	-.039	.014	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.269	.135	2.00	.049	.001	.536	**
BMI	-.003	.014	-0.19	.85	-.03	.025	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.122	.147	-0.83	.41	-.415	.171	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.058	.143	-0.41	.685	-.343	.226	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.068	.186	5.73	0	.698	1.439	***
BNT/BNT-84	1.197	.184	6.49	0	.831	1.564	***
BNT/ChAd-84	.761	.175	4.36	0	.414	1.108	***
Baseline serum anti-NL63 spike IgG	-.007	.16	-0.04	.965	-.325	.311	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.443	.128	3.47	.001	.19	.697	***
Constant	3.563	1.136	3.14	.002	1.304	5.821	***

Mean dependent var	3.759	SD dependent var	0.820
R-squared	0.499	Number of obs	98
F-test	8.662	Prob > F	0.000
Akaike crit. (AIC)	192.588	Bayesian crit. (BIC)	221.023

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.013	.013	-0.97	.336	-.04	.014	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.265	.134	1.97	.052	-.002	.531	*
BMI	-.004	.013	-0.28	.782	-.03	.023	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.11	.149	-0.74	.462	-.407	.186	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.066	.141	-0.47	.643	-.347	.215	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.067	.186	5.73	0	.697	1.437	***
BNT/BNT-84	1.198	.182	6.58	0	.836	1.559	***
BNT/ChAd-84	.773	.176	4.40	0	.424	1.123	***
Baseline serum anti-NL63 spike IgA	-.061	.131	-0.46	.645	-.322	.2	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.459	.132	3.48	.001	.197	.722	***
Constant	3.753	1.014	3.70	0	1.737	5.768	***

Mean dependent var	3.759	SD dependent var	0.820
R-squared	0.500	Number of obs	98
F-test	8.704	Prob > F	0.000
Akaike crit. (AIC)	192.350	Bayesian crit. (BIC)	220.785

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.009	.014	-0.63	.527	-.037	.019	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.291	.136	2.14	.035	.021	.56	**
BMI	-.001	.013	-0.05	.961	-.027	.026	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.156	.152	-1.03	.308	-.459	.147	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.054	.141	-0.38	.705	-.334	.226	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.099	.189	5.81	0	.723	1.475	***
BNT/BNT-84	1.195	.184	6.48	0	.828	1.561	***
BNT/ChAd-84	.789	.175	4.50	0	.44	1.137	***
Baseline mucosal anti-NL63 spike IgG	.127	.105	1.21	.231	-.082	.335	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.447	.131	3.40	.001	.186	.708	***
Constant	2.942	1.021	2.88	.005	.911	4.973	***

Mean dependent var	3.760	SD dependent var	0.829
R-squared	0.512	Number of obs	96
F-test	8.914	Prob > F	0.000
Akaike crit. (AIC)	188.549	Bayesian crit. (BIC)	216.757

*** *p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.011	.014	-0.81	.423	-.04	.017	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.284	.137	2.08	.041	.012	.556	**
BMI	-.001	.014	-0.09	.928	-.028	.026	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.128	.154	-0.83	.408	-.435	.178	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.058	.143	-0.41	.686	-.343	.226	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.094	.191	5.74	0	.715	1.473	***
BNT/BNT-84	1.218	.185	6.58	0	.85	1.585	***
BNT/ChAd-84	.784	.177	4.43	0	.432	1.136	***
Baseline mucosal anti-NL63 spike IgA	.005	.14	0.04	.972	-.274	.284	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.424	.131	3.23	.002	.163	.685	***
Constant	3.396	1.149	2.95	.004	1.111	5.681	***

Mean dependent var	3.760	SD dependent var	0.829
R-squared	0.504	Number of obs	96
F-test	8.621	Prob > F	0.000
Akaike crit. (AIC)	190.179	Bayesian crit. (BIC)	218.387

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.012	.013	-0.93	.353	-.039	.014	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.267	.134	1.99	.05	0	.533	*
BMI	-.003	.013	-0.24	.811	-.029	.023	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.119	.147	-0.81	.421	-.412	.174	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.054	.142	-0.38	.703	-.336	.227	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.067	.186	5.72	0	.696	1.437	***
BNT/BNT-84	1.188	.185	6.44	0	.822	1.555	***
BNT/ChAd-84	.76	.174	4.38	0	.415	1.105	***
Baseline serum anti-229E spike IgG	-.05	.148	-0.34	.735	-.345	.244	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.445	.128	3.48	.001	.191	.698	***
Constant	3.77	1.136	3.32	.001	1.511	6.028	***

Mean dependent var	3.759	SD dependent var	0.820
R-squared	0.500	Number of obs	98
F-test	8.685	Prob > F	0.000
Akaike crit. (AIC)	192.461	Bayesian crit. (BIC)	220.895

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.013	.013	-1.01	.317	-.04	.013	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.242	.134	1.80	.075	-.025	.508	*
BMI	-.006	.013	-0.44	.664	-.032	.02	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.083	.148	-0.56	.578	-.378	.212	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.078	.14	-0.56	.577	-.357	.2	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.076	.185	5.83	0	.709	1.443	***
BNT/BNT-84	1.205	.18	6.68	0	.846	1.563	***
BNT/ChAd-84	.796	.174	4.58	0	.451	1.142	***
Baseline serum anti-229E spike IgA	-.183	.133	-1.37	.174	-.448	.082	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.467	.127	3.66	0	.213	.72	***
Constant	4.253	1.031	4.13	0	2.205	6.301	***

Mean dependent var	3.759	SD dependent var	0.820
R-squared	0.509	Number of obs	98
F-test	9.037	Prob > F	0.000
Akaike crit. (AIC)	190.496	Bayesian crit. (BIC)	218.930

*** *p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.01	.014	-0.71	.479	-.038	.018	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.286	.136	2.11	.038	.016	.557	**
BMI	-.002	.013	-0.12	.902	-.028	.025	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.15	.152	-0.98	.329	-.453	.154	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.056	.141	-0.40	.693	-.337	.225	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.115	.191	5.85	0	.736	1.494	***
BNT/BNT-84	1.2	.185	6.50	0	.833	1.568	***
BNT/ChAd-84	.804	.177	4.54	0	.452	1.157	***
Baseline mucosal anti-229E spike IgG	.105	.106	0.99	.325	-.106	.315	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.439	.131	3.35	.001	.179	.7	***
Constant	2.974	1.046	2.84	.006	.894	5.054	***

Mean dependent var	3.760	SD dependent var	0.829
R-squared	0.509	Number of obs	96
F-test	8.818	Prob > F	0.000
Akaike crit. (AIC)	189.082	Bayesian crit. (BIC)	217.290

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.012	.014	-0.83	.408	-.04	.016	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.279	.136	2.05	.044	.008	.55	**
BMI	-.002	.013	-0.17	.869	-.029	.024	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.119	.151	-0.79	.434	-.42	.182	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.068	.142	-0.48	.632	-.35	.214	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.093	.19	5.75	0	.715	1.47	***
BNT/BNT-84	1.228	.185	6.65	0	.861	1.596	***
BNT/ChAd-84	.777	.176	4.41	0	.426	1.128	***
Baseline mucosal anti-229E spike IgA	-.107	.134	-0.80	.428	-.374	.16	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.426	.131	3.26	.002	.166	.685	***
Constant	3.898	1.121	3.48	.001	1.668	6.127	***

Mean dependent var	3.760	SD dependent var	0.829
R-squared	0.507	Number of obs	96
F-test	8.749	Prob > F	0.000
Akaike crit. (AIC)	189.465	Bayesian crit. (BIC)	217.673

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.012	.013	-0.86	.393	-.038	.015	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.29	.132	2.21	.03	.029	.552	**
BMI	-.001	.013	-0.10	.921	-.027	.024	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.122	.148	-0.82	.412	-.416	.172	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.088	.139	-0.63	.528	-.364	.188	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.01	.182	5.54	0	.647	1.372	***
BNT/BNT-84	1.176	.182	6.46	0	.815	1.538	***
BNT/ChAd-84	.732	.178	4.12	0	.379	1.086	***
Baseline Seromucostatus (Reference: Seronegative/Muconegative)	0	.	.	.	.	.	
Seronegative/Mucopositive	-.26	.211	-1.24	.22	-.679	.159	
Seropositive	.006	.182	0.04	.972	-.354	.367	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.393	.128	3.08	.003	.139	.647	***
Constant	3.502	.893	3.92	0	1.727	5.277	***

Mean dependent var	3.759	SD dependent var	0.813
R-squared	0.499	Number of obs	100
F-test	7.959	Prob > F	0.000
Akaike crit. (AIC)	196.253	Bayesian crit. (BIC)	227.515

*** p<.01, ** p<.05, * p<.1*

Linear regression

Peak Mucosal anti-SARS-CoV2 spike IgG	Coef.	St.Err.	t-value	p-value	[95% Conf	Interval]	Sig
Age	-.012	.013	-0.88	.383	-.038	.015	
Sex: (Reference: Male)	0	.	.	.	.	.	
Female	.281	.132	2.13	.036	.019	.543	**
BMI	-.002	.013	-0.12	.904	-.027	.024	
Comorbidity (Referece: Non-comorbid)	0	.	.	.	.	.	
≥1 Comorbidity	-.115	.147	-0.78	.436	-.408	.177	
Ethnicity (Reference: White)	0	.	.	.	.	.	
Non-white	-.075	.139	-0.54	.591	-.35	.201	
Study Arm (Reference: ChAd/ChAd-84	0	.	.	.	.	.	
ChAd/BNT-84	1.029	.183	5.61	0	.664	1.393	***
BNT/BNT-84	1.196	.182	6.58	0	.835	1.557	***
BNT/ChAd-84	.765	.173	4.42	0	.421	1.11	***
Baseline Mucostatus (Reference: Muconegative)	0	.	.	.	.	.	
Mucopositive	-.073	.16	-0.46	.647	-.391	.244	
Site (Reference: Oxford)	0	.	.	.	.	.	
Southampton	.413	.126	3.27	.002	.162	.663	***
Constant	3.477	.892	3.90	0	1.705	5.249	***

Mean dependent var	3.759	SD dependent var	0.813
R-squared	0.491	Number of obs	100
F-test	8.587	Prob > F	0.000
Akaike crit. (AIC)	195.773	Bayesian crit. (BIC)	224.430

*** p<.01, ** p<.05, * p<.1*