Humoral immune responses to ChAdOx1 nCoV-19 in children

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List of publications

1. Kelly S, Liu X, Theiss-Nyland K, Voysey M, Murphy S, **Li G**, Nyantaro M, Gurung M, Basnet S, Pokhrel B, Bijukchhe SM, Eordogh A, Gombe B, Kakande A, Kerridge S, Kimbugwe G, Kusemererwa S, Lubyayi L, Luzze H, Mazur O, Mujadidi YF, Nabukenya S, Nagumo WR, Nareeba T, Noristani R, O'Reilly P, Roberts A, Shah G, Shrestha S, Shrestha LP, Thapa SB, Kibengo FM, Sharma AK, Elliott A, Shrestha S, Pollard AJ. Optimising DTwP-containing vaccine infant immunisation schedules (OptImms) – a protocol for two parallel, open-label, randomised controlled trials. *Trials*. 2023.24:465.

2. Bijukchhe SM, O'Reilly PJ, Theiss-Nyland K, Gurung M, Pokhrel B, Kelly S, Acharya S, Maharjan S, Shrestha A, Pandey B, Lama M, Shrestha I, Voysey M, Eordogh A, Shrestha S, Feng ES, **Li G**, Shah G, Pollard AJ, Shrestha S. COVID-19 vaccine effectiveness and variants in Nepal: study protocol for a test-negative case-control study with SARS-CoV-2 genetic sequencing. *BMJ Open*. 2023.13:e068334.

3. Mentzer AJ, O'Connor D, Bibi S, Chelysheva I, Clutterbuck EA, Demissie T, Dinesh T, Edwards NJ, Felle S, Feng S, Flaxman AL, Karp-Tatham E, **Li G**, Liu X, Marchevsky N, Godfrey L, Makinson R, Bull MB, Fowler J, Alamad B, Malinauskas T, Chong AY, Sanders K, Shaw RH, Voysey M; Oxford COVID Vaccine Trial Genetics Study Team Group; Snape MD, Pollard AJ, Lambe T, Knight JC. Human leukocyte antigen alleles associate with COVID-19 vaccine immunogenicity and risk of breakthrough infection. *Nat Med*. 2023. 29:147-157.

4. Marchevsky NG, **Li G**, Aley P, *et al*. An exploratory analysis of the response to ChAdOx1 nCoV-19 (AZD1222) vaccine in males and females. *EbioMedicine*. 2022;81:104128.

5. **Li G**, Cappuccini F, Marchevsky NG, *et al*. Safety and immunogenicity of the ChAdOx1 nCoV-19 (AZD1222) vaccine in children aged 6-17 years: a preliminary report of COV006, a phase 2 single-blind, randomised, controlled trial. *Lancet*. 2022. 11;399:2212-2225.

6. **Li G**, Finn A, Pollard AJ. Should we be vaccinating children against COVID-19 in high-income countries? *Expert Rev Vaccines*. 2021. 20:1043-1046.

Declaration of Contributions

I contributed to the study protocol and study documents for the phase II trial described in this thesis. I also conducted study training, enrolment visits and study visits which included collection of blood samples that would later be used for laboratory assays. Total IgG ELISAs and ELISpots were conducted by research assistants at the Jenner Institute. Isotype and subclass ELISAs were optimised by Holly Smith and multiplex bead-based immunoassays were optimised by Dr Sandra Belij-Rammerstorfer. Final isotype and avidity ELISAs and MSD assays were conducted by me with supervision from Dr Emma Sheehan. Most multiplex bead-based immunoassays were conducted by me with assistance from Audrey Song and Dr Susanna Camara. Where assays were conducted by commercial or public health laboratories external to the University of Oxford, this has been indicated in the thesis. Statistical oversight has been provided by Dr Xinxue Liu and Natalie Marchevsky.

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Abstract

COVID-19 vaccination of children and young people is recommended in some countries. Data on immune responses induced by COVID-19 vaccines in children have been reported less widely than for adults.

COV006 was a phase II, single-blind, randomised controlled trial of ChAdOx1 nCoV-19 in healthy participants 6-17 years of age. Children were assigned to four groups to receive two intramuscular doses of $5x10^{10}$ viral particles of ChAdOx1 nCoV-19 or control, 28 or 84 days apart. Study groups were stratified by age (6-11 years and 12-17 years). Due to restrictions in the use of ChAdOx1 nCoV-19 in people under the age of 30, introduced during the study, only participants 12-17 years of age who were randomised to the 28-day interval received their vaccines at the intended interval. The remaining participants received their vaccines at a 112-day interval. The primary outcome was safety, and the secondary outcome was immunogenicity. Exploratory immunology studies were also conducted.

Between February and April 2021, 262 participants were randomised into the study. No serious adverse events related to ChAdOx1 nCoV-19 were recorded. 80% of participants (169 of 210 ChAdOx1 nCoV-19 recipients) reported at least one solicited local or systemic side effect in the first seven days after vaccination. Pain and tenderness were the most common local side effects. Among seronegative participants, anti-SARS-CoV-2 IgG titres were higher in participants 12-17 years of age who received their vaccinations at a 112-day interval compared with 28-day interval, measured at 28 days after the second dose (geometric means 1963 EU/ml, [95% CI 1575-2448] versus 1194 EU/ml [95% CI 908-1568])) and highest in younger participants 6-11 years of age who received their vaccine at

a 112-day interval (geometric mean 2310 EU/ml [95% CI 1700-3138]). Significant boosting of betacoronavirus titres was measured after the first dose of ChAdOx1 nCoV-19 in children but not adults over 18 years of age (ANOVA, $p<0.05$). Children 6-11 years of age generated higher titres of SARS-CoV-2 spike-specific IgG1 than older children and adults (ANOVA, $p=0.02$). SARS-CoV-2 spike-specific FcγR 2a binding correlated most closely with psuedoneutralising antibody titres (Spearman, $r=0.89$).

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Chapter 1. Introduction

1.1 Historical pandemics and their impact on their paediatric population

Historical pandemics have had a significant and widespread impact on the mortality and morbidity of paediatric as well as adult populations. These include pandemics of both bacterial and viral aetiology, several of which have taken place within the last century.

1.1.1 Pandemics of bacterial aetiology

Plague is caused by the flea-borne gram-negative coccobacillus bacteria *Yersinia pestis* and has been linked to three pandemics in humans, the plague of Justinian, the Black Death and the third plague.[1] It has three clinical manifestations, bubonic, septicaemic and pneumonic plague. Bubonic plague is the most common form caused by a bite of an infected flea. Swollen lymph nodes (buboes) may develop accompanied by fever, headache and chills. Pneumonic plague arises from lung infection by *Y. pestis*, from respiratory droplet infection or if bubonic or septicaemic plague are untreated and spread to the lungs. Septicaemic plague can arise as a complication of bubonic or pneumonic forms or in isolation. Early treatment in the form of antibiotics active against enterobacteria, such as fluroquinolones and tetracyclines, can be effective if administered promptly, however if left untreated case fatality rates (CFR) of 30% to 60% have been reported for bubonic plague.[2]

The third plague originated in the province of Yunnan in China in the middle of the 19th century which reached Canton and spread globally via shipping routes through Hong Kong, resulting in over 400 deaths in Europe.[3] Cases were detected on ships docking in London in the late 1890s and international conferences were held in Europe at the time

encouraged regular reporting of cases. Child-specific estimates of morbidity and mortality were not well documented as analysis comparing age groups of cases at the time were not common. However, European sources recorded paediatric cases implicated in within and between household transmission, and associated fatalities of both adults and children. Children playing near the Seine River were thought to be the first cases in a new outbreak of the plague in Paris in 1920, the "peste des chiffoniers".

Plague is now considered a re-emerging disease, with ongoing outbreaks since the 1990s. Madagascar accounts for approximately 75% of cases reported to the World Health Organisation (WHO), and over 2000 cases were reported between 2013-2018,[2,4] with a case fatality rate of 20%, most of which were of the bubonic form.[5] Characterisation of a recent urban plague outbreak in 2017 reported an unusual predominance of pneumonic plague which showed that children under the age of 14 accounted for 26% of confirmed pneumonic cases and 45% of confirmed bubonic cases.[6] However, the authors suggest that a lower median age for bubonic plague cases could be explained by the fact that younger adults are more likely to work in agriculture and suggested that children spend more time close to the floor than adults, leading to greater exposure to flea bites.

Another pandemic of bacterial origin which has occurred in the last century is the seventh cholera pandemic, which was officially declared by the WHO in 1961. An acute diarrhoeal infection caused by consumption of food or water contaminated with *Vibrio cholerae*, mortality rates of cholera range from 1% if treated to 25-50% if untreated.[7] The majority of infected individuals can be managed with oral rehydration solution (ORS) which United Nations Children's Fund (UNICEF)/WHO supply in standard sachets requiring 1 litre of clean water to create standard ORS.

The current pandemic is attributed to the El Tor strain, which was first detected in Makassar, South Sulawesi in 1960 and subsequently spread abroad via shipping routes. The strain reached Africa in 1971 and the Americas in 1991.[8] The WHO estimates that each year there are 1.3-4 million cases, and 21,000 – 143,000 associated deaths, with 1 in 4 cases worldwide affecting children under 5 years of age.[7] Global mortality rates in children under the age of 5 years are estimated to be more than 20 times higher than in those 5 -19 years of age.[9] Mortality and morbidity in children vary with region and time however age-specific data have been gathered from specific outbreaks such as those in Haiti.

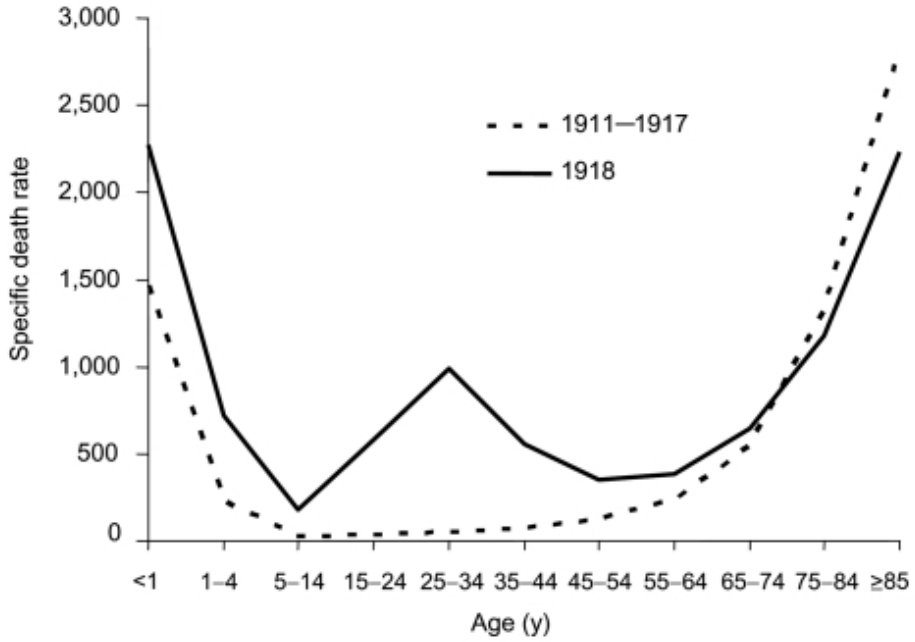
Following the 2010 earthquake and hurricane, cholera re-emerged in the Artibonite area of Haiti after almost a century of no cases being reported. Following extensive investigation by the United Nations and the French Embassy, it was concluded that the source of the outbreak was a river flowing next to a UN military camp, and a pathogenic strain of south Asian type cholera was likely to have been introduced by human activity.[10] Approximately 820,000 cases of cholera and 9782 deaths were reported in Haiti between October 2010 and February 2019. After 3 years with no reported cases of cholera, a resurgence in cases was recorded in late 2022. Between October and December, 13, 672 suspected cases (with 283 deaths at a case fatality rate of 2.05%) were reported by the Haiti Ministry of Public Health and Population. The most affected groups by age were children 1-4 years of age (19%) followed by adults 20 to 29 years of age (15%) and 30 to 39 years of age (15%).[11]

1.1.2 Pandemics of viral aetiology

The WHO's response to the 2020 COVID-19 pandemic was influenced by previous experiences of viral respiratory pathogen outbreaks. Whilst the COVID-19 pandemic led to the greatest burden of disease and mortality in older adults and those with co-morbidities,[12,13] previous pandemics have affected children and adults of different age groups to different degrees.

For example, the 1918/1919 Spanish flu pandemic has been credited as the most influential in determining how the COVID-19 pandemic was approached at a global public health policy level but had a very different impact on different age groups in the population by comparison. Records show that mortality rates from Spanish flu were higher in young adults,[14] peaking at 28 years of age. Mortality rates were also higher in those aged under 5 years and over 65 years, so that plotting mortality data for the pandemic produces graphs with an appearance such as seen in **Figure 1. 1.**

Figure 1.1: "U" and "W" shaped curves showing combined mortality from influenza and pneumonia, by age per 100,000 persons per group, United States, 1911-1918. *The dotted line shows mortality rates for the pre-pandemic years and the solid line for the pandemic year 1918. From Taubenberger J and Morens D. "1918 Influenza: the Mother of All Pandemics". Emerg Infect Dis, 2006. 12(1):15-22.*



Reverse genetics was used to create an influenza virus bearing gene segments obtained from lung tissues preserved from victims of the 1918 pandemic, and molecular evidence now supports the theory that the pandemic resulted from an antigenically novel influenza virus.[15] Pre-pandemic (1900-1917) children aged 5-15 years of age were estimated to represent 11% of total influenza cases in the US, whilst individuals >65 years of age accounted for 6% of annual influenza cases. However, during the 1918 pandemic, the proportion of total cases that were recorded in children 5-15 years of age jumped to 25% with those >65 years of age falling to 0.6%.[16] This led many countries (such as the UK and United States) to introduce a policy of school closures as well as banning children from public places such as theatres. However, some larger US cities such as New York kept schools open whilst instituting enhanced 'medical inspection' of children to enable early

isolation of symptomatic children.[17] These inspections were also a source of epidemiological data for the local government as the pandemic progressed. The observed difference in burden of disease by age group is now felt to be consistent with the theory that older adults possessed prior immunity against the 1918 strain, possibly induced by exposure to a related viral protein which individuals >65 years of age had previous exposure to, but to which children had not.

By contrast, the 2003 severe acute respiratory syndrome (SARS) outbreak attributed to a novel coronavirus SARS-CoV in South-East Asia demonstrated a milder clinical course in children under 12 years of age than in adults. Clinical cases in children reported a shorter time to resolution of illness and lower levels of documented community transmission.[18] No deaths were reported in children under 12 years of age and no cases of vertical transmission were reported in neonates. Longitudinal analysis of paediatric clinical samples showed that the predominant serum cytokine profile in patients represented a type 1 T helper lymphocyte response with correspondingly limited increases in interleukin 6 and tumour necrosis factor alpha levels.[19] At the time, it was hypothesised that the lower burden of disease in children could be due to greater exposure to seasonal coronaviruses leading to higher levels of pre-existing immunity and hence cross-reactive antibodies providing a greater degree of protection in children. However, this hypothesis does not appear to have been explored further in this cohort of paediatric patients.

The 2009 H1N1 (swine flu) outbreak was designated a pandemic by the WHO in September 2009.[20] The 2009 virus was shown to contain genetic elements from four sources – classical and Eurasian swine as well as human and avian influenza strains. Children and young adults under 18 years of age were disproportionately affected, with

more severe outcomes in children under 5 years of age. 70 paediatric deaths were reported in the UK, with a mortality rate of 6 per million population[21] and the highest mortality rate in children under 12 months of age and from ethnic minority backgrounds.

In September 2009, the European Medicines Agency (EMA) recommended to the European Commission that two vaccines be authorised for the H1N1 swine flu pandemic. These were Focetria (MF59-adjuvanted, inactivated vaccine, Novartis) and Pandemrix (AS03-adjuvanted, inactivated vaccine, GlaxoSmithKline).[22] Prior immunogenicity studies of trivalent inactivated influenza vaccines in children had demonstrated superiority of a two-dose schedule compared with just one in adults.[23] Given the greater burden of disease in children and vulnerable adults, both vaccines were recommended in a two-dose schedule at an interval of three weeks, for children from six months of age as well as pregnant women. The 2009 H1N1 vaccination programme implemented in the US was estimated to have prevented approximately 20 deaths in children.[24] However, following similar vaccination campaigns across Europe, including the UK where at least 850,000 doses were estimated to have been given to children between 6 months and 16 years, pharmacovigilance monitoring found that Pandemrix vaccination was associated with an increased risk of narcolepsy.[25] Renewal of authorisation for these two vaccines was not pursued by their respective manufacturers and the WHO downgraded the swine flu pandemic status 12 months later.

An outbreak of a second novel coronavirus, Middle East Respiratory Syndrome coronavirus (MERS) has also affected several countries in the Middle East. Again, associated with a range of clinical outcomes from asymptomatic infection to severe acute respiratory distress with respiratory failure and death, MERS was first isolated from a

human case in Saudi Arabia in 2012, with the natural host thought to be the dromedary camel. Likened to SARS, mortality rates in admitted adult patients were considerably higher with a case fatality rate of 60% reported in initial cases.[26] Comparative epidemiology of adult and paediatric MERS cases found that paediatric cases were rare (1.7%), more likely to be asymptomatic and less likely to be fatal than adult cases.[27] Children were more likely to report contact with an infected case and because of this, it was felt that they were at risk primarily within a household setting and their role in transmission of MERS was limited; instead a role for healthcare-associated infections was significant in the MERS outbreak.[28] Asymptomatic infection rates were found to vary from 7% to 66% in epidemiological studies[27] and without extensive household testing it was unclear at the time the extent to which children contributed to this.

In summary, historical outbreaks and pandemics have illustrated how differences between adult and children in terms of their social behaviours and immune characteristics suggest they need to be considered separately to adults in an outbreak context. Children reside in educational institutions with not only high levels of social mixing but also giving the opportunity for them to be observed over time. They possess immune systems with different levels of exposure to circulating viruses than adults which respond differently to viral challenges and may have different responses to pandemic vaccines. Whilst all of these factors should be considered, the questions posed in this thesis will focus on age-specific responses to vaccines.

1.2 The SARS-CoV-2 pandemic

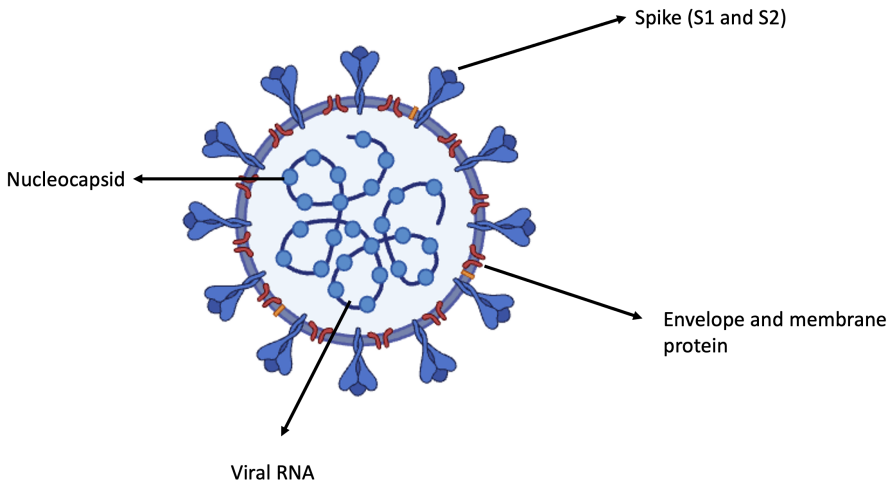
The WHO was informed of cases of pneumonia of unknown aetiology in Wuhan City, Hubei Province, China, on 31 December 2019[29] and shortly thereafter it was announced

that the genetic sequence of a novel coronavirus had been detected in clinical samples gathered from Chinese patients. The first sequences of the virus were published on 10th January 2020 by the Wuhan Center for Disease Control and Prevention together with additional Chinese public health stakeholders and the University of Sydney, Australia and were deposited on GenBank.[30] Using this information, the *Coronaviridae* Study Group of the International Committee on Taxonomy of Viruses assessed the placement of the virus within the existing nomenclature system and recognised it as belonging to a sister clade of human and bat severe acute respiratory syndrome coronaviruses, designating it SARS-CoV-2, with the infectious disease that it causes subsequently termed COVID-19. A public health emergency of international concern was subsequently announced on 30th January 2020, and due to rapid global spread, a pandemic was declared on 11th March 2020.[31]

1.2.1 The Virus

SARS-CoV-2 is a *Betacoronavirus,* within the single-strand positive-sense ribonucleic acid (RNA) virus family *Coronaviridae,* order *Nidovirales.* The reference strain (Wuhan-Hu-1) originated from a clinical sample taken from a patient in China on 26th December 2019 and is 29 903 nucleotides in length.[32] The gene order is similar to that of other known coronaviruses – 5' replicase ORF1ab-S-E-M-N-3'. The subsequent downstream open reading frames (ORFs) encode spike (S), envelope (E), membrane (M) and nucleocapsid (N) genes. The coronavirus ORF1ab gene is known to contribute to 16 non-structural proteins. The 5' and 3' terminal sequences of the reference strain are also typical of those found in betacoronaviruses,[33] possessing lengths of 265 and 229 nucleotides respectively. A schematic diagram of the structure of the virus is shown in **Figure 1.2**.

Figure 1.2: Schematic of SARS-CoV-2 structure[1]



Following the 2003 SARS-CoV outbreak, a consensus had developed around the use of the spike protein of SARS-CoV as a target for vaccine and therapeutic development.[34] The spike protein was known to play a vital role in viral attachment and host cell entry, as the spike protein required cleavage into S1 and S2 units by proteases to become fusion competent. The receptor binding domain of SARS-CoV S1 was also known to bind to host cell receptors and was also responsible for viral fusion with host cells. Amongst all the structural proteins of SARS-CoV, spike protein was recognised as the main antigenic domain inducing immune responses and neutralising antibodies.

1.2.2 Mode of transmission

Crucial to the implementation of effective infection control policy was understanding the method of transmission of SARS-CoV-2. The extent to which SARS-CoV-2 is primarily

[1] Drawn with help from biorender.com

transmitted through close contact in droplets versus longer distance airborne transmission via aerosols has been debated extensively, partly due to variation in the terminology and size definition of respiratory particles that could transmit SARS-CoV-2 and the time needed for an evidence base to emerge.[35] For example, the size threshold set for a respiratory droplet by the World Health Organisation is >5μm, [36] below which droplets are referred to as droplet nuclei which can remain in the air for a longer period and be transmitted over longer distances. By contrast, exposure scientists and industrial hygienists refer to the size of an inhalable aerosol at 100μm.[37] This had implications for the infection prevention and control advice produced by the WHO. Although initial advice focused on prevention of infection via the droplet route (including the need for face masks and adequate hand hygiene), subsequent advice also includes precautions to be taken in indoor areas as the evidence base for airborne transmission has grown.[38]

Early estimates of the basic reproduction number (R_0), i.e. the number of SARS-CoV-2 cases that would result from one index case in a fully susceptible population, ranged from 2-3.5 in China,[39] with UK estimates in a similar range.[40] This compared with a typical R_0 of 1-2 for seasonal influenza in a UK flu season.[41]

1.2.3 Incubation period and clinical characterisation

An accurate assessment of the incubation period of SARS-CoV-2 was important in determining advice on quarantine, testing strategies and to inform modelling. Before the emergence of novel variants, the mean incubation period was estimated at between 5.1 to 6.5 days in length.[42] The emergence of variants is discussed in Chapter 4. However, the incubation period has been shown to be significantly reduced in cases of Omicron infection versus cases of other variants of concern in young people 18-29 years of age.

Individuals infected with SARS-CoV-2 experienced a spectrum of symptoms from asymptomatic infection to critical illness. Accurate monitoring of the global situation was facilitated by the publication of case definitions outlined in **Table 1.1.**

Table 1.1 World Health Organisation COVID-19 case definitions 22nd July 2022.
Adapted from "WHO COVID-19 case definition"

Suspected case of SARS-CoV-2 infection	A	*Clinical criteria* Acute onset of fever AND cough OR Acute onset of three or more of following: fever, cough, general weakness/fatigue, headache, myalgia, sore throat, coryza, dyspnoea, nausea/diarrhoea/anorexia OR *Epidemiological criteria* Contact of a probably or confirmed case, or linked to a COVID-19 cluster[a]
	B	A patient with severe acute respiratory illness (defined as acute respiratory infection with history of fever or measured fever of greater or equal to 38C and cough, with onset within the last 10 days and requiring hospitalisation)
	C	A person with no clinical signs or symptoms OR meeting epidemiological criteria but with a positive professional/self-test SARS-CoV-2 antigen rapid test
Probable case of SARS-CoV-2 infection	A	A patient who meets clinical criteria AND is a contact of a probable or confirmed case, or linked to a COVID-19 cluster
	B	Death, not otherwise explained, in an adult with respiratory distress preceding death AND who was a contact of a probable or confirmed case or linked to a COVID-19 cluster
Confirmed case of SARS-CoV-2 infection	A	A person with a positive Nucleic Acid Amplification Test (NAAT), regardless of clinical criteria OR epidemiological criteria
	B	A person meeting clinical criteria AND/OR epidemiological criteria with a positive professional/self-test SARS-CoV-2 antigen rapid test

[a]COVID-19 cluster defined as a group of symptomatic individuals linked by time, geographic location and common exposure, containing at least one nucleic acid

amplification test-confirmed case or at least two epidemiologically linked, symptomatic persons who are rapid-antigen test positive

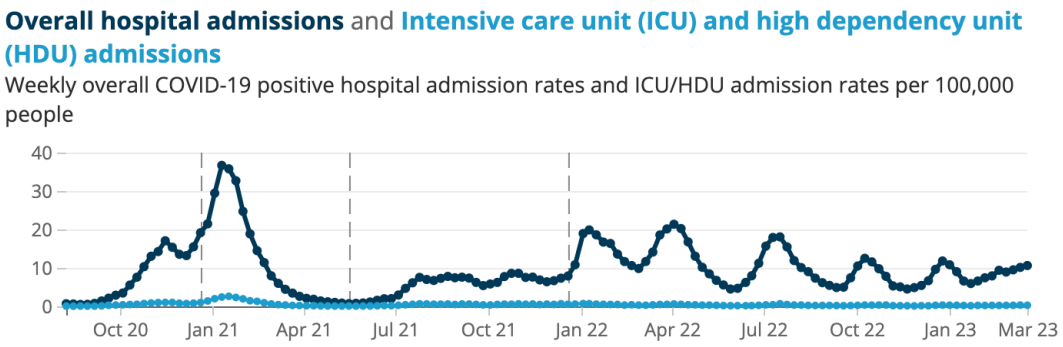
To facilitate harmonisation of clinical research between countries, the WHO Research and Development programme devised a minimum set of outcome measures for clinical studies. This resulted in the WHO Clinical Progression scale (Table 2, below), to objectively score the patient's clinical progress as they moved through the healthcare system.[43]

Table 2.2 WHO Clinical Progression scale. *Adapted from "A minimal common outcome measure set for COVID-19 clinical research". WHO Working Group on the Clinical Chracterisation and Management of COVID-19 infection. Lancet Infect Dis. 2020. 20(8):e192-e197.*

Patient State	Descriptor	Score
Uninfected	Uninfected; no viral RNA detected	0
Ambulatory mild disease	Asymptomatic; viral RNA detected	1
	Symptomatic; independent	2
	Symptomatic; assistance needed	3
Hospitalised: moderate disease	Hospitalised; no oxygen therapy*	4
	Hospitalised; oxygen by mask or nasal prongs	5
Hospitalised: severe diseases	Hospitalised; oxygen by NIV or high flow	6
	Intubation and mechanical ventilation, pO_2/FiO_2 ≥150 or SpO_2/FiO_2 ≥200	7
	Mechanical ventilation pO_2/FIO_2 <150 (SpO_2/FiO_2 <200) or vasopressors	8
	Mechanical ventilation pO_2/FiO_2 <150 and vasopressors, dialysis, or ECMO	9
Dead	Dead	10

During the initial and subsequent waves of infection during 2020 and 2021, national healthcare systems were placed under severe pressure by demand that the COVID-19 pandemic placed on critical care and intensive care beds, particularly by patients requiring respiratory support and oxygen. In the UK, the number of HDU/ITU beds occupied by confirmed COVID-19 patients grew from 22 on March 10th 2020, to 1796 on March 31st 2020, in a country where adult critical care bed capacity in 2020 was estimated to be 4,130.[44] **Figure 1.3** illustrates the burden that COVID-19 positive patients continued to place on the UK NHS system throughout 2020 and 2021.

Figure 1.3 – Number of COVID-19 patients admitted to hospital in the UK between April 2020 and July 2022 across England, Ireland, Scotland and Wales. *Wales figures were suspected cases whilst the other three nations reported confirmed cases[2].*



The majority of clinical data from the first wave of COVID-19 came from severe cases which had been admitted to intensive care. In the UK, of 10,834 patients admitted to critical care between February and August 2020, 70.1% were male, with a median age of 60 years and 32.6% were of non-white ethnicity.[45] 39.4% were clinically obese and 8.2% reported a serious comorbidity in their clinical history.

[2] *https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/articles/coronaviruscovid19latestinsights/overview . Accessed 19th July 2024*

1.2.4 Mortality and morbidity

Age, sex and ethnicity emerged as risk factors for severe COVID-19 disease and death.[46] Estimates of CFR were, and remain heavily dependent upon, the level of testing for SARS-CoV-2 that was being undertaken in healthcare settings and in the community in each country at a point in time. Initial estimates of the CFR from COVID-19 disease came from China and ranged from as high as 12% in heavily affected areas in Wuhan to 1% in less affected areas in China.[47]

A recent review of global data suggests that worldwide, CFR peaked at approximately 7% during April 2021.[48] The crude mortality rate as well as excess mortality rate in the UK was notably higher than in neighbouring European countries with similar demographic characteristics[49] and it has been proposed that the number of SARS-CoV-2 tests being conducted in the community in the UK may have contributed to these results. Analysis has confirmed that the relationship between the number of tests performed, and CFR was negative in the UK during 2020.[50]

In the United Kingdom, 92.3% of deaths were in individuals aged 60 years of age and over, and 58.3% of deaths in those 80 years of age and over. Deaths were higher amongst men and after age-standardising for the population, mortality rates remained higher in non-white ethnic groups.[51] Although children were initially thought to be at lower risk of infection due to the predominance of severe cases amongst older adults who represented the majority of those admitted to hospital with COVID-19 disease, serological studies in the UK have shown that seroprevalence of anti-nucleocapsid antibody was similar in adults and children after the first wave in 2020, supporting the theory that children and adults had

a similar risk of getting infected but that children had a higher likelihood of asymptomatic disease.[52] As of May 2024, over 775 million confirmed cases and over 7 million deaths have been reported globally.[53] It is estimated that 0.4% of these deaths occurred in individuals under 20 years of age.[54]

1.3 Development of a vaccine for adults

1.3.1 Adenoviral structure and mechanism of action as a vaccine platform

Adenoviruses are non-enveloped, double stranded DNA icosahedral viruses with a linear genome varying in length from 26-48 kilobase pairs (kbp) depending on the native species.[55]The genome capsid consists primarily of the major coat protein hexon, which forms icosahedron facets. Hexon is a polypeptide of over 900 amino acids in length and accounts for approximately 60% of the virion mass. The vertices consist of penton base and fibres, whilst minor coat proteins are located on both the inner and outer surface of the capsid and change with species. Hexons form trimers and each icosahedral unit comprises 4 hexon trimers with one penton molecule, in total 13 polypeptides. Trimeric fibres attach to the outer surface of the penton base, composed of three domains – a head, shaft and N-terminal tail. The C-terminus head is responsible for attachment to the receptor of the host cell membrane. Adenoviruses encode a large quantity of DNA-binding proteins within the virion itself. These include proteins V, VII and μ, which are basic and thought to act as DNA condensing agents.

The vaccine antigen requires incorporation into the viral genome at a well-characterised location, and in adenoviral-vectored vaccines this is the E1 locus.[56] Simultaneous insertion

of a restriction site at the adenoviral E1 locus leaves the ChAd virus replication deficient as the E1 region of the genome is essential for viral replication. Because ChAds are non-enveloped, the vaccine antigen is not expressed on the surface of the virion but is expressed at high levels after the vector enters the target cells (myocytes) of the vaccine recipient.

Viral vectors are effective inductors of the innate, humoral and cellular immune responses. Pathogen-associated molecular patterns of adenoviruses are recognised by cell pattern-recognition receptors, such as toll-like receptors TLR2/4 in the plasma membrane of immune cells, including dendritic cells, T cells, neutrophils, monocytes and epithelial cells.[57] Viral vectors infect host cells and lead to express of vaccine antigen on the infected cell surface membrane which can be recognised by B cell receptors and initiate T-independent B-cell activation. Phagocytosis of cells infected with the viral vector can lead to presentation of antigen by antigen presenting cells via MHC molecules, stimulating the proliferation of CD4+ and CD8+ T cells.

1.3.2 ChAdOx1 CoV-19

ChAdOx1 nCoV-19 was developed for prevention of ancestral COVID-19 disease directed against the Wuhan strain. It is a recombinant replication-defective chimpanzee adenovirus vector expressing the SARS-CoV-2 spike (S) surface glycoprotein.[58] This is driven by the human cytomegalovirus major immediate early promoter that includes intron A and a human tPA leader sequence. ChAdOx1 nCoV-19 was previously shown to be immunogenic in mice, ferrets, non-human primates and pig models.[59] Post-vaccination challenge in rhesus macaques showed that a single vaccination significantly reduced viral

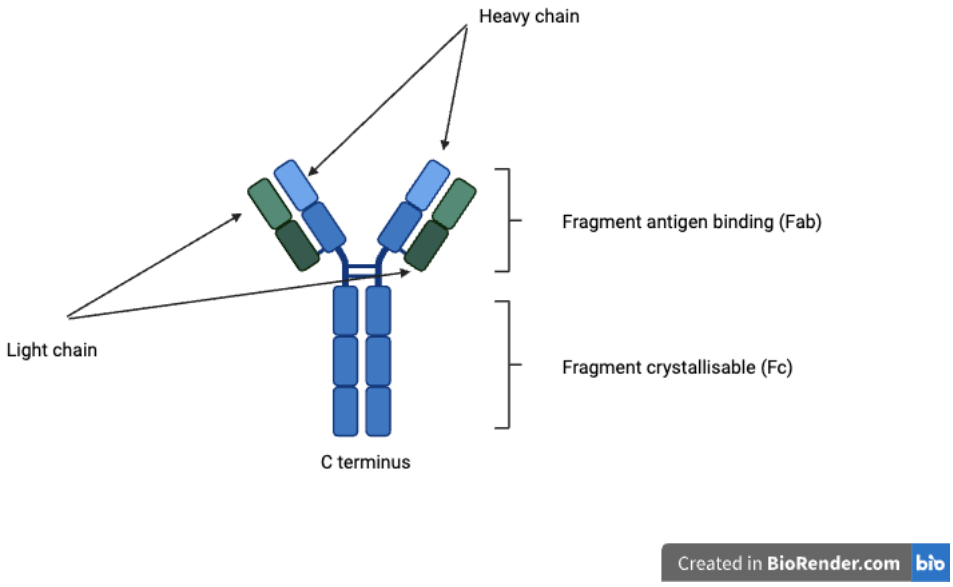
load in the bronchoalveolar lavage fluid and respiratory tract tissue of vaccinated animals compared with controls.[60]

1.3.3 Characterising antibody responses to vaccination

1.3.3.1 Antibody structure

Antibodies are large molecules with a molecular weight approximately 150kDa, and consist of two different polypeptide chains in duplicate.[61] The heavy chain (H) is approximately 50 kDa in size and the light (L) chain is approximately 25 kDa in size. Two heavy chains are linked together by a disulphide bond and the heavy chains are each linked to a light chain by a disulphide bond. Antibodies are 'Y' shaped molecules which consist of three broad regions – two fragment antigen binding regions (Fab) which are identical in structure and whose function is to bind to antigen (**Figure 1.4**). The third region (Fc, fragment crystallisable) is involved in binding to effector molecules.

Figure 1.4 Schematic illustration of antibody molecule



There are 5 classes of antibody, IgM, G, A, D and E which have the same four chain antibody structure but differ in the amino acid composition and structure of the heavy chains, which are named μ, γ, α, δ and ε respectively.

The differences are greatest in the Fc region which leads to differing profiles of effector functions. The carboxy (or C) terminal half of the light chain and two-thirds of the heavy chain does not differ significantly between different immunoglobulin molecules. However, the N terminal of approximately 100 amino acid residues in both chains demonstrates considerable variability between molecules. As different immunoglobulin molecules recognise and bind to different antigens and these are the regions of structural variability between molecules, these hypervariable regions are referred to as complementarity determining regions.

1.3.3.2 Antibody affinity and avidity

Affinity can be defined as the strength of a single interaction between an antibody binding site and epitope on a target antigen (i.e. monovalent binding). Antibody avidity represents the overall strength of antibody-antigen interactions and can be modified by several factors including multivalent binding. Whilst traditionally, antibody affinity was quantified ex-vivo in solution by its equilibrium constant, there are now several different approaches to quantifying antibody affinity and avidity in greater detail.

Surface plasmon resonance[62,63] immobilises the antigen on a dedicated sensor chip surface and the analyte (antibody in solution) flows over the surface bearing the antigen. Interaction is observed using real-time detection of mass change on the chip surface.

Microfluidic Diffusional Sizing (MDS)[64] can be used to measure the affinity of the interaction between antibody and a protein to which it binds, which again allows determination of dissociation constant and antibody concentration.

A modified approach to enzyme-linked immunosorbent assay (ELISA) can also be used to measure avidity, using sodium thiocyanate after the step where antigen-antibody complexes have been formed and providing a quick way to determine the avidity index of a given sample. [65,66]

1.3.3.3 Fcγr activity

FC gamma receptors are transmembrane glycoprotein molecules which can bind to the Fc portion of IgG molecules. In humans, three groups of receptors have been described across a variety of cell types,[67,68] linking the activities of the adaptive immune system with the innate immune system. They bind antibodies that are complexed with antigens and trigger intracellular signalling pathways which active immune cells such as macrophages, neutrophils and NK cells. Receptors can be considered as activating or inhibitory, depending on the signalling properties of their intracellular domains. Immunoreceptor tyrosine activation motifs (ITAM) are associated with receptors I, IIa, IIIa whereas IIb mediates signalling activity through an immunoreceptor tyrosine inhibitory motif (ITIM). Although some cells express a limited range of receptors (e.g. B cells and FcγRIIb) most other immune effector cells express a balance of inhibitory and activating receptors to create a balanced response **(Table 1.3).** Expression can also be modulated by cytokines, with pro-inflammatory cytokines increasing the expression of activating receptors.

Table 1.3 Expression patterns of FcγR in human effector cells

	I	IIa	IIb	IIIa	IIIb
Neutrophil	Ind	+	+	-	+
Eosinophil	Ind	+	+	_	Ind
Monocyte	+	+	+	_	_
Macrophage	+	+	+	_	_
Dendritic cell	Ind	+	+	Ind	_
NK cell	-	-	-	+	_
B cell	-	-	+	_	_
T cell	-	-	_	_	_
Basophil	Ind	+	+	_	_
Platelet	-	+	_	_	_

Adapted from Bournazos, Gupta and Ravetch (2020)[69]

Phagocytosis internalises cells and enables antigen presentation on both MHC class I and MHC class II molecules. It may also trigger cytokine release. Depending on the cell type, FcγR cross-linking may induce respiratory burst and degranulation of effector cells or phagocytosis of IgG-opsonised virions or infected cells that harbour the replicating virus.

Different IgG subclasses have differing affinities for FcγRs which can affect the overall effectiveness of a vaccine by altering the quality of the immune response. Therefore, differences in IgG profile together with FcγR expression may provide insight in differences in vaccine-mediated protection mechanisms between vaccines and vaccine platforms.[70]

1.3.4 Clinical trials in adults

The clinical development programme for ChAdOx1 nCoV-19 included five clinical trials[71–74] prior to initiation of recruitment for a paediatric trial (COV006). A phase 2/3 study of two doses of the viral vector ChAdOx1 nCoV-19 vaccine in adults began recruitment in Oxford, UK in April 2020.[71] Interim analyses of efficacy data from these trials enabled Conditional Marketing Authorisation (CMA) for ChAdOx1 nCoV1-19 for adult use on 30th December 2020. As of March 2021, one month after paediatric recruitment began, over 58,670 adults had been enrolled and received at least one dose of ChAdOx1 nCoV-19 or control vaccine (of which 36,580 had received ChAdOx1 nCoV-19 intramuscularly). [75] No safety concerns were highlighted at this point.

1.4 Was a paediatric vaccine needed?

1.4.1 Burden of acute SARS-CoV-2 disease in children

Whilst the burden of disease in adults was immediately apparent from the pressures on the healthcare system, delays in establishing a widespread polymerase chain reaction (PCR) testing programme in the UK community as well as the closure of schools on 10th March 2020 meant that assessing the impact of the pandemic on children was challenging. Together with safety data from the adult ChAdOx1 nCoV-19 trials showing that the vaccine demonstrated an acceptable safety profile in participants over 18 years of age, it was argued that a phase 2 paediatric ChAdOx1 nCoV-19 trial was needed and COV006

began recruitment in February 2021. By Spring 2021, several arguments, were put forward to support the eventual introduction of a paediatric vaccination programme.[76] Some of the evidence listed below, eventually used to justify a paediatric vaccination programme, emerged as the trial was ongoing.

Although initial estimation of the case fatality rate in children indicated that it was much lower than in adults (approximately 0.0008% based on UK data compared with 0.05% in adults based on global data),[77,78] approximately 4% of children admitted with SARS-CoV-2 infection required intensive care admission and intubation, in a high-income country setting.[79] Hospitalisation rates were difficult to assess as the proportion of children who were asymptomatic with SARS-CoV-2 infection was estimated at between 35-90%, and children were more commonly asymptomatic than adults.[80] Although national reporting of mortality rates from COVID-19 in children was challenging due to varying availability of PCR and serological testing, analysis by the United Nations Inter-agency Group for Child Mortality Estimation has shown that worldwide, there was no excess mortality in people under 25 years of age associated with the pandemic in 2020/2021.[81]

1.4.2 Role in transmission

The role that children played in the transmission of coronavirus in the wider community, and the role of vaccination in reducing transmission from children to vulnerable adults, remained uncertain at the time[82] but was widely cited in support of vaccine introduction. There was concern that educational settings might play a role in spreading of SARS-CoV-2 infection in the community. However, a systematic review has shown that children had lower odds of infection at school than at home or in other community settings.[83] A meta-

analysis has also shown that children are no more likely than adults to transmit SARS-CoV-2 to close contacts at home.[84]

1.4.3 PIMS-TS

Very rarely, younger children infected by SARS-CoV-2 acutely were observed to subsequently develop paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 (PIMS-TS or multisystem inflammatory syndrome in children, MIS-C) in the following 6-8 weeks.[80] The symptoms were similar to toxic shock syndrome and were characterised by multi-organ dysfunction requiring high dependency care, although milder cases including abdominal pain, vomiting and diarrhoea were also reported. As of December 2021, 5,973 patients meeting MIS-C case definition were reported to the Centers for Disease Control and Prevention[85] and mortality rates were estimated at 1-2% .

1.4.4 Economic and social factors

Benefits of vaccination included prevention of acute SARS-CoV-2 disease and post-infectious complications as well as possible reduction of transmission to vulnerable individuals in the community. Furthermore, the impact of school exclusions or closures, as a result of cases of coronavirus infection, on education and mental health of children was cited as a reason for vaccine introduction.[86]

1.5 Previous paediatric trials of ChAdOx1

The ChAd viral vector platform had already been subject to extensive clinical development prior to the COVID-19 pandemic, including in trial participants under the age of 18 years of age. Heterologous prime-boost ChAd-vectored malaria vaccines had previously demonstrated acceptable safety and tolerability profiles in infants and children from 10

weeks to 6 years of age in trials of the ChAd63 ME-TRAP.[87] Overall, more than 400 children from infancy to 6 years of age had received a ChAd-vectored vaccine in malaria vaccine trials. More recently, trials of an Ebola vaccine ChAd3-EBO-Z had demonstrated acceptable safety and tolerability profiles in children 1-17 years of age at a dose similar to or greater than that of the then newly licensed adult dose of ChAdOx1 nCoV-19, $5x10^{10}$vp.[88] These studies are summarised in **Table 1.4** below.

Table 1.4: Summary of previous clinical trials of ChAd-vectored vaccines in children and infants

Country	Infectious disease target	Vector	Cohort	Schedule	Route and dose	Immunogenicity	AEFIs
Mali, Senegal[88]	Ebola	ChAd3-EBO-Z	600 children (1-5 years, 6-12 years, 13-17 years)	ChAd3-EBO-Z /MenACWY-TT	1 x 10^{11} VP IM	Anti-glycoprotein Ebola virus IgG Mean values were highest in 1-5 years, followed by 13-17 years and then 6-12 years of age cohorts	Fever reported in 32% of ChAd3-EBO-Z; 4-20% of 13-17 year olds; 1-25% of 6-12 year olds; 23-50% of 1- 5 year-olds Injection site pain 31% of 13-17 year olds; 41% of 6-12 year olds; 55% of 1-5 year olds No grade 3 solicited AEs, 2 SAEs not related to vaccine
Burkina Faso[89]	Malaria	ChAd63 ME-TRAP	700 healthy malaria exposed children (5-17 months)	ChAd63 ME-TRAP/ D56 MVA ME-TRAP OR 2 doses of rabies vaccine	5 x 10^{10} VP IM (MVA 1 x 5^{10} VP)	T cell response median 326 SFU/106 PBMC (CI 290-387) 72% seropositive after prime; 98% seropositive after boost	Measured fever in 30; reported fever in 45% Most common SAE pneumonia (12 in ChAd63 ME-TRAP arm, 10 in control arm)
Gambia[90]	Malaria	ChAd63 ME-TRAP	65 infants and neonates (1, 8 or 16 weeks of age)	Chad63 ME-TRAP/ D56 MVA ME-TRAP and EPI OR EPI only	5 x 10^{10} VP IM (MVA 1 x 5^{10} VP)	T cell mean response increases 16weeks (154-1436 SFC), 8weeks (283-1759 SFC),	Fever documented in 73% in ME-TRAP with EPI versus 80% in EPI only group at 16 weeks

						1 week (254-755 SFC): 93% seropositivity at D63	Diarrhoea in 53% of 16/52 versus 33% of 1 week-old infants 1 case of SIDS not related to vaccine
Gambia and Burkina Faso [91,92]	Malaria	ChAd63 ME-TRAP	138 children (from 10 weeks of age)	ChAd63 ME-TRAP/ D56 MVA ME-TRAP	All ages except 5-17 months received 1 x 10^{10} VP IM and 5 x10^{10} VP IM	10 week-old infants had significantly higher antibody responses post boost in higher dose ChAd63 ME-TRAP prime	Approximately 10% of 2-6 year olds reported a fever

AE = adverse event; EPI= Expanded Programme on Immunisation; IM = intramuscular; MVA = modified vaccinia virus Ankara; SAE = serious adverse event; SIDS = sudden infant death syndrome; SFU = spot forming units; VP = viral particles

The difference between the studies listed in **Table 1.3** and the proposed ChAdOx1 nCOV-19 paediatric study was that a ChAd-vectored vaccine would be used in a homologous prime-boost (first and second dose) regimen. Therefore, reactogenicity following the second dose of ChAdOx1 nCoV-19 in children would have to be carefully monitored as some vaccines such as measles-mumps-rubella and varicella are known to cause increased injection site reactions after a second dose.[79] There were also concerns that anti-vector immunity might adversely affect the immunogenicity of the vaccine.

The vaccine was widely deployed during the COVID-19 pandemic, when the ChAdOx1 viral vector was administered, for the first time, as a homologous vaccine regimen to children and young adults. Whether there may be age-related differences in immune response is of interest as vaccines against further emerging diseases are being developed using the same vaccine platform which could be deployed in paediatric populations in the future.

1.6 Aims

Paediatric COVID-19 vaccine development lagged that for adults. Immunobridging studies are now permitted to infer vaccine efficacy in children. However, much of our understanding of B-cell and childhood antibody responses comes from previous studies in infants and younger children under 2 years, focusing on the ages during which they receive their childhood vaccines through the Expanded Programme for Immunisation.

The focus of this DPhil is to investigate whether there are age-related differences in immune response to the chimpanzee adenoviral vectored COVID-19 vaccine ChAdOx1 nCoV-19 in children, by comparing humoral immune responses in younger children (6-11 years), older children (12-17 years) and adults in vaccine trials. This will be achieved with the following four aims:

Aim 1:

To evaluate the safety and immunogenicity of one and two doses of COVID-19 vaccine ChAdOx1 nCoV-19 in children aged 6-17 years of age (COV006).

Aim 2:

To evaluate the effect of pre-existing immunity to circulating human coronaviruses on immune responses to one and two doses of COVID-19 vaccine ChAdOx1 nCoV-19 in children aged 6-17 years of age.

Aim 3:

To evaluate the effect of age (comparing primary and secondary school-aged children with adult vaccine recipients) on antibody isotypes and Fc functions of antibodies generated in response to one and two doses of ChAdOx1 nCoV-19.

Aim 4:

To evaluate the effect of age (comparing children aged 6-11 years and 12-17 years with adult vaccine recipients) on avidity of spike-specific antibodies generated in response to ChAdOx1 nCoV-19.

Chapter 2. Materials and methods

This chapter outlines the clinical and laboratory methods which were used to address the main aims of the thesis as outlined in Chapter 1. It will be referred to throughout the results chapters where relevant.

2.1 Clinical trial design of COV006

A phase 2, single-blind, randomised, controlled trial was conducted at four study sites in the UK (Centre for Clinical Vaccinology and Tropical Medicine, University of Oxford, Oxford; University Hospital Southampton NHS Foundation Trust, Southampton; St George's University of London, London; and University Hospitals Bristol and Weston NHS Foundation Trust, Bristol). An outline of the study design is shown below in **Table 2.1.**

Table 2.1 Overview of study design for COV006

<table>
<tr><td>Clinical phase</td><td>Phase II</td></tr>
<tr><td>Trial design</td><td>Single-blind, randomised, controlled, multi-centre</td></tr>
<tr><td>Participants</td><td>Healthy children aged 6-17 years of age</td></tr>
<tr><td>Sample size target</td><td>300 children
<table>
<tr><th>Group</th><th>N</th><th>Age (years)</th><th>Schedule</th></tr>
<tr><td>1</td><td>75</td><td>12-17</td><td>ChAdOx1 nCoV-19 or MenB with homologous boost at D28</td></tr>
<tr><td>2</td><td>75</td><td>12-17</td><td>ChAdOx1 nCoV-19 or MenB with homologous boost at D84</td></tr>
<tr><td>3</td><td>75</td><td>6-11</td><td>ChAdOx1 nCoV-19 or MenB with homologous boost at D28</td></tr>
<tr><td>4</td><td>75</td><td>6-11</td><td>ChAdOx1 nCoV-19 or MenB with homologous boost at D84</td></tr>
</table></td></tr>
<tr><td>Trial period</td><td>12 months post first vaccination per participant</td></tr>
<tr><td>Primary objective</td><td>To assess the safety, tolerability and reactogenicity profile and tolerability of a prime boost regimen (with an interval between prime and boost of 28 or 84 days) of the candidate vaccine ChAdOx1 nCoV-19 (5.0 x10^{10}vp /5.0 x10^{10}vp) in children 6-17 years of age</td></tr>
<tr><td>Secondary objective</td><td>To assess cellular and humoral immunogenicity of ChAdOx1 nCoV-19 (5.0 x10^{10}vp /5.0 x10^{10}vp) given as homologous prime boost (at 28 and 84 days post prime) in children 6-17 years of age</td></tr>
</table>

Healthy participants aged 6–17 years of age were recruited through social media, websites, and local and national media. All participants, or their parents on their behalf, initially completed an online screening form. Participants who had a history of chronic respiratory conditions, laboratory-confirmed COVID-19 (either a history of a positive result on a validated test or known seropositivity before enrolment), or previously received capsular group B meningococcal vaccine were excluded.

Written informed consent obtained from participants aged 16 years of age or older, or from parents or guardians if aged between 6 and 15 years. Written assent was obtained from participants aged 11 years or older. This study was approved by the Medicines and Healthcare products Regulatory Agency (MHRA) and the South-Central Berkshire Research Ethics Committee. The protocol and amendments were approved by the UK National Health Service Health Research Authority before implementation. The trial was done in compliance with the principles of the Declaration of Helsinki and the International Council for Harmonisation Good Clinical Practice guidelines.

2.1.1 Study vaccinations

The vaccine ChAdOx1 nCoV-19 was supplied by AstraZeneca (Investigational Medicinal Product Dossier, version 1.0; Jan 28th, 2021), stored at -80°C and thawed at room temperature. ChAdOx1 nCoV-19 was administered intramuscularly at a dose of 5×10^{10} viral particles, as per the standard adult dose. Capsular group B meningococcal vaccine was supplied by the UK Health Security Agency (previously known as Public Health England) and administered intramuscularly at a standard dose of 0·5 ml.

2.1.2 Blinding and randomisation

Computer-generated randomisation lists were prepared by the study statisticians. Participants were randomly assigned (4:1:4:1) to receive a two-dose series of ChAdOx1 nCoV-19 or capsular group B meningococcal vaccine as a control with a 28-day interval, or a two-dose series of ChAdOx1 nCoV-19 or capsular group B meningococcal vaccine with an 84-day interval. An active control was used to maintain masking of participants who had local or systemic side-effects. Randomisation was stratified by age group (6–11 years and 12–17 years) and study site using a block size of 10, chosen to align with the study group sizes. The

trial staff administering the vaccine prepared vaccines out of sight of the participants and syringes were covered with an opaque material until ready for administration to ensure masking of participants. Participants were observed for 30 minutes after vaccination. Clinical investigators involved in endpoint assessment and the laboratory team remained masked to ChAdOx1 nCoV-19 or capsular group B meningococcal vaccine group allocation.

2.1.3 Amendment to the study design

During the recruitment window for participants 6–11 years of age, the UK Government was advised by the Joint Committee on Vaccination and Immunisation that individuals younger than 30 years of age who had not yet received a first dose of the ChAdOx1 nCoV-19 vaccine should be given an alternative COVID-19 vaccine following safety concerns of vaccine-induced thrombosis and thrombocytopenia syndrome. This recommendation led to the cessation of further recruitment of participants aged 6–11 years, although recruitment targets for participants aged 12–17 years had already been met. Second-dose vaccination was then paused pending an MHRA review. Only participants aged 12–17 years randomly assigned to the 28-day interval groups had received second doses by this time. By April 2021, further safety data in adults who had received second doses of ChAdOx1 nCoV-19 in the UK became available for review by the MHRA. The MHRA then authorised administration of second vaccine doses to those participants aged 12–17 years randomly assigned to 84-day interval groups and to all those aged 6–11 years. As the originally intended day 84 window for second doses had passed, the second dose of vaccine was given to those participants aged 12–17 years randomly assigned to 84-day interval groups and to all those aged 6–11 years at day 112. Hereafter, 84-day interval refers to the planned interval group to which participants were randomly assigned and 112-day interval to the interval at which participants in this group received the doses.

2.1.4 Final study sampling schedule

Following the amendments outlined in 2.1.3, blood samples for serum and peripheral blood mononuclear cells were taken at the time points outlined below in Figure 2.1. This included visits at baseline, 28 days post first dose, day of boost and 28 days post second dose across all age groups.

The final visit occurred at D364 across all groups. Safety blood samples were taken for a subset of up to 40 participants aged 6–11 years at day 2 and day 7 following the second dose (up to 20 participants per timepoint). Participants completed electronic diaries as outlined in section 2.2.

Figure 2.1 - Outline of study procedures. *An overview of differences in study procedures between groups in COV006. *Group 3 participants were allocated 50/50 to attend V5 at D140 or 364 to avoid more than 5 blood draws per participant.*

			Study timeline							
Group	**Age**	**Study event**	**D0**	**D28**	**D56**	**D84**	**D112**	**D140**	**D182**	**D364**
1	12 - 17 years		V1	V2	V3				V4	V5
		Randomisation	✓							
		Vaccination	💉	💉						
		Clinical visit (with blood draw)	🩸	🩸	🩸				🩸	🩸
		E Diary	📅	📅	📅				📅	📅
2	12 - 17 years		V1			V2	V3	V4		V5
		Randomisation	✓							
		Vaccination	💉				💉			
		Clinical visit (with blood draw)	🩸			🩸	🩸	🩸		🩸
		E Diary	📅			📅	📅	📅		📅
3	6 - 11 years		V1	V2		V3	V4(a)	V5*		V5*
		Randomisation	✓							
		Vaccination	💉				💉			
		Clinical visit (with blood draw)	🩸	🩸		🩸	🩸	🩸		🩸
		E Diary	📅	📅		📅	📅	📅		📅
4	6 -11 years		V1			V2	V3(a)	V4		V5
		Randomisation	✓							
		Vaccination	💉				💉			
		Clinical visit (with blood draw)	🩸			🩸	🩸	🩸		🩸
		E Diary	📅			📅	📅	📅		📅

2.1.5 Sample processing

Serum samples were processed at individual sites. Blood was allowed to clot for at least 30 minutes to one hour at room temperature and transferred to a fridge at 4°C. Within 24 hours, samples were centrifuged at 3000g for 10 minutes and the serum aliquoted and stored at -80°C, before shipping to the Churchill Centre for Vaccinology and Tropical Medicine for testing.

Lithium heparin samples were transported at ambient temperature to the Jenner Institute for processing within 4 hours of being taken. PBMCs were isolated for fresh ELISpots and remaining PBMCs frozen down at 500,000 cells/ml in liquid nitrogen.

2.2 Primary outcomes – clinical

The primary objective of the trial was to assess the safety, tolerability and reactogenicity profile of a prime boost (two dose) regimen of ChAdOx1 nCoV-19 in children aged 6-17 years.

2.2.1 Reactogenicity and safety reporting

Participants completed electronic diaries for solicited adverse events for the first 7 days after vaccination and for unsolicited adverse events until 28 days after vaccination. Safety blood samples were taken for a subset of participants aged 6–11 years at day 2 and day 7 following the second dose. Laboratory and clinical adverse events were graded on a scale of 0–4. Study follow-up was 12 months from date of first vaccination.

Table 2.2a Severity grading criteria for solicited local adverse events

Adverse Event	Grade	Intensity
Pain at injection site	1	Pain that is easily tolerated
	2	Pain that interferes with daily activity
	3	Pain that prevents daily activity
	4	A&E visit or hospitalization
Tenderness	1	Mild discomfort to touch
	2	Discomfort with movement
	3	Significant discomfort at rest
	4	A&E visit or hospitalization
Erythema at injection site*	1	2.5 - 5 cm
	2	5.1 - 10 cm
	3	>10 cm
	4	Necrosis or exfoliative dermatitis
Induration/Swelling at injection site	1	2.5 – 5 cm and does not interfere with activity
	2	5.1 - 10 cm or interferes with activity
	3	>10 cm or prevents daily activity
	4	Necrosis

Table 2.2b. Severity grading criteria for solicited systemic adverse events

Grade	Intensity
Grade 0	None
Grade 1	Mild: transient or mild discomfort (<48 hours). No interference with activity. No medical intervention or therapy required.
Grade 2	Moderate: mild to moderate limitation in activity – some assistance may be needed. No or minimal medical intervention/therapy required.
Grade 3	Severe: marked limitation in activity, some assistance usually required. Medical intervention/therapy required.
Grade 4	Potentially like-threatening. Required assessment in A&E or hospitalisation

*erythema less than 2.5cm is an expected consequence of skin puncture and was therefore not be considered an adverse event

2.2.2 Self-reported SARS-CoV-2 cases

Participants were asked to self-report positive SARS-CoV-2 PCR results or lateral flow tests carried out in the community. The trial team made a note of whether the participant was asymptomatic or symptomatic at the time of testing and the date of testing. No regular asymptomatic testing of participants was conducted.

2.3 Secondary outcomes – immunological

Serum and heparinised samples were taken at the timepoints illustrated in **Figure 2.1**. Serum samples were processed at trial sites and stored at -80C before being transported to the Churchill Centre for Vaccinology and Tropical Medicine. Heparinised blood samples were transported to the Jenner Institute within 4 hours of collection at room temperature and peripheral blood mononuclear cells were isolated for fresh *ex-vivo* ELISpot assays. Remaining cells were cryopreserved at a concentration of 5 x 10^6 per ml. Serum aliquots were sent to Monogram laboratories for pseudo neutralisation assays. All three assays listed below were conducted at all timepoints, except that no ELISpot assays were conducted at D84.

2.3.1 Total IgG enzyme-linked immunosorbent assay

An in-house standardised total IgG enzyme-linked immunosorbent assay (ELISA) against trimeric SARS-CoV-2 spike protein was optimised using a reference plasma pool was obtained using serum donated from healthcare workers (Protective Immunity from T cells in Healthcare workers; PITCH study). A further positive control was obtained from National Institute for Biological Standards and Control (NIBSC) donor sample 20/130 and a negative control from human donor serum (Sigma). The coating protein for the assay (SARS-CoV-2 spike trimer, Wuhan) was sourced from Native Antigen. Briefly, Nunc Immuno ELISA plates

were coated with spike trimer at a concentration of 1ug/ml (diluted in DPBS) and incubated at 4°C for at least 16 hours and no more than 72 hours. Plates were washed four times with PBS/0.05% Tween (PBS-T) and blocked with casein for 60 minutes at room temperature. Samples and controls were plated in triplicate as per the layout in **Figure 2.2**. Values in rows D and E show wells which contained the reciprocal dilution of the serum pool, which formed the standard curve. Positive control 1 was a 1:1600 dilution of the standard pool and therefore equivalent to standard 5. Positive control 2 was a 1:1600 dilution of the NIBSC positive control.

Figure 2.2 Total IgG ELISA plate layout. *Samples were plated in triplicate for each dilution. Pink boxes illustrate standard concentrations and yellow boxes control wells.*

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Pos Control 1
B												
C												
D	100	200	400	800	1600	3200	6400	12800	25600	51200	Blank	
E	100	200	400	800	1600	3200	6400	12800	25600	51200		
F	Sample 12	Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	Pos Control 2
G												
H												

Following a 2-hour incubation at room temperature, secondary antibody was added (goat anti-human IgG conjugated to alkaline phosphatase, Merck) at a concentration of 1:1000 for a 1-hour incubation at room temperature. Plates were washed four times with PBS-T and 100 µl of p-Nitrophenyl Phosphate (PNPP) added per well. Plates were developed until the control and standard 5 had reached an optical density (OD) of 1 (0.9-1.3). Following development, plates were read at OD_{405} using the Bio-tek ELx800 microplate reader with Gen5 ELISA software. Plates were passed if the blank <0.2 and standard 1 OD >3.5 and the curve fit pre-specified QC parameters for a 4-parameter logistic model. The standard point corresponding to positive control 1 (standard 5) was assigned a value of 1 ELISA Unit (EU)

and plates passed if the control was interpolated as 1600 (1500-2300). Samples were repeated if the sample OD_{405}>3.5 or if the CV between sample repeats was >20%.

2.3.2 Interferon-γ ELISpot

An *ex-vivo* enzyme-linked immunosorbent spot (ELISpot) assay to quantify antigen-specific interferon-γ release by peripheral blood mononuclear cells was used to assess cellular immunogenicity. Briefly, Multiscreen IP ELISpot plates (Merck Millipore) were coated in anti-IFN-γ antibody (10μg/ml) the day before the blood draw occurred. On the day of venepuncture, freshly isolated peripheral blood mononuclear cells (PBMCs) were isolated, counted and resuspended in R10 (Roswell Park Memorial Institute [RPMI] medium with 1:10 concentration of fetal calf serum, FCS) within four hours of sampling. Cells were plated out in duplicate under the conditions in **Figure 2.3**, at a concentration of 250,000 cells per well. R10 was used as a negative control; peptide pools from influenza, Epstein Barr Virus and cytomegalovirus (FEC) and phytohaemagglutinin-L (PHA-L) were used as positive controls. Peptide pools from S1 (150 peptides) and S2 (120 peptides) regions of SARS-CoV-2 (ProImmune) were designed to cover the length of the spike construct, with 15mer peptides overlapping by 10aa, resulting in 258 peptides. Each pool (**Figure 2.3** rows D-G) contained 20 peptides. Due to the small volumes of participant serum obtained, pools 4-12 were further combined with 60 peptides in some pools. The mean spot-forming units (SFU) per well were multiplied by 4 to scale from 250,000 plated cells to SFU/ million cells.

Figure 2.3 ELISpot plate layout. *Samples were plated in duplicate for each condition. R10 = RPMI with 1:10 fetal calf serum; FEC = peptide pool from influenza, Epstein Barr Virus and cytomegalovirus; PHA = phytohaemagglutinin; SEB = staphylococcal enterotoxin B*

	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6	
	1	2	3	4	5	6	7	8	9	10	11	12
A	R10	R10	R10	R10	R10	R10	R10	R10	R10	R10	R10	R10
B	R10	R10	R10	R10	R10	R10	R10	R10	R10	R10	R10	R10
C	FEC	FEC	FEC	FEC	FEC	FEC	FEC	FEC	FEC	FEC	FEC	FEC
D	Pool 1-3	Pool 1-3	Pool 1-3	Pool 1-3	Pool 1-3	Pool 1-3	Pool 1-3	Pool 1-3	Pool 1-3	Pool 1-3	Pool 1-3	Pool 1-3
E	Pool 4-6	Pool 4-6	Pool 4-6	Pool 4-6	Pool 4-6	Pool 4-6	Pool 4-6	Pool 4-6	Pool 4-6	Pool 4-6	Pool 4-6	Pool 4-6
F	Pool 7-9	Pool 7-9	Pool 7-9	Pool 7-9	Pool 7-9	Pool 7-9	Pool 7-9	Pool 7-9	Pool 7-9	Pool 7-9	Pool 7-9	Pool 7-9
G	Pool 11-12	Pool 11-12	Pool 11-12	Pool 11-12	Pool 11-12	Pool 11-12	Pool 11-12	Pool 11-12	Pool 11-12	Pool 11-12	Pool 11-12	Pool 11-12
H	PHA/SEB	PHA/SEB	PHA/SEB	PHA/SEB	PHA/SEB	PHA/SEB	PHA/SEB	PHA/SEB	PHA/SEB	PHA/SEB	PHA/SEB	PHA/SEB

2.3.3 Pseudotype neutralisation assay, Monogram

A pseudotype neutralisation assay (PNA) was conducted by Monogram Biosciences using a lentivirus-based SARS-CoV-2 pseudovirus expressing SARS-CoV-2 spike protein on its surface. Participant serum samples were heat inactivated and diluted 1:40 in cell culture medium. Neutralising antibody titres were quantified by incubating (for 1 hour at 37°C) a standarised concentration of pseudovirus with 3-fold serial dilutions of participant sera. Following incubation, human embryonic kidney 293 ACE2-transfected cells were added to the sample, incubated for 60-80 hours at 37°C and assayed for luciferase expression. Neutralisation titres were reported as the reciprocal of the serum dilution conferring 50% inhibition of pseudovirus infection.

2.3.4 Serostatus evaluation, external assays

Serostatus at baseline was determined by an anti-nucleocapsid assay (Roche, Elecsys), using recombinant nucleocapsid antigen in a double-antigen sandwich format. 20 µl of participant serum was incubated with biotylated and ruthenylated nucleocapsid antigen, streptavidin-labelled and then quantified using electrochemiluminescence. A cut-point ELISA (PPD) for anti-nucleocapsid status was used to determine serostatus conversion at all timepoints after baseline.

2.4 Exploratory immunological outcomes

2.4.1 MSD multiplexed immunoassay

Total IgG titres against circulating human coronavirus and SARS-CoV-2 antigens were measured using Meso Scale Discovery (MSD) V-PLEX COVID-19 serology kits. Coronavirus Plate 3 consisted of a pre-defined panel of 9 antigens with a BSA control, coated on to a 10-spot MULTI-SPOT® 96-well plate. Serum controls for the antigens supplied at pre-defined concentrations by the manufacturer are shown in **Table 2.3.**

Table 2.3 Values for serology controls in MSD arbitrary units for Lot A00731-2

Antigen	Concentration of IgG			Unit of measure
	Control 1.1	Control 1.2	Control 1.3	
SARS-CoV-2 Nucleocapsid	19.4	3.31	1.05	AU/ml
SARS-CoV-2 Spike (Wuhan)	16.7	4.13	1.48	
SARS-CoV-2 S1 RBD	7.18	2.12	0.545	
SARS-CoV-1 Spike	1.27	0.431	0.385	
HCoV-HKU1 Spike	7.67	1.48	2.39	
HCoV-OC43 Spike	12.8	4.03	10.6	
HCoV-NL63 Spike	1.30	0.25	0.344	
HCoV-229E Spike	7.59	2.20	2.97	
MERS-CoV-Spike	1.12	0.129	0.70	

Plates were blocked by addition of 150 µl of MSD Blocker A to each well for 30 minutes at room temperature with 700 rpm shaking. This was followed by three PBS-T washes. Standards, controls, and samples were plated as outlined below in **Figure 2.4.**

Figure 2.4 MSD assay plate layout. *Samples were plated in duplicate for each dilution. Standards 1-8 were 1:4 serial dilutions and controls 1-3 were plated neat from MSD.*

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 1		Control 1		Control 2		Control 3		Sample 1		Sample 2	
B	Standard 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7	
C	Standard 3		Sample 8		Sample 9		Sample 10		Sample 11		Sample 12	
D	Standard 4		Sample 13		Sample 14		Sample 15		Sample 16		Sample 17	
E	Standard 5		Sample 18		Sample 19		Sample 20		Sample 21		Sample 22	
F	Standard 6		Sample 23		Sample 24		Sample 25		Sample 26		Sample 27	
G	Standard 7		Sample 28		Sample 29		Sample 30		Sample 31		Sample 32	
H	Standard 8		Sample 33		Sample 34		Sample 35		Sample 36		Sample 37	

The serum standard was plated at a 1:4 serial dilution and controls were plated neat as supplied by the manufacturer. Samples were plated at dilutions between 1:500 to 1:100,000, diluted with MSD dilution buffer. A lower limit of 1:500 was chosen as lower dilutions resulted in readings out of quantifiable range (see below). Following a 2-hour sample incubation at room temperature with 700 rpm shaking, plates were washed three times with PBS-T and 50 µl of detection buffer added per well. Plates were covered with foil and incubated for a further one hour before washing with PBS-T three times and PBS only, once. 150 µl of MSD read buffer was added to each well and plates were read immediately using the MESO QuickPlex SQ 120MM reader and Methodical Mind software. Antigen pass criteria were standard and control well recovery of 70-130 % and co-efficient of variation <20 %. Sample pass criteria were mean titre < standard 6 and > standard 3 on the standard curve, and co-efficient of variation <30 %. Raw plate data were exported to MSD Workbench v4.0 for analysis. Workbench converted mean fluorescence values (MFV) to arbitrary units (AU)/ml by multiplying the MFV by the serum dilution.

2.4.2 Standardised antibody subclass and isotype ELISA

Anti-spike (Wuhan) subclass titres (IgG1,IgG3) and isotype (IgA and IgM) titres were measured using an in-house ELISA. Three individual standard serum pools for IgG1, IgG3/IgM and IgA were produced in-house from COVID-19 vaccine trial vaccinees. The coating protein for the assay (SARS-CoV-2 spike trimer, Wuhan) was sourced from Native Antigen. Briefly, Nunc Immuno plates were coated with spike trimer at a concentration of 2 μg/ml for IgG1, IgG3 and IgM, or 5 μg/ml for IgA, for at least 16 hours at 4°C. Plates were then washed four times with PBS-T and blocked with casein for 60 minutes at room temperature before being tapped dry. Samples were plated in triplicate as below in **Figure 2.5** from a starting dilution of 1:50. Rows G and H show the reciprocal of the dilutions of the standard serum pool used to generate a standard curve for each isotype/subclass. The values in D1-F1 and D12-F12 give the reciprocal of the dilution of the serum pool used to make controls 1-3.

Figure 2.5 Subclass ELISA plate layouts. *Samples were plated in triplicate for each dilution. Pink boxes show control dilutions with the standard curve from G1/H1 to G10/H10.*

IgG1:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
C												
D	800											800
E	800	Sample 13	Sample 14	Sample 15	Sample 16	Sample17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	800
F	800											800
G	100	200	400	800	1600	3200	6400	12800	25600	51200	Casein	Casein
H											Casein	Casein

IgG3:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
C												
D	1600											1600
E	1600	Sample 13	Sample 14	Sample 15	Sample 16	Sample17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	1600
F	1600											1600
G	200	400	800	1600	3200	6400	12800	25600	51200	102400	Casein	Casein
H											Casein	Casein

IgA:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
C												
D	800											800
E	800	Sample 13	Sample 14	Sample 15	Sample 16	Sample17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	800
F	800											800
G	100	200	400	800	1600	3200	6400	12800	25600	51200	Casein	Casein
H											Casein	Casein

IgM:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
C												
D	6400											6400
E	6400	Sample 13	Sample 14	Sample 15	Sample 16	Sample17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	6400
F	6400											6400
G	800	1600	3200	6400	12800	25600	51200	102400	204800	409600	Casein	Casein
H											Casein	Casein

Plates were incubated at 37°C with 300 rpm shaking for 2 hours. Plates were then washed four times with PBS-T and secondary antibody added (goat anti-human IgG1/IgG3/IgM/IgA conjugated to alkaline phosphatase, Southern Biotech) diluted 1:1000 in casein. Secondary antibody was added to the samples and incubated for a further hour at 37°C with 300 rpm shaking before washing four times with PBS-T and addition of PNPP. Following

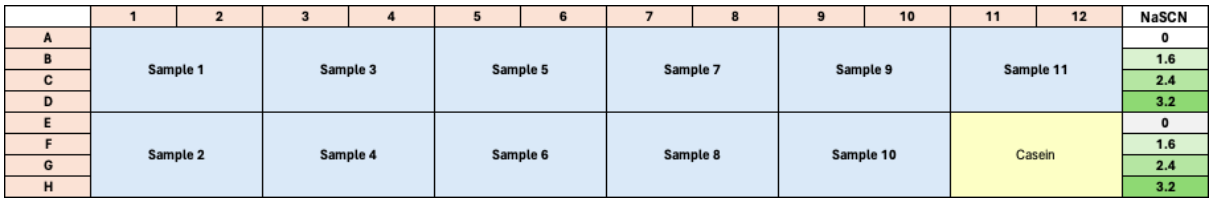
development, plates were read at OD_{405} using the Bio-tek ELx800 microplate reader with Gen5 ELISA software. Plates were deemed a 'pass' if the OD_{405} of the controls and standard 4 ranged between 0.8-1.2, the value of blank wells was <0.15 and the EU of the controls lay within pre-specified QC limits. Samples were assigned a pass if the OD_{405} for that dilution was recorded as between 0.2-2.0 and with CV <20 %.

2.4.3 Standardised avidity ELISA

ELISA plates were coated, washed and blocked as in section 2.2.1. Serum samples were plated in multiples of eight as outlined in **Figure 2.6**. Serum dilutions were determined by calculating the concentration of serum required to give a total IgG ELISA OD reading of 1. Plates were coated with Native Antigen SARS-CoV-2 spike trimer at a concentration of 1 µg/ml overnight (at least 16 hours). Plates were then washed four times with PBS-T and blocked with casein for 1 hour at room temperature. Plates were then tapped dry. Serum samples were diluted to contain an antibody titre of 1 EU. This was done to standardise antibody levels and to prevent the influence of antibody titre on antibody avidity. Following a 2-hour sample incubation at room temperature, plates were washed four times with PBT-T and 50µl sodium thiocyanate was added to each row at the concentrations listed in green in **Figure 2.6.** Plates were incubated for 15 minutes at room temperature and washed four times with PBS-T. Addition of secondary antibody and development buffer section was repeated as for the total IgG ELISA in section 2.3.1. Following development, plates were read at OD_{405} using the Bio-tek ELx800 microplate reader with Gen5 ELISA software. Reading of plates continued until the OD405 of the samples in the 0M row (i.e. A and E) reached 1. Plates were considered passed if the OD405 of the control wells was <0.2. Samples were considered passed if the OD of the 0M row was between 0.8-1.2 and the CV was <20%. The IC50 was

defined as the molarity of sodium thiocyanate which gave 50% of the control (0M) OD405 signal.

Figure 2.6 Avidity ELISA plate layout. *Samples were plated in duplicate for each NaSCN condition.*



2.4.4 Luminex multiplexed immunoassay

Antigen-specific Fc-receptor binding profiles were generated using a custom magnetic multiplexed immunoassay on the Luminex platform. Antigens were coupled to Bio-Plex Pro Magnetic COOH beads (Bio-Rad, at a concentration of 1.25 x 10^7/ml) using the Bio-Rad Bio-Plex Amine Coupling Kit. Antigens were coupled to the following bead regions below in **Table 2.4** at a concentration of 10µg antigen/ml per 10^6 beads:

Table 2.4. Luminex bead regions with corresponding antigens

Bead region	Antigen
26	SARS-CoV-2 nucleocapsid
29	Omicron spike
35	HKU1 spike
37	229E spike
43	OC43 spike
46	NL63 spike
55	SARS-CoV-2 Wuhan spike

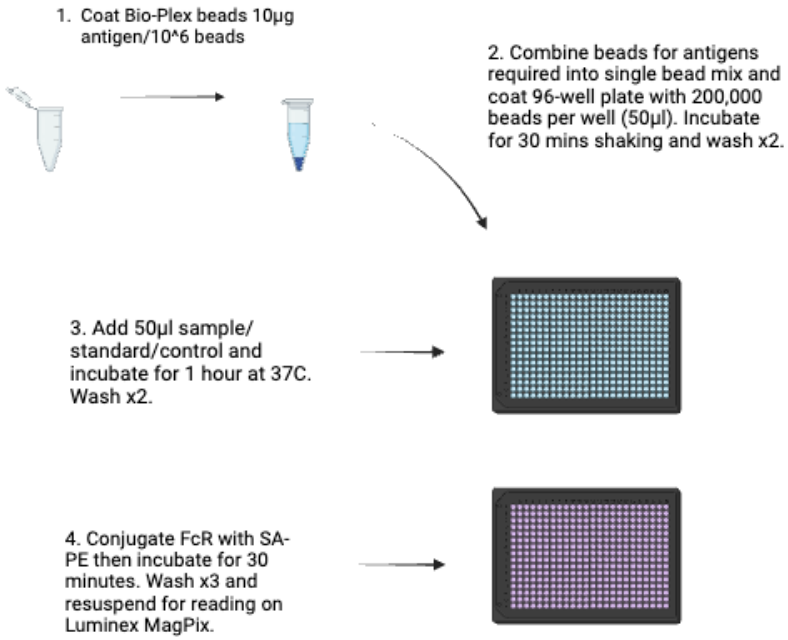
96-well plates (Greiner) were coated with coupled beads at a concentration of 1000 beads in 50 µl per well for each of the above bead region/antigen pairings, for 30 minutes at 700 rpm shaking at room temperature. Plates were washed twice using a Luminex magnetic plate with 100 microlitres of 0.1% bovine serum albumin in phosphate buffered saline with tween (BSA/PBS-T). Serum samples were prepared at dilutions of 1:200 in 0.1% BSA/PBS-T. The reference plasma pool was obtained from participants of the PITCH study. The standard curve was a 1:2 dilution series with an initial dilution of 1:300, and the internal control a 1:3000 dilution prepared with a 1:10 dilution of standard 1. Samples and standards were plated in duplicate as below:

Figure 2.7. Luminex assay plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 1	Sample 1	Sample 7	Sample 7	Sample 13	Sample 13	Sample 19	Sample 19	Sample 25	Sample 25	Sample 31	Sample 31
B	Sample 2	Sample 2	Sample 8	Sample 8	Sample 14	Sample 14	Sample 20	Sample 20	Sample 26	Sample 26	Sample 32	Sample 32
C	Sample 3	Sample 3	Sample 9	Sample 9	Sample 15	Sample 15	Sample 21	Sample 21	Sample 27	Sample 27	Sample 33	Sample 33
D	Sample 4	Sample 4	Sample 10	Sample 10	Sample 16	Sample 16	Sample 22	Sample 22	Sample 28	Sample 28	Sample 34	Sample 34
E	Sample 5	Sample 5	Sample 11	Sample 11	Sample 17	Sample 17	Sample 23	Sample 23	Sample 29	Sample 29	Sample 35	Sample 35
F	Sample 6	Sample 6	Sample 12	Sample 12	Sample 18	Sample 18	Sample 24	Sample 24	Sample 30	Sample 30	Sample 36	Sample 36
G	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7	Standard 8	Standard 9	Standard 10	Blank	Internal control
H	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7	Standard 8	Standard 9	Standard 10	Blank	Internal control

Plates were incubated for 1 hour at 37°C, 700 rpm shaking, before washing twice again with 0.1%BSA/PBS-T using a Luminex magnetic plate. FcR antigens IIa, IIb, IIIa or IIIb, Sino-Biological) were coupled to streptavidin-phycoerythrin (SA-PE) in a foil-covered rotator for 10 minutes. 50µl of receptor-SA-PE solution were added to each well and incubated for 30 minutes, room temperature at 700 rpm shaking. One FcR antigen was tested per plate, therefore each set of 36 serum samples was repeated four times to obtain a full FcR profile.

Figure 2.8 Schematic diagram of Luminex protocol[3]



Plates were read using the Luminex MAGPIX® system. Median mean fluorescence intensity values were exported using xPONENT software. Plates were passed if the R2 of the curve was >0.99 and standard 4 and the interpolated mean values were within predicted range.

2.5 Statistical analysis and data management

2.5.1 Analysis of clinical outcomes

Correlations between solicited and unsolicited symptoms were assessed using Spearman's rank correlation co-efficient for non-parametric analyses. The number of self-reported SARS-CoV-2 infections were used as endpoints for exploratory survival analyses.

[3] Drawn with biorender.com

2.5.2 Analysis of immunological outcomes

Isotype and subclass ELISA results below the limit of assay detection were assigned values listed in **Table 2.5.**

Table 2.5 Lower limits of detection for isotype and subclass ELISAs in ELISA Units

Assay	Lower limit of detection (EU/ml)	Value assigned if below limit of detection (EU/ml)
IgG1	18	18
IgG3	8	8
IgA	12	12
IgM	12	12

MSD results below the lower limit of detection (below S6 on the standard curve at a 1:500 dilution) were assigned a value corresponding to the average S6 value for that antigen across all plates x 500.MSD results above the upper limit of detection (above S3 on the standard curve at a 1:100,000 dilution) were assigned a value corresponding to the average S3 value for that antigen across all plates x 100,000. These are shown in **Table 2.6.**

Table 2.6 Lower and upper values assigned to MSD assay in Arbitrary Units

MSD antigen	Value assigned if below limit of detection (AU/ml)	Value assigned if above limit of detection (AU/ml)
229E	14.69	183223
NL63	2.42	30489
HKU1	15.25	184263
OC43	23.71	308080
SARS-CoV-2 S	34.32	420085

Antibody and MFI data were log transformed prior to performing comparisons and data distributions assessed for normality prior to analyses. Univariate analyses (comparisons of age groups at a single timepoint) were conducted using one-way analysis of variance (ANOVA) testing with a Bonferroni correction. Bivariate analyses (comparison of age groups at multiple timepoints) were conducted using two-way ANOVA testing with a Bonferroni correction. Data were tested by group for normality using the Shapiro-Wilk test and examination of respective quantile-quantile (QQ) plots. Some groups with smaller numbers of assays did not pass normality testing although the raw data approximated to a normal distribution. Therefore, the results of significance testing have been interpreted with caution. Analyses were not adjusted for matching to enable the maximal use of data generated.

2.5.3 Analysis software

Raw MSD and Luminex data were exported using Methodical Mind and Luminex® software. Analyses were performed using R version 2022.12.0+353 and Graphpad Prism version 10.

Chapter 3. Safety and immunogenicity[4]

3.1 Introduction

By June 2022, ChAdOx1 nCoV-19 was approved for use in more than 180 countries[93] following phase 2/3 trials reporting acceptable safety, tolerability, immunogenicity, and efficacy of a two-dose schedule in adults aged 18 years and older. In the UK setting, real-world data showed 91% efficacy against hospitalisation-associated infection by the delta (B.1.617.2) variant, 74% efficacy against symptomatic infection by the alpha (B.1.1.7) variant, and 67% efficacy against symptomatic infection by the delta variant.[94,95] The WHO advised that children aged 12 years and older who were at risk of severe COVID-19 should be offered BNT162b2 (Pfizer-BioNTech).[96]

An increasing number of countries had approved COVID-19 vaccination with mRNA vaccines to children aged 5 years and older (such as Canada, Germany, Italy, the UK, and the USA). ChAdOx1 nCoV-19 had not previously been evaluated in a paediatric population. Eight licensed vaccines to prevent COVID-19 were approved by WHO for Emergency Use Listing (EUL): seven for use in adults aged 18 years and older, and one for use in adults aged 16 years and older. A further 28 vaccines were approved for use in at least one country. Peer-reviewed clinical trial data describing safety and immunogenicity of EUL vaccines in children and adolescents had been published for four of these vaccines (CoronaVac [SinoVac],[97] BBIBP-CorV [SinoPharm],[98] BNT162b2 [Pfizer–BioNTech],[99] and mRNA-1273[Moderna][100]). Immunobridging studies showed that that two standard adult doses of

[4] Passages of this chapter have previously been published in Li G, Cappuccini F, Marchevsky NG, *et al*. "Safety and immunogenicity of the ChAdOx1 nCoV-19 (AZD1222) vaccine in children aged 6-17 years: a preliminary report of COV006, a phase 2 single-blind, randomised, controlled trial" *Lancet*. 2022, 399(10342):2212-2225. doi: 10.1016/S0140-6736(22)00770-X. PMID: 35691324

either BNT162b2 or mRNA-1273 generated neutralising antibody titres in adolescents 12–15 years of age comparable with adults 18–25 years of age. Use of fractional dose of 10μg BNT162b2 in children 5–11 years of age had an acceptable reactogenicity profile and induced neutralising antibody titres comparable with those in adolescents at the age of 12–15 years receiving an adult dose.[101] Similarly, phase 1/2 data of the BBIBP-CorV vaccine supported phase 3 evaluation of the adult regimen (two 4μg doses, four weeks apart) in children. [98]

As outlined in Chapter 1, chimpanzee adenoviral-vectored vaccines had previously been administered to more than 400 children from infancy to 6 years of age in trials of a malaria vaccine ChAd63 ME-TRAP.[76] More recently, trials of an Ebola vaccine ChAd3-EBO-Z showed an acceptable safety and tolerability profile in children 1-17 years of age at a similar dose of the licensed adult dose of ChAdOx1 nCoV-19 (5x10^10 viral particles).[75] Given the importance of having multiple options for immunisation of children and adolescents against SARS-CoV-2, the safety, tolerability, and immunogenicity of ChAdOx1 nCoV-19 in children aged 6–17 years was evaluated with a phase II clinical trial, COV006. The aim of this chapter is to report the primary outcomes of the trial.

3.2 Methods

3.2.1 Sample selection

All samples were obtained from routine visits during the clinical trial. For the trial visit schedule, see Chapter 2, section 2.1. Total IgG ELISAs were conducted blinded, on all samples available at each timepoint from participants who received two doses of study vaccine. Results have been grouped by serostatus at baseline and trial arm (vaccine received and interval between first and second doses). Results have been included whether a participant self-reported a SARS-CoV-2 infection during the duration of the study or not. Samples for ELISpot, pseudovirus neutralisation and anti-nucleoprotein assays were selected in the same way. Survival analyses were conducted using self-reported SARS-CoV-2 infection as the endpoint. The follow-up period commenced 14 days after receiving a second vaccine and participants were censored if they withdrew from the trial or if they received a COVID-19 vaccine in the national COVID-19 immunisation programme.

3.2.2 Immunology assays

The results of three immunological assays are reported in this chapter. Please refer to the following section for each assay:

1. Total IgG enzyme-linked immunosorbent assay: Chapter 2, section 2.2.1
2. T cell ELISpots: Chapter 2, section 2.2.2
3. Pseudovirus neutralisation assay: Chapter 2, section 2.2.3

3.2.3. Statistical methods

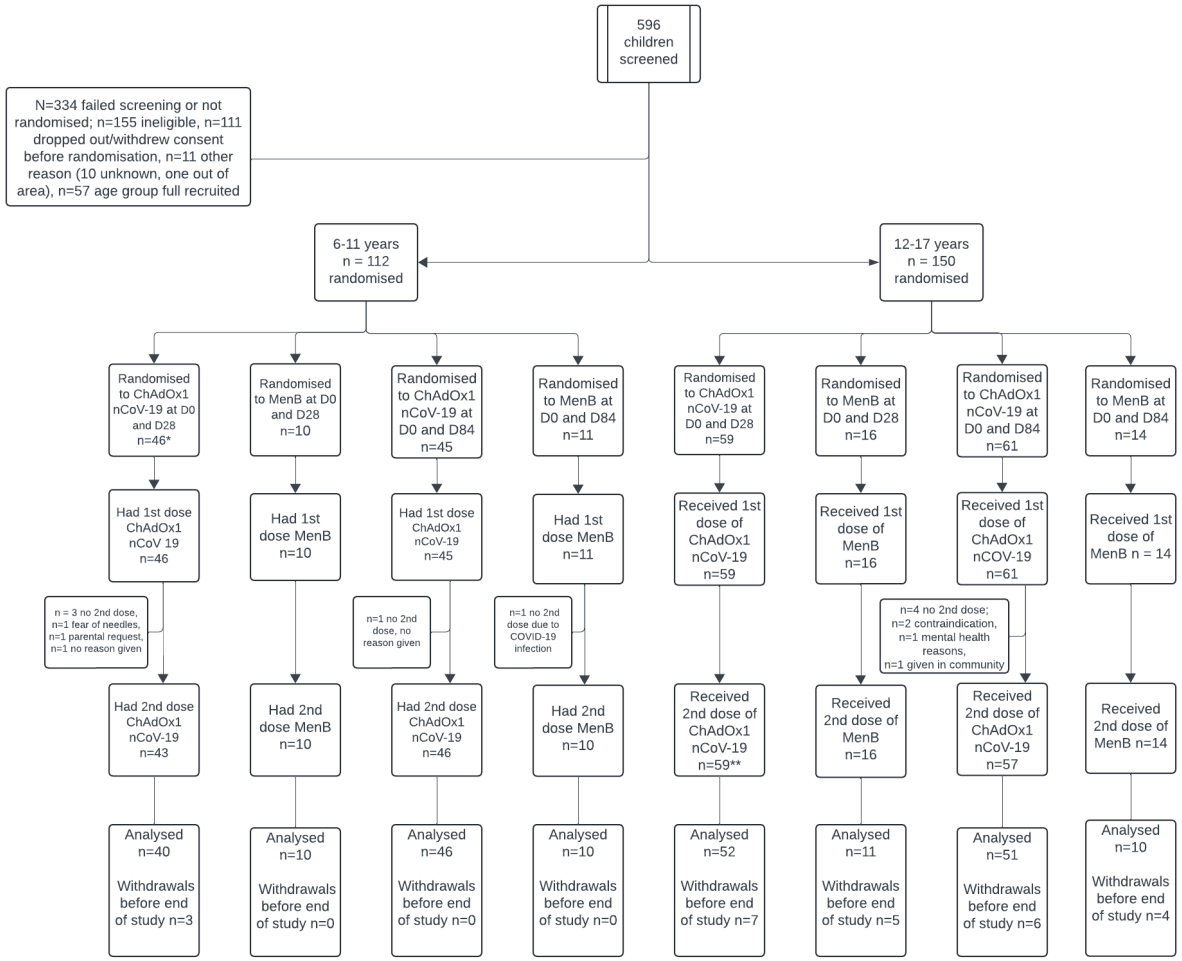
Two-way ANOVA was used to compare results of serology assays in this chapter, grouping by age group and time point. For further details of the statistical analyses used in this chapter, please see Chapter 2, section 2.5.

3.3 Results

3.3.1 Recruitment of children to phase II clinical trial

596 children were screened for enrolment into COV006 between February and April 2021. 262 participants were randomised to a trial arm and 230 remained enrolled at the end of the study follow-up period (364 days). A consort diagram **(Figure 3.1)** outlines reasons for screening failure, enrolment by age category, numbers in each randomisation arm and reasons for withdrawals in each arm if known.

Figure 3.1. CONSORT diagram for COV006



*one 12-17 year old participant wrongly randomised to 6-11 year age group at short interval

**one participant oringally randomised to short interval ChAdOx1 arm received a delayed second dose at the long interval dose timepoint due to meeting temporary eligibility criteria

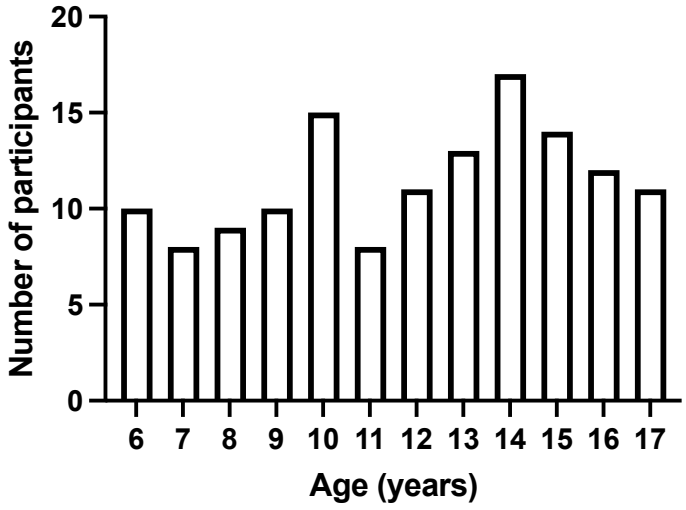
3.3.2 Summary of trial participants

Table 3.1 outlines the breakdown of COV006 recipients by age and sex. **Figure 3.2** shows the age of COV006 participants represented by a histogram. There were slightly greater numbers of children 10 and 14 years of age recruited into the trial but the mean age and ratio of male to female participants was balanced.

Table 3.1. Characteristics of participants recruited to COV006

N	*Age group (years)*	*Mean age (years)*	*Sex*	
			Female	*Male*
112	*6-11*	*8.7*	*55/112*	*57/112*
150	*12-17*	*14.6*	*74/150*	*76/150*

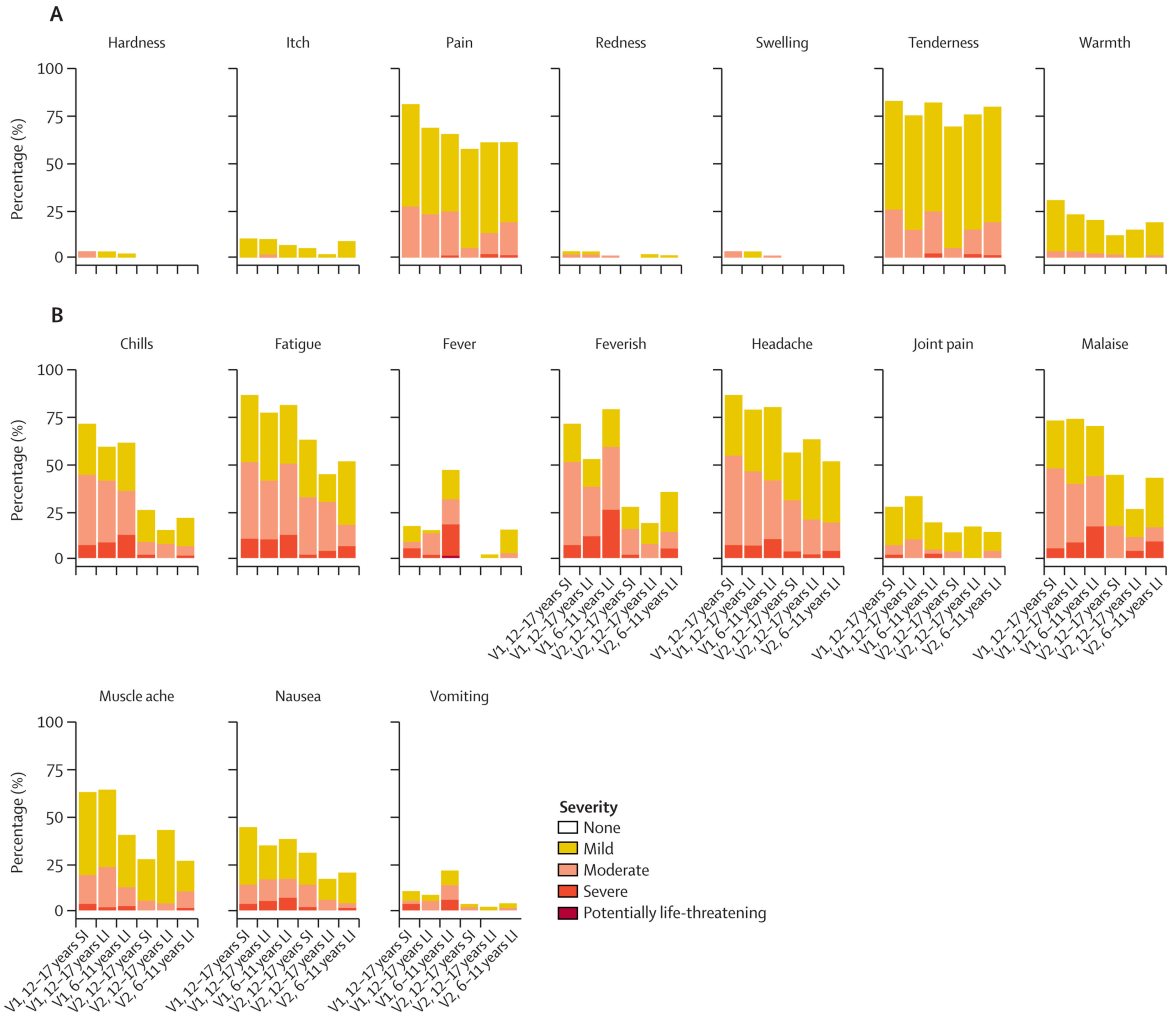
Figure 3.2. Age distribution of participants recruited to COV006



3.3.3 ChAdOx1 nCOV-19 demonstrates an acceptable reactogenicity profile in children

239 participants completed a symptom diary following their first vaccination and 192 participants following their second vaccination. **Figure 3.3** shows the severity grading score for each local and symptom, systemic symptom, for day 0 to day 7 post vaccination after first and second vaccine doses. Both local and systemic symptoms peaked on day 1 post vaccination and were less severe after the second dose than after the first dose. Pain (reported by 138/239) and tenderness (176/239) were the most reported local adverse events on day 1 after a first dose. Fever, chills, headache, malaise and fatigue were the most reported systemic events on day 1 after a first dose. 19 (16%) of 120 participants aged 12–17 years and 42 (47%) of 90 participants aged 6–11 years reported fever (temperature ≥38°C) after receiving the first dose, reducing to 1 (1%) of 113 in the older age group in both interval groups and 12 (15%) of 80 in the younger age group after receipt of the second dose at 112 days. One participant aged 6–11 years receiving ChAdOx1 nCoV-19 reported a temperature of 40°C after a first dose which resolved within 24 hours.

Figure 3.3 7-day reactogenicity data for local (A) and systemic (B) adverse events following first and second doses of ChAdOx1 nCoV-19, by age group.



Data presented are maximum severity across the first 0–7 days following the respective dose. V1=first dose. V2=second dose. SI=short interval (28-day interval). LI=long interval (112-day interval).

3.3.4 No significant adverse events related to vaccination were reported

Five serious adverse events were reported in the ChAdOx1 nCoV-19 arms, however none of them were deemed related to vaccination by the DSMC. These comprised one episode of tonsillitis, one episode of appendicitis, one diagnosis of a benign pituitary tumour, one diagnosis of an ovarian mass and one episode of testicular torsion.

3.3.5 ChAdOx1 nCoV-19 is immunogenic in seronegative and seropositive children with higher measures of immunogenicity generated in younger children 6-11 years of age or when there is a longer interval between doses

ChAdOx1 nCoV-19 was immunogenic in COV006 participants of all ages. A geometric mean fold rise (GMFR) of 506 in total anti-spike IgG was measured in participants 6-11 years of age, 28 days after the first dose (D28/D0). A GMFR of 352 was measured in participants 12-17 years at the same timepoint (D28/D0). Geometric mean titres (GMTs) at 28 days after a second dose were similar in participants 6-11 years of age and 12-17 years of age who received the vaccines at a 112-day interval (2310 EU 95% C.I. 1700-3138; 1963 EU 85% C.I. 1575-2448) but lower in those 12-17 years of age who received a second dose at a shorter D28 interval (1194 EU, 95% C.I. 908-1568) (**Table 3.2; Figure 3.4**). Titres increased in all study arms between the timepoint 28 days after a second dose of vaccine and the final endpoint of the study, including the control arms. This could be explained by SARS-CoV-2 community acquired infection and is investigated further in section 3.3.9 with the use of anti-nucleocapsid antibody data to determine which participants were and were not infected.

Table 3.2 In-house SARS-CoV-2 (Wuhan) anti-spike ELISA results in baseline seronegative ChAdOx1 nCOV-19 recipients and MenB recipients, comparing geometric mean titres (GMTs) 28 days after receiving a first dose, second dose and at the end of the study (D364). *95% confidence interval of GMTs are given in brackets followed by the number of samples available at each timepoint.*

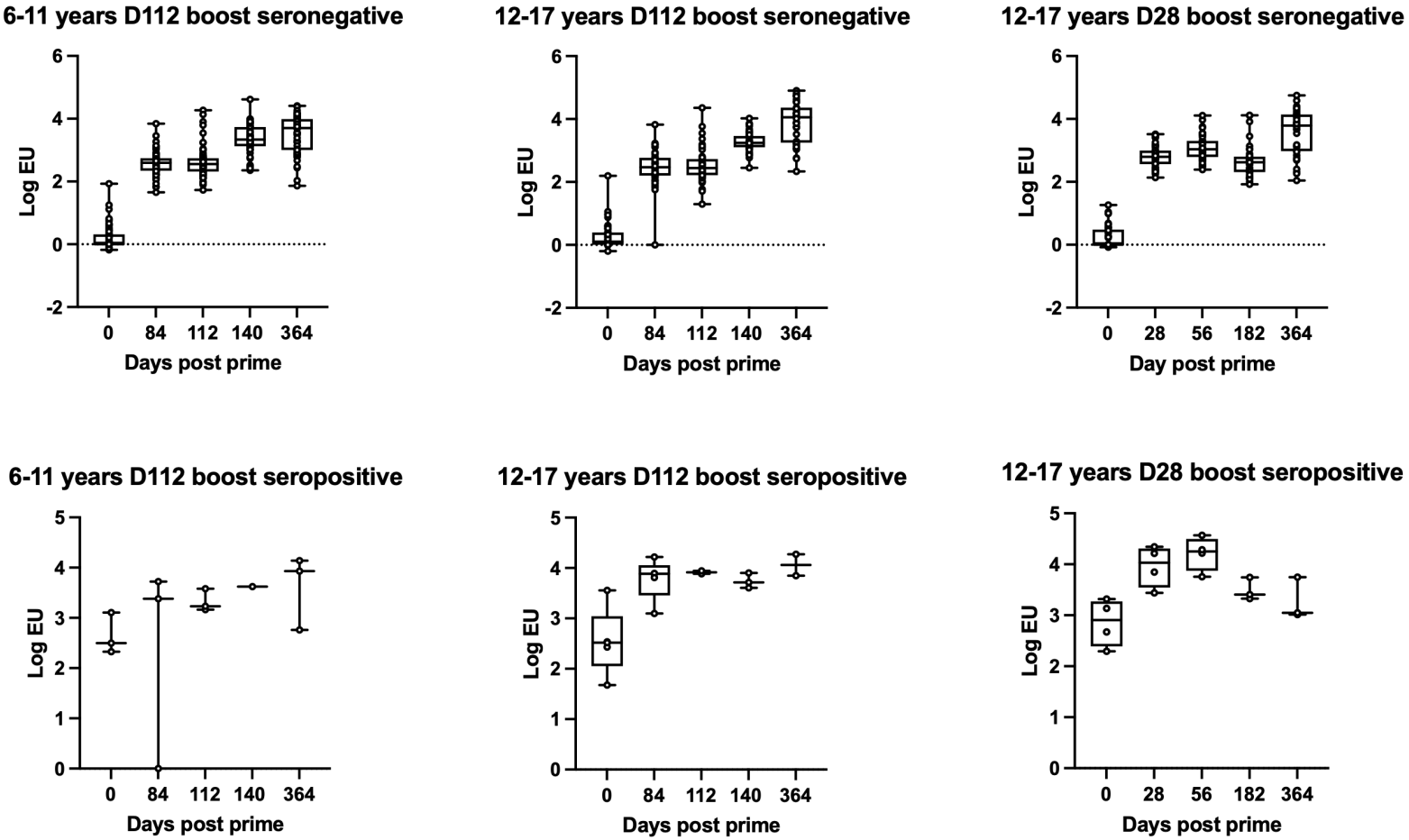
Age group (years)	Interval (days)	Arm	GMT D0 (EU/ml)	GMT D28 post first dose (EU/ml)	GMT D28 post second dose (EU/ml)	GMT D364 (EU/ml)
6-11	112	ChAdOx1 nCOV-19 (N=91)	1.5 (1.27-1.84), 77	759 (560 – 1030), 39	2310 (1700-3138), 48	3109 (2127-4544) 54
		MenB (N =21)	1.9 (0.99-3.76), 16	1 (1-1), 10	2 (1-2), 9	54 (8-379), 9
12-17	112	ChAdOx1 nCOV-19 (N=61)	1.85 (1.4-2.39), 63		1963 (1575-2448), 45	8265 (5291-12911), 45
		MenB (N=14)	1.2 (0.99-1.45), 12		4.5 (1-17), 11	9321 (1296-67041), 7
12-17	28	ChAdOx1 nCOV-19 (N=59)	1.8 (1.4-2.28), 47	634 (496-810), 45	1194 (908-1568), 45	3738 (2172-6433), 45
		MenB (N=16)	2.5 (1.28-5.3), 13	2 (1-4), 14	4 (2-10), 13	3293 (779-13916), 9

Table 3.3 shows results for seropositive individuals. Although sample numbers were small, the data show that there is an increase in anti-spike total IgG after both a first and second dose of vaccine in seropositive individuals, however the magnitude of increase after a second dose appears to be less than for seronegative individuals.

Table 3.3 In-house SARS-CoV-2 (Wuhan) anti-spike ELISA results in baseline seropositive ChAdOx1 nCOV-19 recipients, comparing geometric mean titres (GMTs) 28 days after receiving the first and second dose). *95% confidence interval of GMTs are given in brackets followed by the number of samples available at each timepoint.*

Age group (years)	Interval (days)	GMT on day of second dose (EU)	GMT D28 post second dose (EU)
6-11	112	2121 (591-7615), 1	4209, 1
12 -17	112	8220 (3373-20031), 2	5516 (2355-12920), 3
12-17	28	9170 (2071-40612), 4	16120 (4714, 55127), 4

Figure 3.4 In-house SARS-CoV-2 (Wuhan) anti-spike IgG ELISA in baseline seronegative and seropositive COV006 participants who received two doses of ChAdOx1 nCoV-19. EU = EU/ml



Comparisons were also made of cellular immune responses. **Table 3.4** and **Figure 3.5** show IFN-γ ELISpot results from baseline seronegative COV006 participants. Cellular responses were detected in both age groups after a first vaccination. 28 days after the first dose, cellular responses in participants 6-11 years of age were 253 spot forming cells (SFCs) per million (10^6) peripheral blood mononuclear cells (PBMCs) (95% CI 177-360) compared with 295 SFCs/10^6 PBMCs in participants 12-17 years of age (95% CI 228-380), with a geometric mean ratio (GMR) of 0.86. Geometric mean (GM) cellular responses remained above baseline levels but declined by 28 days after the second dose in both 6-11 years of age (to 104 SFCs/10^6 PBMCs) and 12-17 years of age (256 SFCs/10^6 PBMCs). Cellular responses 28 days after the second vaccine dose were higher in 12-17 years who received the doses at a 28-day interval (256 SFCs/10^6 PBMCs, 95% CI 188-349) rather than a 112-day interval (137 SFCs/10^6 PBMCs, 95% CI 96-195) with a GMR of 1.86. Cellular responses at 28 days after second dose at a 112-day interval were higher in children 12-17 years of age (287 SFCs/10^6 PBMCs, 95% CI 195-421) than children 6-11 years of age (229 SFCs/10^6 PBMCs, 95% CI 171-305) with a GMR of 1.25.

Table 3.4 In-house ELISpot results in baseline seronegative ChAdOx1 nCOV-19 recipients, comparing geometric mean spot forming cell counts (SFCs) 28 days after receiving the first and second dose. *95% confidence interval of geometric mean counts are given in brackets followed by the number of samples available at each timepoint.*

Age group (years)	Interval (days)	Arm	GM D0 (SFC/10^6 PBMCs)	GM D28 post first dose (SFC/10^6 PBMCs)	GM D28 post second dose (SFC/10^6 PBMCs)	GM D364 (SFC/10^6 PBMCs)
6-11	112	ChAdOx1 nCOV-19 (N=91)	26 (22-31), 73	253 (177-360), 34	104 (79-137), 39	229 (171-305), 50
		MenB (N =21)	21 (16-28), 18	22 (13-36), 9	19 (12-32), 6	66 (30, 147), 8
12-17	112	ChAdOx1 nCOV-19 (N=61)	42 (33-55), 46		137 (96-195), 35	287 (195-421), 40
		MenB (N=14)	42.5 (24-76), 11		30 (12-77), 8	226 (11-464), 6
12-17	28	ChAdOx1 nCOV-19 (N=59)	33 (25-43), 43	295 (228-380), 45	256 (188-349), 42	217 (143-329), 40
		MenB (N=16)	33 (20-54), 14	23 (15-34), 14	22 (15-32), 14	262 (86-800), 9

Similar to anti-spike IgG titres, pseudovirus SARS-CoV-2 neutralisation antibody titres peaked at day 29 after the second dose of ChAdOx1 nCoV-19, shown in **Table 3.5** and **Figure 3.6.** Titres were higher in younger children 6-11 years of age than older children 12-17 years of age 28 days after receiving a second dose at a 112-day interval (IC50 2436 95% CI 767-4104 versus IC50 415 95% CI 306-524) with a GMR of 5.86.

Table 3.5 Pseudovirus neutralisation assay results in baseline seronegative ChAdOx1 nCOV-19 recipients, comparing half maximum inhibitory concentrations (IC50) 28 days after receiving the first and second dose. *95% confidence interval of mean concentrations are given in brackets followed by the number of samples available at each timepoint.*

Age group (years)	Interval (days)	Arm	Mean concentration D0 (IC50)	Mean concentration D28 post first (IC50)	Mean concentration D28 post second dose (IC50)	Mean concentration GMT D364 (IC50)
6-11	112	ChAdOx1 nCOV-19 (N=91)	0.92 (-0.81-2.4), 80	462 (-89 - 1014), 35	2436 (767 – 4104), 49	1410 (1033-1788), 37
		MenB (N =21)	0,0	0,0	0,0	2.5, 1
12-17	112	ChAdOx1 nCOV-19 (N=61)	1.2 (-1.2-3.6), 50		415 (306-524), 44	783 (-792, 2357), 4
		MenB (N=14)	0 (0), 12		1064 (-1009, 3137), 10	0,0
12-17	28	ChAdOx1 nCOV-19 (N=59)	0,0	172 (-7, -297), 46	246 (113, 381), 47	1191 (710-1673), 17
		MenB (N=16)	0,0	2 (-2-6), 15	4 (-5-13), 14	2819 (274, 5364), 6

Figure 3.5 In-house SARS-CoV-2-specific IFN-γ ELISpot counts in baseline seronegative COV006 participants who received two doses of ChAdOx1 nCoV-19. *SFC = spot forming cells/10^6 peripheral blood mononuclear cells*

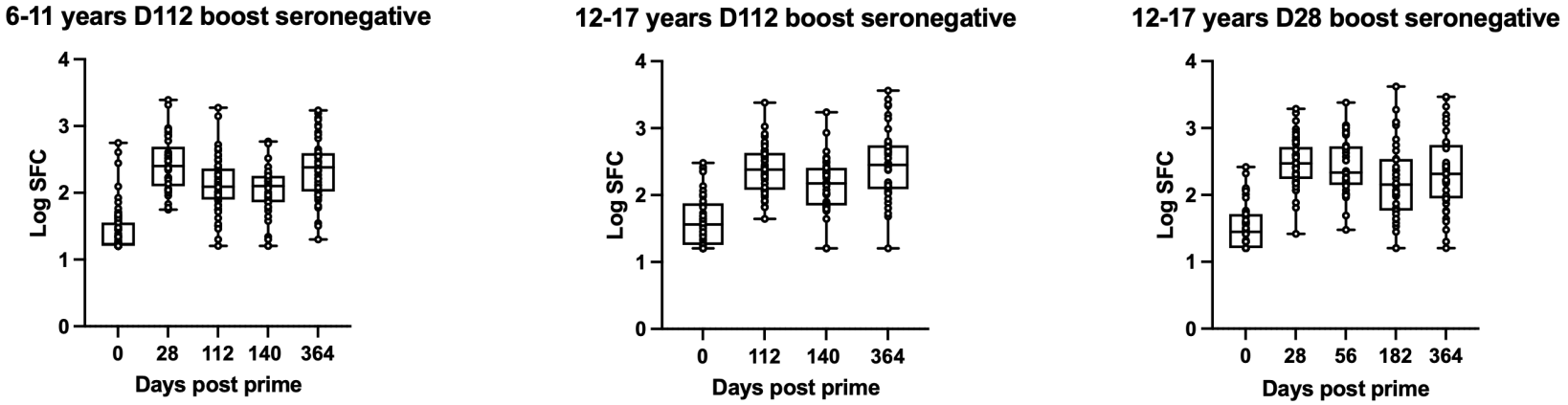
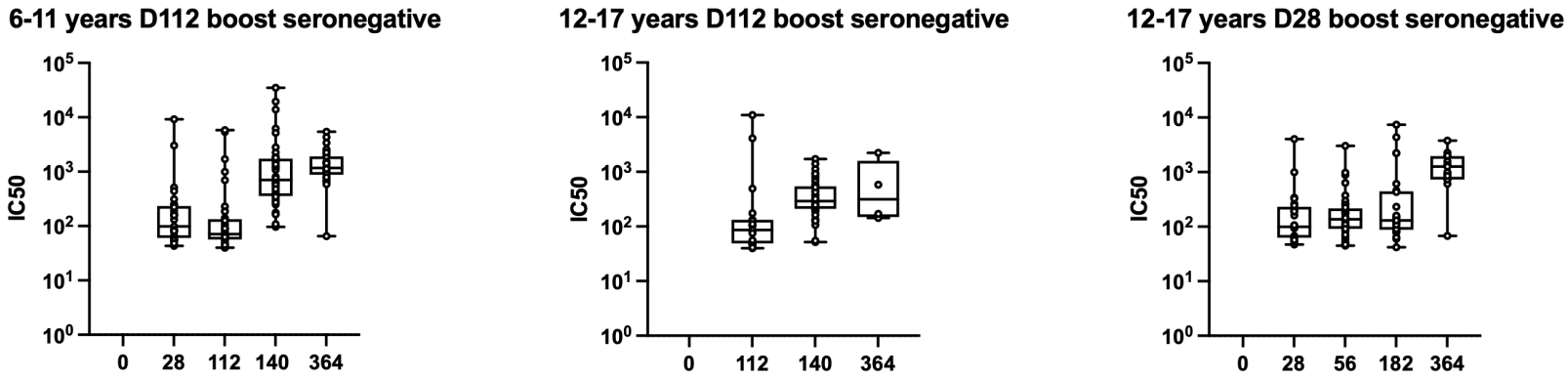


Figure 3.6 SARS-CoV-2 pseudotype neutralisation assay results in baseline seronegative COV006 participants who received two doses of ChAdOx1 nCoV-19. *IC50 = half maximal inhibitory concentration*



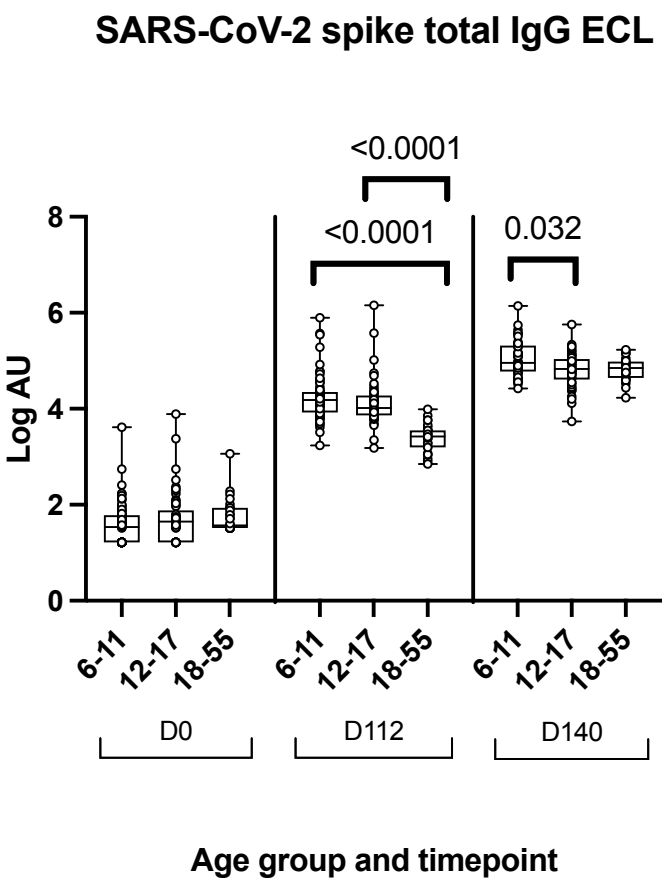
3.3.6 Immunogenicity is significantly higher in children than adults after one dose of ChAdOx1 nCoV-19 but this difference disappears after two doses

A subgroup of adult participants in an adult trial of ChAdOx1 nCoV-19 received a two-dose priming series at a 112-day (+/- 28 day) interval, similar to COV006 participants. However, the only comparable immunogenicity data for this subgroup were results from an external electrochemiluminescence assay. **Table 3.6** compares GMTs in children and adults who received their priming series at a comparable interval, at three timepoints – baseline, 112 days after first dose (day of second dose) and 140 days after first dose (28 days after second dose). **Figure 3.7** shows that after one dose younger children 6-11 years of age and older children 12-17 years of age generate significantly higher anti-spike IgG titres than adults 18-55 years of age, but after two doses the only significant difference is between younger and older child age groups. Therefore, a significant difference between adults and children emerges after one dose but is no longer present after two doses.

Table 3.6 Electrochemilluminescence data comparing anti-spike IgG titres across children and adults. *Participants received first dose at D0 and second dose at D112.*

Timepoint	6-11 years GMT (log AU)	12-17 years GMT (log AU)	18-55 years GMT (log AU)
D0	1.6, n=84	1.7, n=53	1.8, n=27
D112	4.2, n=70	4.1, n=49	3.4, n=27
D140	5.0, n=49	4.8, n=46	4.8, n=26

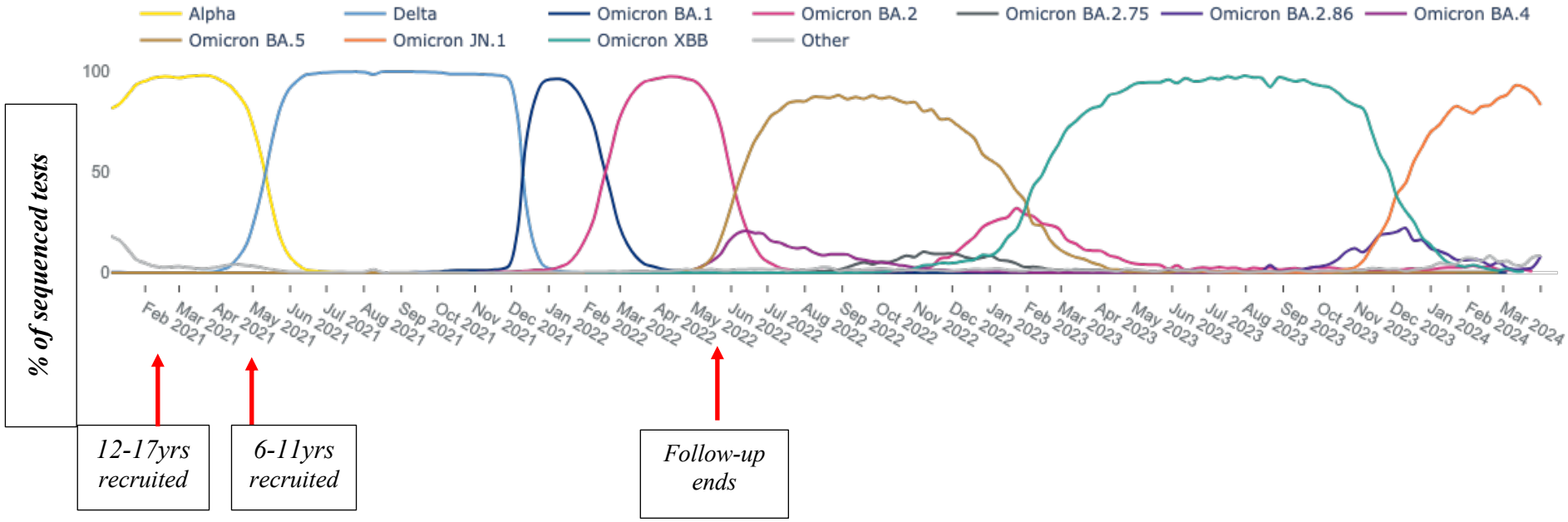
Figure 3.7 Scatter plot of electrochemilluminescence results comparing anti-spike IgG titres between children and adults across timepoints. *Participants received first dose at D0 and second dose at D112. Significance testing conducted using two-way ANOVA. AU = arbitrary units*



3.3.7 Waves of COVID relative to COV006 recruitment

Although COV006 was not adequately powered to calculate efficacy of the vaccine, participants did self-report positive test results for SARS-CoV-2 and therefore the data on infections could be used to conduct survival analysis with positive SARS-CoV-2 test results as an endpoint. **Figure 3.8** illustrates which SARS-CoV-2 variants were in circulation in the UK during the trial follow-up period as this could impact upon the interpretation of data on self-reported infections from participants.

Figure 3.8. Dominant SARS-CoV-2 variants circulating in the UK during recruitment period for COV006 *Adapted from ukhsa-dashboard.data.gov.uk/topics/covid-19 accessed 19th July 2024*



3.3.8 Survival analysis shows no difference in infection rates between ChAdOx1 nCoV-19 and MenB recipients

A survival analysis was conducted using self-reported SARS-CoV-2 infection as the endpoint, with censoring for COVID-19 vaccines administered outside the trial (as the COVID-19 vaccination programme for school-aged children was rolled out in the UK) or withdrawal from the study. The follow-up period began two weeks after receipt of the second ChAdOx1 nCoV-19 vaccine. **Figure 3.9** shows that the confidence intervals for the control and ChAdOx1 nCoV-19 arm overlap. The analysis suggests that in the COV006 study cohort, there was no difference between ChAdOx1 nCoV-19 or MenB recipients with regards to the proportion of self-reported SARS-CoV-2 infection, i.e. ChAdOx1 nCoV-19 did not confer any protection against either symptomatic or asymptomatic infection in children.

Table 3.7 compares the number of asymptomatic and symptomatic self-reported SARS-CoV-2 infections in the ChAdOx1 nCoV-19 and MenB arms. Although the percentage of symptomatic infections in the ChAdOx1 nCoV-19 arm (74%) is lower than in the MenB arm (92%), the sample numbers are too small to draw a conclusion.

Figure 3.9 Survival curve comparing time to self-reported SARS-CoV-2 infection in ChAdOx1 nCoV-19 and MenB recipients in COV006. *Survival time calculated from 14 days following second dose of vaccine with censoring for study withdrawal or COVID-19 vaccination separate from the study in the national immunisation programme. Shaded area shows 95% confidence interval. Chad=0 represents the control arm, chad=1 represents ChAdOx1 nCOV-19 recipients.*

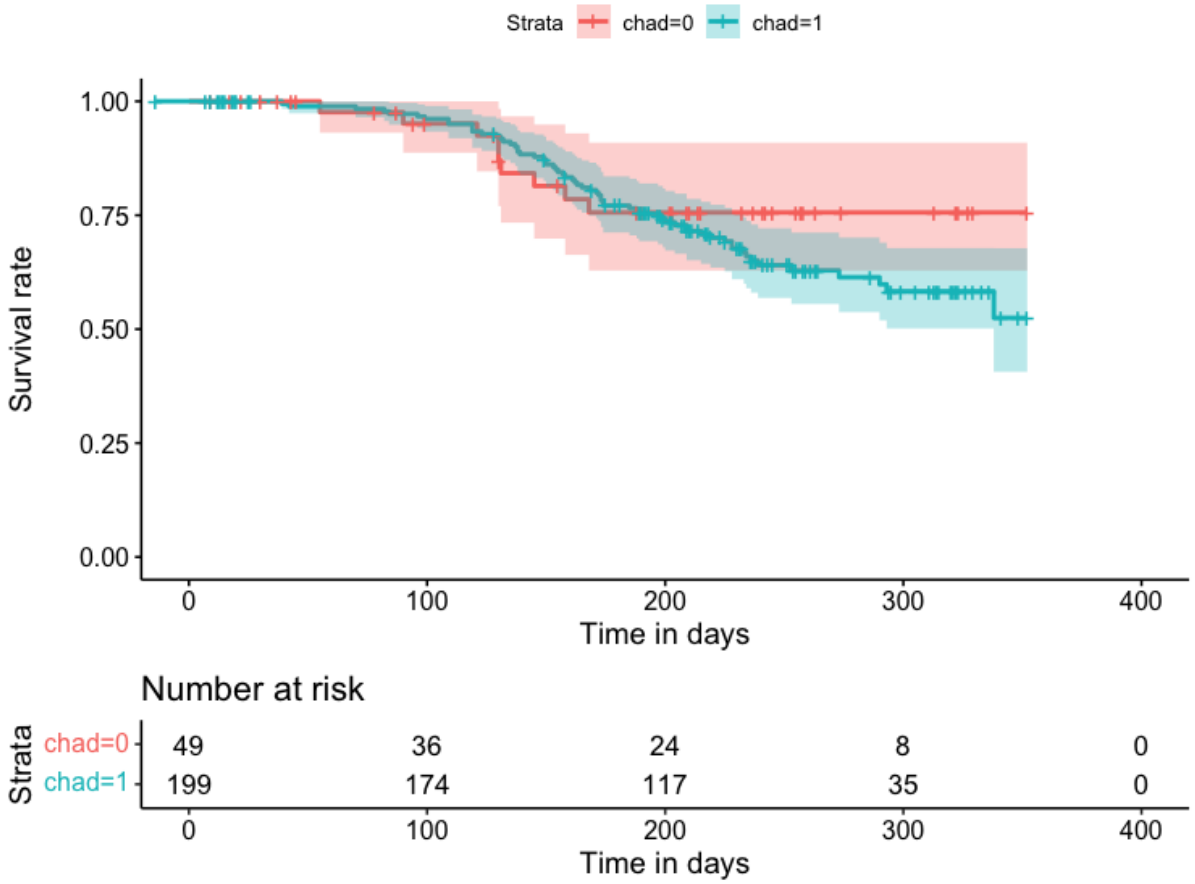


Table 3.7 Comparison of symptomatic versus asymptomatic self-reported SARS-CoV-2 infections in COV006 participants

	Symptomatic	Asymptomatic	Total
ChAd	68	24	92
MenB	12	1	13
Total	80	25	105

3.3.9 Anti-N data also show no difference in infection rates between ChAdOx1 nCoV-19 and MenB recipients

Children experience greater rates of asymptomatic SARS-CoV-2 infection than adults. To adjust for potential under-reporting of SARS-CoV-2 infection episodes, anti-N IgG titres were assayed at all timepoints where samples were available. Participants were defined as experiencing breakthrough infection if their anti-N titre had at least doubled between D28 post second dose and the final timepoint in the study (D364). Participants who did not have a result at D28 post second dose or D364 were not included in this analysis. **Table 3.8** shows that SARS-CoV-2 infection appear to occur at similar rates in the ChAdOx1 nCoV-19 and MenB arms, therefore supporting the results of the survival analysis which suggest that ChAdOx1 nCoV-19 does not protect children against SARS-CoV-2 infection.

Table 3.8 Comparison of breakthrough infection in ChAdOx1 nCoV-19 and MenB recipients in COV006, defined by anti-N titre *Breakthrough infection defined as a greater than 2-fold increase in anti-N titre between 28 days after second dose and final timepoint in the study (D364). Numbers denote number of participants i.e. one participant may have experienced more than one breakthrough infection*

	Infection by D364	No infection by D364	Status unknown D364	Total
ChAdOx1 nCoV-19	40	46	19	105
MenB	5	8	13	26
Total	45	54	32	131

3.4 Discussion

These are the first trial results describing immunisation of children and adolescents with ChAdOx1 nCoV-19 at different dosing intervals, showing that the vaccine is well-tolerated and immunogenic in children and adolescents 6-17 years of age, when given as a two-dose regimen. Reactogenicity was lower after the second dose regardless of dosing interval, a finding also observed in adult studies.[71] Spike-specific cellular immune responses were observed following the first dose. Humoral immunity was greater in the longer dosing interval arm than the shorter interval arm, and greater in younger participants than older participants dosed 112 days apart. Although this study was not powered to formally assess efficacy, when using self-reported SARS-CoV-2 episodes as a clinical endpoint ChAdOx1 nCoV-19 does not appear to protect children against SARS-CoV-2 infection.

Consistent with previous clinical trials of ChAd-vectored vaccines in children, ChAdOx1 nCoV-19 had an acceptable tolerability profile. Fever rates of 16% in participants aged 12-17 years after a first dose are comparable with those reported after routine childhood immunisations and in an adult trial of ChAdOx1 nCoV-19 where it was reported at 18%.[72] No SAEs or AESIs related to study vaccine administration occurred. Other trials have also assessed safety and reactogenicity of COVID-19 vaccines in adolescents. A phase 2 trial of BNT162b2 in adolescents reported higher fever rates after a second dose, consistent with adult findings,[102] but not observed in our study. Fever rates of 4-5% were reported in a phase 2 trial of CoronaVac in adolescents[97] and 12% in a phase 2 trial of BBIBP-CorV.[98] However, fever rates were significantly higher in younger participants aged 6-11 years than 12-7 years of age who received ChAdOx1 nCoV-19 (47%), which is consistent with findings from a phase 2/3 study of BNT162b2 where fever rates approaching 100% post administration of two adult doses in participants aged 5-11 years are described.[101] Fractional doses of

BNT162b2 are now recommended for younger children, with a more acceptable reactogenicity profile. [103]

Participants in this study generated at least as great a magnitude of anti-spike IgG response as adults aged 18-65 years after two doses of ChAdOx1 nCoV-19 given at an 8–12-week interval. As already described in adults, a greater humoral response was measured after a longer dosing interval of ChAdOx1 nCoV-19.[104] Younger children 6-11 years of age produced greater anti-spike IgG titres and neutralising antibody concentrations than older children 12-17 years of age after both a first and second dose of vaccine with a 112-day interval. In seronegative participants, an increase in anti-spike IgG titres was measured after the second dose of ChAdOx1 nCoV-19 regardless of dosing interval, but no rise in titres was seen in seropositive participants after a second dose. This suggests that when vaccine supplies are limited, receiving only one dose of ChAdOx1 nCoV-19 may be sufficient to confer protection in children and adolescents with previous COVID-19 infection. The level of IgG titres observed in our trial are similar to those seen in a study conducted in the US, Peru and Chile where receiving two adult doses four weeks apart was associated with 74% efficacy against symptomatic PCR-positive SARS-CoV-2 infection in adults 18-64 years of age.[105]

Immunogenicity data from paediatric trials of other COVID-19 vaccines have also been published. Two separate phase 1/2 trials of CoronaVac and BBIBP-CorV in children 3-17 years of age showed that two standard adult doses of vaccine given 28 days apart were as immunogenic in children as in adults, as assessed by neutralising antibody titres.[97,106] Greater immunogenicity in younger children 5-11 years of age has been observed after vaccination with adult doses of BNT162b2, such that fractional doses of 10 μg are now recommended for use in this age group.[107] Phase 2/3 trials of BNT162b2 in children 6-15 years of age [99,108]

reported preliminary efficacy results comparable with adult trials. However real-world data suggest that protection against mild disease conferred by fractional dosing regimens may be less than that of adult doses.[109]

ChAdOx1 nCoV-19 produces significant increases in spike-specific T cell responses in our cohort and this is the first study to describe the generation of cellular immunity in response to COVID-19 vaccination in healthy children. A direct comparison to responses measured in adults aged 18-65 years after ChAdOx1 nCoV-19 vaccination is not possible due to the different spike peptide pooling strategy used in the IFN-γ ELISpot assay carried out in this study. Whilst humoral immunogenicity differed significantly between younger and older age groups, T cell responses were comparable between children 6-11 years of age and 12-17 years of age after both first and second doses. As previously observed in the adult population,[104] a second dose of vaccine did not further increase spike-specific T cell responses regardless of the interval between doses. Vaccination against the ancestral Wuhan SARS-CoV-2 strain induces memory T cells which have the ability to recognise conserved epitopes in variants of concern (VOCs).[110] Vaccination-associated protection against severe COVID-19 disease including hospitalisation caused by VOCs persists despite falling levels of neutralising antibodies, suggesting that non-neutralising antibodies and T cell responses may confer a degree of protection against severe disease.[111] In addition, early induction of SARS-CoV-2-specific T cell activity correlates with less severe disease in natural infection.[112] Whilst these findings support a role for T cell activity in conferring protection against severe disease, the role that cellular immunity plays in the child and adolescent population which is at low risk of severe disease requires further investigation.

Anti-vector immunity has been hypothesised to negatively impact the immunogenicity of ChAd-vectored vaccines. Published data have shown that similar levels of anti-vector immunity were seen regardless of vaccination interval and age group.[76] Therefore, anti-vector immunity is unlikely to be the main reason explaining the observed difference of the ChAdOx1 nCoV-19 immunogenicity between arms. Consistent with findings in adults, there was no strong correlation between the level of anti-vector immunity measured immediately before administering a second dose and humoral and cellular measures of immunogenicity 28 days after the second vaccination.[113] These results allow comparison of both humoral and cellular immunity with results from adult studies and indicates that ChAdOx1 nCoV-19 is at least as immunogenic in the age groups studied and therefore could be expected to provide a degree of clinical protection similar to that observed in the adult efficacy trials and real-world effectiveness studies.

The results above also suggest that it is not possible to draw conclusions about the efficacy of ChAdOx1 nCoV-19 in children. The ancestral vaccine has been shown to demonstrate reduced protection against Omicron variants, however the dominant variant in circulation during the follow-up period of COV006 was the delta variant during the summer of 2021, which the ancestral vaccine has been shown to provide some protection against.[95]

Following conditional approvals and emergency use authorisation of ChAdOx1 nCoV-19 in adults, rare cases of thrombosis with thrombocytopenia syndrome (TTS) emerged in association with first-dose administration of the ChAdOx1 nCoV-19, with an incidence rate of 8.1 per million administered vaccine doses among adults.[114] TTS is now listed as a rare side effect of the vaccine and several countries restrict the use of ChAdOx1 nCoV-19 in younger age groups. The risk of TTS in children cannot be directly extrapolated from adult

data as the epidemiology of thrombotic disorders differs between children and adults.[115] TTS has also been reported following administration of the Ad26.COV2.S vaccine[116] and mRNA COVID-19 vaccines, as well as after SARS-CoV-2 infection[117]. Other side-effects associated with COVID-19 vaccination include myocarditis which has been observed in predominantly adolescent males after receiving BNT162b2 and mRNA-1273, most commonly within 4 days of receiving a second dose of vaccine.[118,119] Policy makers have had to weigh up the risks of any paediatric COVID-19 vaccination programme against the likely benefits.

Most adolescents eligible for COVID-19 vaccination in high-income countries were offered BNT162b2 as advised by the WHO. However, clinical trial data for other COVID-19 vaccines in children were needed as countries are resorting to the use of alternative vaccines due to supply constraints; BBIBP-CorV and CoronaVac were administered to children under 16 years of age in low-, middle- and high-income countries.[120]

These results have several limitations, including a small sample size and limited ethnic diversity; 93% of the trial participants were of Caucasian ethnicity. Although ChAdOx1 nCoV-19 efficacy does not vary significantly with ethnicity in adults, a higher prevalence of PIMS-TS/MIS-C in Black, Hispanic and Asian and Pacific Islander children has been reported.[121] The cohort of healthy volunteers for COV006 excluded children with significant medical comorbidities, who are at increased risk of severe COVID-19 disease and may be prioritised for vaccination. The relatively limited number of study visits may have affected the accuracy of the analyses of survival and anti-N data which were conducted. The high rate of asymptomatic infection means that it was not possible to know when infections occurred between visits which were sometimes six months apart. Another factor to consider is that the vaccine targeted the ancestral spike protein (Wuhan) whereas the dominant variant

circulating at the time in the UK during the study follow-up period was Delta or Omicron BA.1 This may have reduced the apparent efficacy of the vaccine.

The assays conducted only enable a description of how immune responses in children differ to adults. Further cellular assays such as a memory B-cell ELISpot would improve understanding of how younger children are able to generate higher titres of total IgG. In addition, anti-vector immunity was cited as a concern regarding the use of a novel homologous prime-boost regimen in children and anti-vector assays were not done here, but the results have been published elsewhere and shown not to correlate with overall immunogenicity.[63]

Future SARS-CoV-2 vaccine studies in children will need to be guided by the direction of vaccination policy in children. Where vaccination of children is focused on protecting those with co-morbidities and most vulnerable to severe infection, clinically relevant endpoints must be chosen and studies powered appropriately in study cohorts composed of the target populations. Where transmission of SARS-CoV-2 may be of greater concern and driving vaccination initiatives, regular serum sampling and nasal swabbing of participants will be required for meaningful trial results.

Chapter 4. Impact of immunity to seasonal human coronaviruses

4.1 Introduction

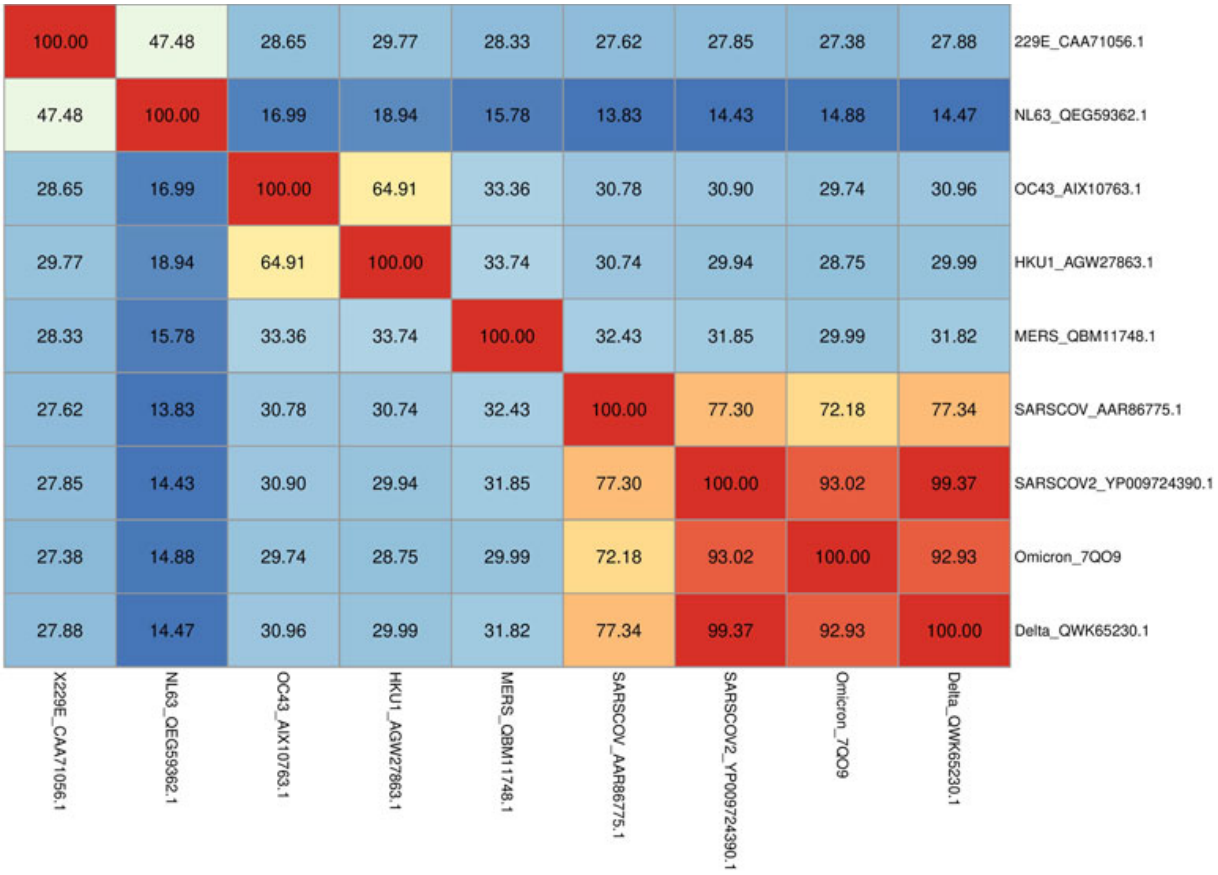
Novel variants of the SARS-CoV-2 virus emerged as the pandemic evolved in 2020. The first case of the Alpha variant (B.1.1.7) globally was sequenced in the UK in September 2020 [122] possessing several mutations to the spike protein including the deletion of H69/V70 (histidine and valine at position 69/70) which mediated greater cell-cell fusion of B.1.1.7 than wild-type Wuhan SARS-CoV-2,[123] N501Y (substitution of asparagine to tyrosine in amino-acid position 501) which increased the affinity of the spike protein for cellular receptors[124] and E484K (substitution of glutamine acid by lysine at position 484) which was associated with reduced neutralising activity of human post-vaccination and convalescent sera as well as reduced vaccine effectiveness.[125] The second wave of COVID-19 disease in Autumn 2020 in the UK was partly attributed to the greater transmissibility of this variant, with the percentage of people testing positive for SARS-CoV-2 during the period of Alpha variant dominance peaking in January 2021.

The Delta (B.1.617.2) variant with nine amino acid mutations in the spike protein was dominant in the UK from May to December 2021. Four substitutions were of particular concern – D614G (aspartic acid to glycine), T478K (threonine to lysine), L452R (leucine to arginine) and L681R (proline to arginine), all associated with improved transmissibility as well as decrease in neutralising antibody titres, evasion of neutralising antibody activity or decrease in the sensitivity of the virus to neutralising antibodies.[126] The next variant of concern to be dominant in the UK, Omicron (originally B1.1.529 and reclassified now into BA lineages), was first reported to the WHO in November 2021 after initial detection in Botswana with a significantly greater degree of mutations to the spike construct than seen

with previous variants. More than 50 mutations have now been detected, particularly to the SARS-CoV-2 receptor binding domain (RBD). Whilst the virus continues to evolve, as of April 2024, all WHO designated variants of interest (VOI) originate from the original Omicron lineage, including XBB.1.5, XBB.1.16, EG.5, BA.286 and JN.1.[127]

The *Coronavirinae* subfamily includes four genera – alpha and beta coronaviruses which infect mammals and humans, and gamma and delta coronaviruses which commonly infect birds. Multiple sequence alignment and examination of spike protein sequence homology has shown that similarity is greatest between SARS-CoV-2 and other betacoronaviruses (e.g. OC43 and HKU1) as shown in **Figure 4.1**[128,129] and is thought to underlie pre-existing reactivity to SARS-CoV-2 s in unexposed individuals.[130] NL63 cross-reactivity with SARS-CoV-2 spike has been demonstrated and may be related to the fact that both viruses utilise the ACE2 receptor to facilitate viral entry into host cells.[131]

Figure 4.1. Percentage identity matrix showing sequence homology between HCoVs
Adapted from Cicaloni et al. A Bioinformatics Approach to Investigate Structural and Non-Structural Proteins in Human Coronaviruses. Front Genet. 2022.14;13:891418.



Pre-pandemic serum reactivity against SARS-CoV-2 has been associated with the presence of memory T cells capable of recognising SARS-CoV-2 epitopes as well as epitopes from circulating human coronaviruses. Natural infection with SARS-CoV-2 has been shown to increase titres of total IgG against beta-coronaviruses (OC43, HKU1) but not alphacoronaviruses (NL63, 229E).[132] Induction of cross-reactive immunity to betacoronaviruses has also been positively correlated with severity of COVID-19 disease.[133,134] However, the extent to which such cross-reactive immunity may have clinical consequences is unknown. A paediatric cohort study comparing serum samples pre- and post-pandemic showed that higher baseline titres of binding antibody to HCoVs were not associated with a reduced risk of becoming infected with SARS-CoV-2 in the community.[135]

The phenomenon of immune imprinting, whereby exposure to one strain of a virus results in priming of B cell memory and limits the future development of memory B cells against antigenically related variants, is of relevance to the development of variant SARS-CoV-2 vaccines to ensure they are effective against future strains. Immune imprinting has been demonstrated in mice as immunisation of them with HCoV proteins prior to SARS-CoV-2 vaccination has been shown to impede the generation of SARS-CoV-2 neutralising antibody.[136] Whether such immune imprinting is also seen in humans and how it is associated with differences in the immune response to COVID-19 vaccination has relevance to the overall understanding of vaccine response across different age groups, which could impact vaccine design as children and adults are thought to have different levels of pre-existing exposure.

This chapter will investigate whether there is evidence of immune imprinting in children following ChAdOx1 nCoV-19 vaccination by measuring levels of HCoV spike-specific antibody before and after vaccination and whether this is related to age-specific differences in HCoV immunity.

4.2 Methods

4.2.1 Sample selection

Quantification of total IgG against circulating human coronavirus antigens and SARS-CoV-2 antigens by MSD multiplexed immunoassay was performed on the sera of COV006 seronegative participants who received two doses of ChAdOx1 nCoV-19 and had provided serum samples at three timepoints: D0 baseline (day of first vaccine dose), D112 (day of second dose) and D140 (28 days after second dose). These were compared with adult subjects recruited into a clinical trial of ChAdOx1 nCoV-19 in adults who also provided serum samples at three timepoints, however the interval between the first and second dose was shorter in adults. Sample selection for the multiplexed Luminex immunoassay were chosen in a similar manner.

Participant samples were excluded if the participant self-reported a SARS-CoV-2 infection during the trial follow-up period.

4.2.2 Sample collection and storage

All samples used for assays in this chapter were obtained from serum samples collected at study visits as outlined in **Figure 2.1**. Following collection, serum samples were processed on site as per **Chapter 2, section 2.1.5**. Serum samples were stored at the CCVTM and IDRM at -80C before being thawed for use. Although the study protocol recommended maximum volumes for blood collection (5ml serum and 5ml lithium heparin sample at each visit for children 6-11 years of age, and 5ml serum and 10ml lithium heparin for each visit for children 12-17 years of age), samples were often below this range.

4.2.3 Assays

For details of the assays used in this chapter, please refer to the following sections:

1. MSD multiplexed immunoassay – Chapter 2, section 2.4.1
2. Luminex multiplexed immunoassay – Chapter 2, section 2.4.3

4.2.4 Statistical analysis

Statistical approaches are outlined in Chapter 2, section 2.5.2. One-way ANOVA was used to compare results between age groups at the same timepoint or within an age group at two different timepoints. Two-way ANOVA was used to compare between groups at more than one timepoint. Although subjects were chosen because of matching of samples across timepoints, results were not available at all timepoints due to technical challenges. Therefore, no adjustment for matching was made during analysis.

4.2 Results

4.3.1 Sample numbers

The age group and booster interval breakdown of participants who provided samples for the above assays is shown below in Table 4.1.

Table 4.1: Sample numbers for MSD assay

Age (years)	Interval between vaccines (days)	No. of PIDs
6-11	112	27
12-17	112	25
Adult	28	40

Fcγr binding profiles were assessed using a custom Luminex multiplexed immunoassay on samples obtained from 49 COV006 participants **(Table 4.2)** and 20 adult sample from participants in an adult ChAdOx1 nCoV-19 study.

Table 4.2 Sample numbers for Fcγr profiles

Age (years)	Interval between vaccines (days)	No. of participants (N)
6-11	112	23
12 to 17	112	26
18 to 55	112	20

4.3.2 Immunity to HCoVs increases with age in childhood and is lower than in adults

To examine whether the trend between age and HCoV immunity reported in the published literature for paediatric cohorts in different country settings were also true in a UK setting, the relationship between HCoV spike binding titres for OC43, HKU1, 229E and NL63 and age was examined. Correlation between age in years and anti-spike total IgG was positive and statistically significant for 229E spike ($r=0.3341$; $p<0.001$) and was positive but not statistically significant (using a threshold of Spearman's Rank Correlation Coefficient $r>0.3$; $p<0.05$) for HKU1, NL63 and OC43 spike (**Figure 4.2, Table 4.3**).

Figure 4.2 Relationship between age and baseline HCoV immunity and COV006 participants*. Linear regression lines demonstrating the association between age (in years) at recruitment and baseline (D0) HCoV total IgG titres against (a) 229E (b) NL63 (c) OC43 and (d) HKU1 spike. AU values were determined by MSD multiplexed immunoassay in SARS-CoV-2 seronegative COV006 partcipants (n=124) and adult ChAdOx1 nCoV-19 participants (n=40). Brown shaded areas show 95% confidence intervals of regression lines. Full statistical analysis is reported in Table 4.1.*

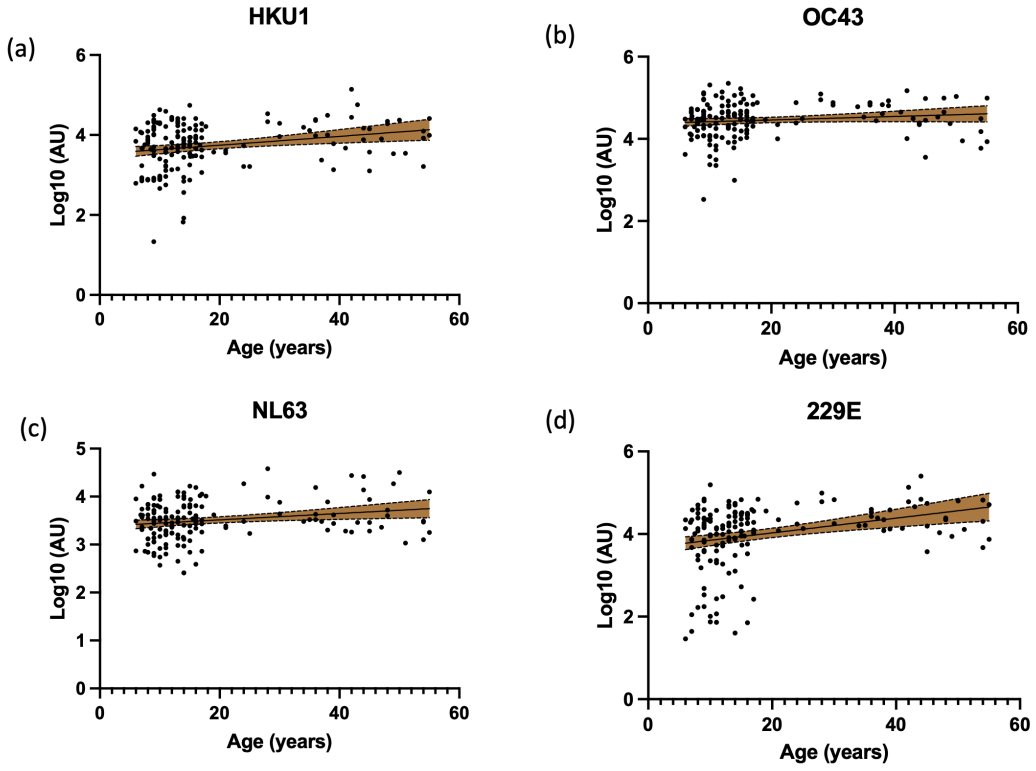


Table 4.3. Associated statistics for Figure 4.2. *Spearman's Rank Correlation Coefficients with 95% confidence intervals to describe the relationship between age and HCoV titre for each of 229E, NL63, OC43 and HKU, with linear regression values.*

	229E	NL63	OC43	HKU1
Spearman r	0.3341	0.1566	0.1800	0.2216
95% Confidence interval	0.1862-0.4672	-0.0012-0.3067	0.0230-0.3284	0.0662-0.3665
p value	<0.0001	0.0453	0.0211	0.0043

4.3.3 HCoV-specific Fcγr binding profiles demonstrate age-specific profiles

HCoV-specific total IgG titres at baseline (D0) did not differ between age groups for OC43 (**Figure 4.3a**), HKU1 (**Figure 4.4a**) or NL63 (**Figure 4.5a**). 229E anti-spike total IgG was higher in the adult age group than in children aged 12-17 years (p=0.0298) or children aged 6-11 years (p=0.02) (**Figure 4.6a**). However, HCoV spike-specific Fcγr-binding profiles revealed age-specific trends which were not observed in the total IgG titres alone. Whilst baseline MFI values for OC43 spike-specific Fcγr 2a binding antibody (**Figure 4.3b**), HKU spike-specific Fcγr 2a binding antibody (**Figure 4.4b**) and NL63 spike-specific Fcγr 2a binding antibody (**Figure 4.5b**) did not differ by age group, similar to the trends in total IgG titres (**Figure 4.3a, Figure 4.4a, Figure 4.5a**), baseline MFI values for HCoV spike-specific Fcγr 2a, 3a and 3b binding antibody were significantly higher in the older age group (18-55 years) than either the older or younger child groups. The same trend was observed for 229E (**Figure 4.6**), however this was difficult to interpret as baseline total IgG values were also highest in the adult age group and therefore it is unclear whether the age differences in Fcγr binding profiles were a reflection of the total IgG titres.

Figure 4.3. Total IgG and Fcγr binding profile for OC43. *(a) Baseline OC43 anti-spike total IgG across age groups in years (6-11 n=36, 12-17 n=37, 18=55 n=40) at baseline compared with (b-e) OC43 spike-specific Fcγr receptor profile at baseline (sample size for each age group minimum n=10, maximum n=26). AU = arbitrary unit, MFI= mean fluorescence intensity*

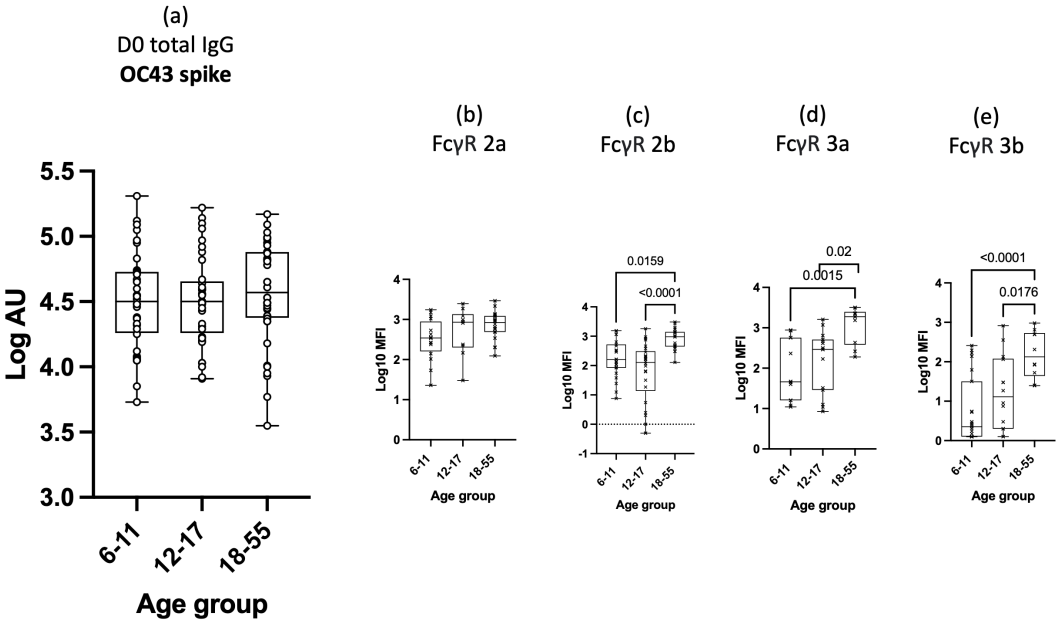


Figure 4.4 Total IgG and Fcγr binding profile for HKU1. *(a) Baseline HKU1 anti-spike total IgG across age groups in years (6-11 n=36, 12-17 n=37, 18=55 n=40) at baseline compared with (b-e) HKU1 spike-specific Fcγr receptor profile at baseline (sample size for each age group minimum n=10, maximum n=26) AU = arbitrary unit, MFI= mean fluorescence intensity*

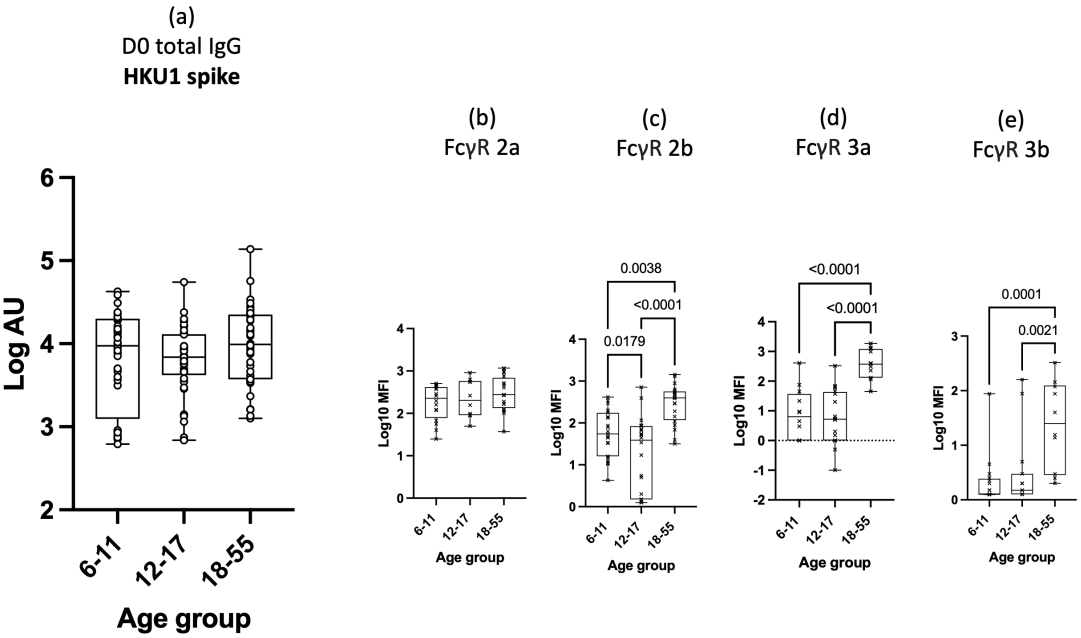


Figure 4.5. ***Total IgG and Fcγr binding profile for NL63.*** *(a) Baseline NL63 anti-spike total IgG across age groups in years (6-11 n=36, 12-17 n=37, 18=55 n=40) at baseline compared with (b-e) NL63 spike-specific Fcγr receptor profile at baseline (sample size for each age group minimum n=10, maximum n=26). AU = arbitrary unit, MFI= mean fluorescence intensity*

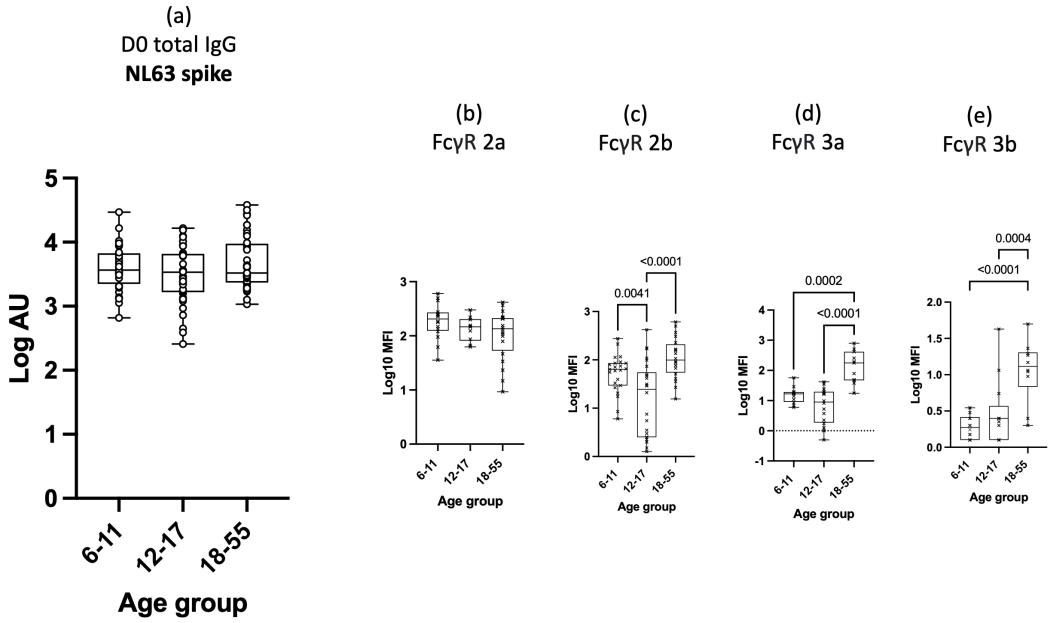
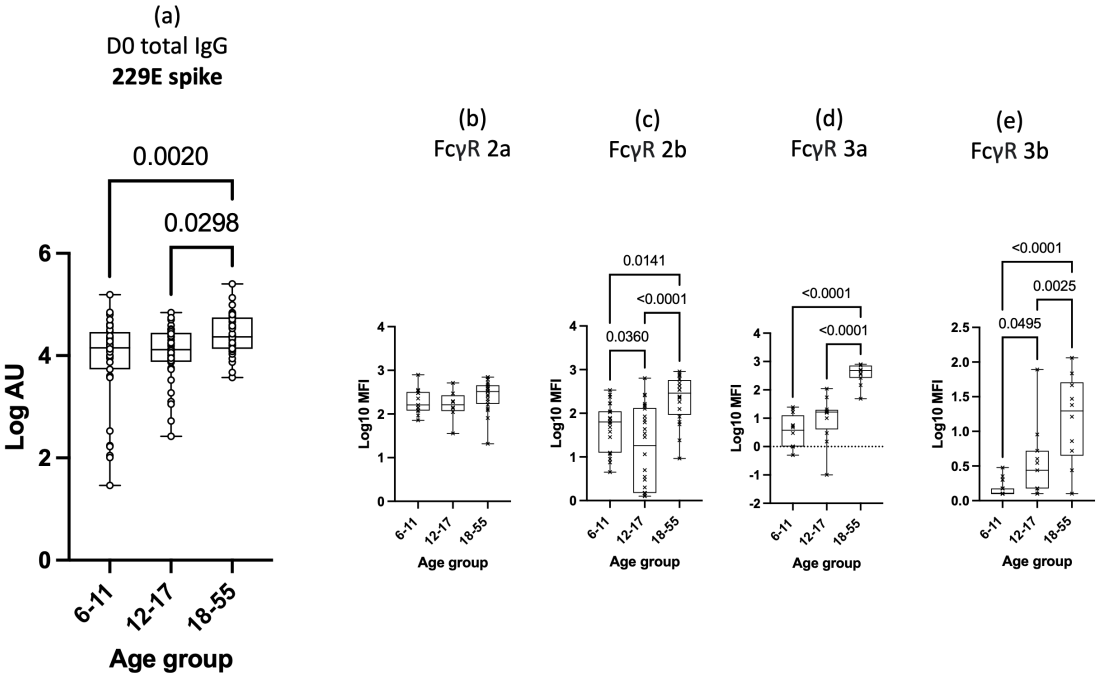


Figure 4.6. Total IgG and Fcγr binding profile for 229E. *(a) Baseline 229E anti-spike total IgG across age groups in years (6-11 n=36, 12-17 n=37, 18=55 n=40) at baseline compared with (b-e) 229E spike-specific Fcγr receptor profile at baseline (sample size for each age group minimum n=10, maximum n=26). AU = arbitrary unit, MFI= mean fluorescence intensity*



4.3.4. No association between HCoV IgG on day of vaccination (first or second dose) and ChAdOx1 nCoV-19 response in children but association measured in adults

To investigate whether baseline immunity to HCoVs was associated with the response to either a first or second dose of vaccine, correlation co-efficients were calculated between total IgG for each HCoV on day of vaccination and vaccine response 28 days later. No significant association between HCoV spike-specific total IgG titre on the day of vaccination (either first or second dose) and ChAdOx1 nCoV-19 response as measured by SARS-CoV-2 anti-spike IgG 28 days after vaccination was found in either the 6-11 years or 12-17 years age group (**Figure 4.7, Table 4.4**). A statistically significant association was measured between OC43 titre on day of first vaccination in adults (18-55 years) and anti-spike IgG titre 28 days after vaccination ($r=0.32$, $p=0.04$), but not following a second vaccination.

Table 4.4 Associated statistics for Figure 4.7. P values and 95% confidence intervals for correlation co-efficients (Spearman, illustrated in **Figure 4.7**) between HCoV titres at time of vaccination and anti-spike total IgG responses 28 days after receiving a (a) first and (b) second dose of ChAdOx1 nCoV-19

(a)

6-11 years

		OC43	NL63	229E	SCoV2 D28
P values	HKU1	0.01	0.20	0.10	0.74
	OC43		0.03	0.14	0.60
	NL63			0.37	0.25
	229E				0.46

		OC43	NL63	229E	SCoV2 D28
95% CI	HKU1	0.1083 to 0.6703	-0.1306 to 0.5162	-0.0615 to 0.5655	-0.4168 to 0.3085
	OC43		0.0229 to 0.6203	-0.0963 to 0.5413	-0.2759 to 0.4458
	NL63			-0.1923 to 0.4682	-0.1650 to 0.5343
	229E				-0.2379 to 0.4778

12-17 years

		OC43	NL63	229E	SCoV2 D28
P values	HKU1	0.03	0.00	0.05	0.61
	OC43		0.02	0.11	0.52
	NL63			0.04	0.70
	229E				0.43

		OC43	NL63	229E	SCoV2 D28
95% CI	HKU1	0.0186 to 0.6111	0.1730 to 0.6998	-0.0058 to 0.5957	-0.4072 to 0.2542
	OC43		0.0411 to 0.6251	-0.07510 to 0.5490	-0.4256 to 0.2333
	NL63			0.0020 to 0.6006	-0.2730 to 0.3902
	229E				-0.2077 to 0.4473

18-55 years

		OC43	NL63	229E	SCoV2 D28
P values	HKU1	0.01	0.14	0.01	0.54
	OC43		0.01	0.07	0.04
	NL63			0.00	0.08
	229E				0.16

		OC43	NL63	229E	SCoV2 D28
95% CI	HKU1	0.1177 to 0.6537	-0.09175 to 0.5164	0.0848 to 0.6343	-0.2266 to 0.4077
	OC43		0.1304 to 0.6610	-0.0380 to 0.5550	0.0011 to 0.5814
	NL63			0.3529 to 0.7748	-0.0454 to 0.5498
	229E				-0.1026 to 0.5084

(b)

6-11 years

		OC43	NL63	229E	SCoV2 D140
P values	HKU1	0.00	0.01	0.61	0.23
	OC43		0.06	0.40	0.60
	NL63			0.45	0.83
	229E				0.83

		OC43	NL63	229E	SCoV2 D140
95% CI	HKU1	0.2158 to 0.7791	0.1092 to 0.7322	-0.4740 to 0.2991	-0.1684 to 0.5742
	OC43		-0.03114 to 0.6599	-0.5235 to 0.2380	-0.2971 to 0.4758
	NL63			-0.2545 to 0.5106	-0.3532 to 0.4257
	229E				-0.3516 to 0.4272

12-17 years

		OC43	NL63	229E	SCoV2 D140
P values	HKU1	0.06	0.10	0.01	0.39
	OC43		0.02	0.01	0.42
	NL63			0.00	0.83
	229E				0.86

		OC43	NL63	229E	SCoV2 D140
95% CI	HKU1	-0.02648 to 0.6826	-0.0837 to 0.6507	0.1168 to 0.7521	-0.2495 to 0.5551
	OC43		0.0857 to 0.7381	0.1454 to 0.7645	-0.2613 to 0.5463
	NL63			0.4272 to 0.8660	-0.3746 to 0.4518
	229E				-0.3821 to 0.4448

18-55 years

		OC43	NL63	229E	SCoV2 D56
P values	HKU1	0.00	0.03	0.01	0.39
	OC43		0.00	0.00	0.23
	NL63			0.00	0.23
	229E				0.64

		OC43	NL63	229E	SCoV2 D56
95% CI	HKU1	0.2634 to 0.7321	0.0221 to 0.5951	0.1314 to 0.6616	-0.1901 to 0.4464
	OC43		0.4065 to 0.7987	0.2542 to 0.7275	-0.1365 to 0.4894
	NL63			0.3687 to 0.7820	-0.1349 to 0.4906
	229E				-0.2523 to 0.3926

4.3.5 First dose of ChAdOx1 nCoV-19 associated with statistically significant increase in betacoronavirus titres in children

HCoV spike total IgG titres were measured on the day of receiving the vaccine (both first and second dose) followed by 28 days later **(Figure 4.8).** A statistically significant increase in OC43 specific total IgG was found in children 12-17 years of age following a first dose ($p=0.0306$) corresponding to a D28 GMT of 4.7 AU and fold change of 1.76. The D28 GMT for SARS-CoV-2 spike total IgG in this age group was 4.31 AU.

A statistically significant increase in HKU1 specific total IgG was measured in children 6-11 years of age ($p=0.0148$) following a first dose, corresponding to a D28 GMT of 4.17 AU and a fold change of 3.36. The D28 GMT for SARS-CoV-2 spike total IgG in this age group was 4.65 AU. No other evidence of an increase in HCoV titres associated with vaccination was observed for any other age/HCoV pairing.

Figure 4.7. Correlation matrices showing relationship between HCoV total IgG titres on day of first dose vaccination) and vaccine response 28 days later (SARS CoV-2 spike total IgG) across age groups (6-11 years n=27 panel (a), 12-17 years n=25 panel (b), 18-55 years n=50 (panel c)), and between HCoV total IgG titres on day of second vaccination) and vaccine response 28 days later (SARS CoV-2 spike total IgG) across age groups (6-11 years n=27 panel (d), 12-17 years n=25 panel (e), 18-55 years n=50 (panel f)) *Numbers denote Spearman's rank correlation co-efficient.*

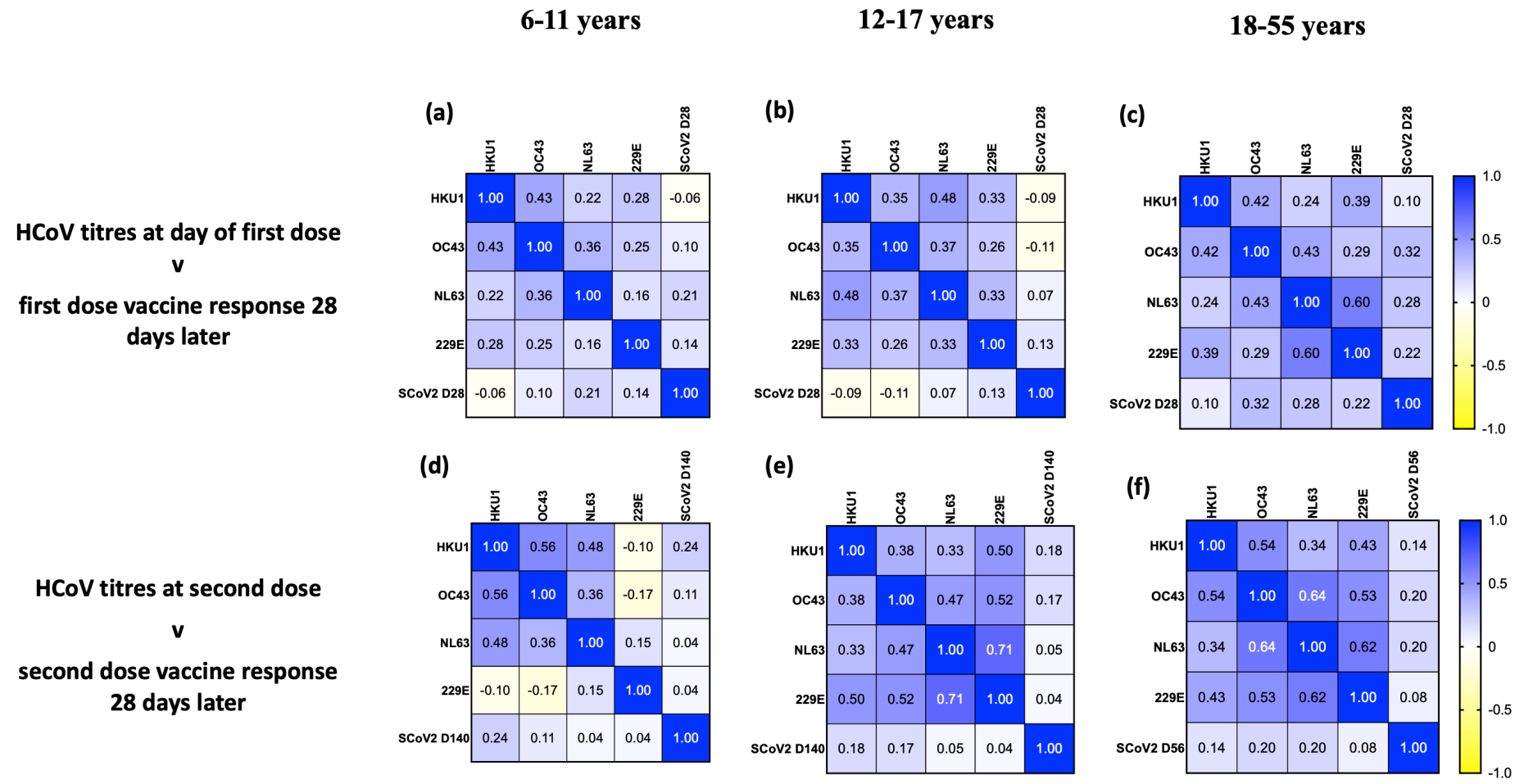
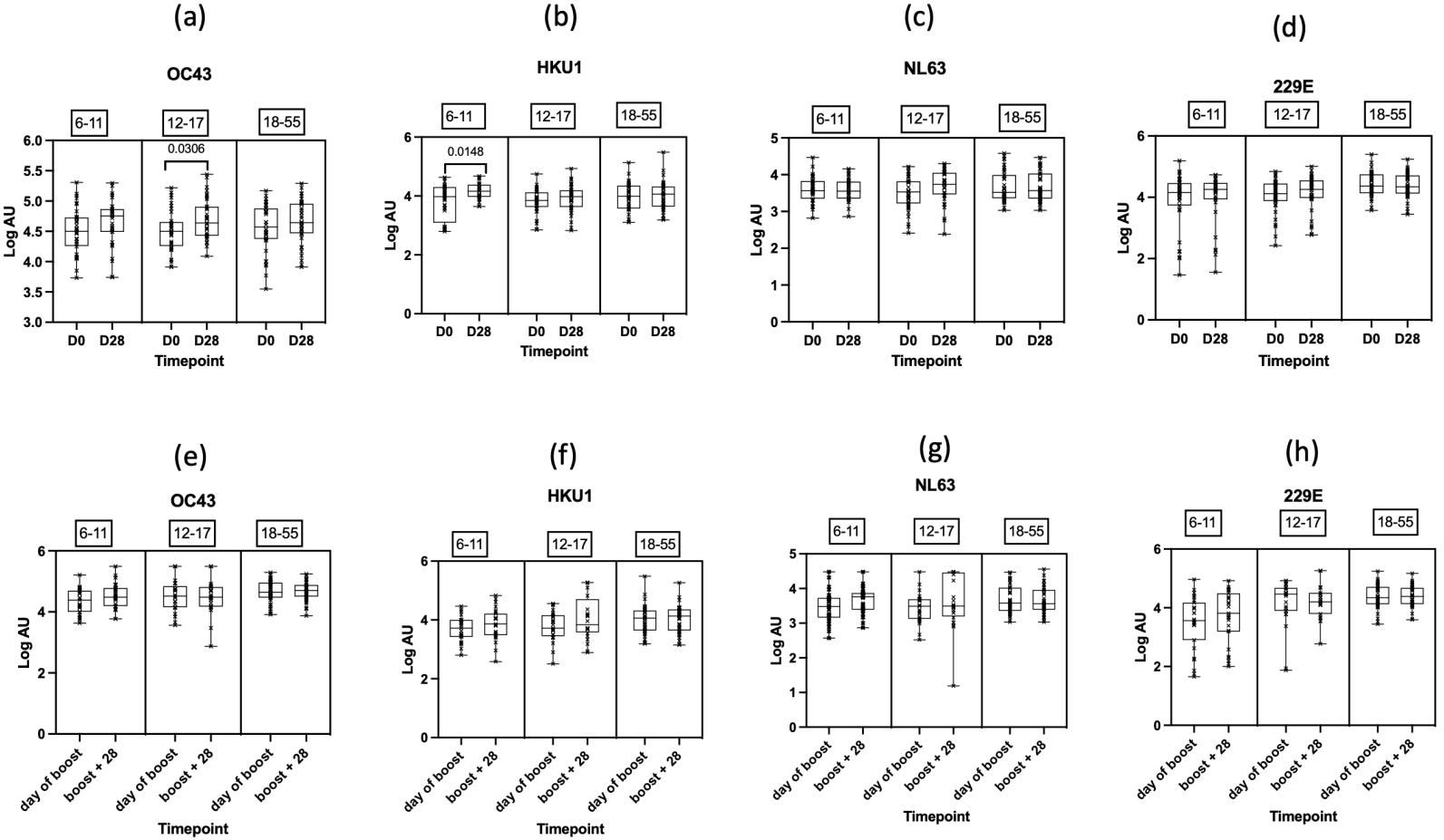


Figure 4.8 Comparison of HCoV titres at baseline and 28 days after receiving a first dose of ChAdOx1 nCoV-19 in seronegative participants across age groups (6-11 years n=27, 12-17 years n=25, 18-55 years n=50) panels (a-d) and comparing day of boost and 28 days after boost across age groups (panels e-g). Significance testing conducted using ANOVA, only significant (p<0.05) results are shown. *AU = arbitrary unit*



4.4 Discussion

These results show that serum antibody titres against circulating seasonal HCoVs increase from 6 to 55 years of age, and in this population sample this association reaches statistical significance for HCoV 229E. This is the first time that an age-dependent HCoV-specific Fcγr binding profile has been reported, with higher titres of HCoV-specific Fcγr 2b, 3a and 3b binding antibody found in adults than in children. In adults, but not children, OC43 total IgG titre at the time of first dose but not second dose was positively associated with ChAdOx1 nCoV-19 vaccine response. Finally, a first dose of vaccine was associated with a statistically significant increase in total anti-spike IgG antibody titres for betacoronaviruses in children (OC43 in children 12-17 years of age and HKU1 in children 6-11 years of age) but not in adults, demonstrating how the relationship between HCoV exposure and SARS-CoV-2 vaccination differs with age.

These findings are consistent with longitudinal seroepidemiology studies of seasonal HCoVs, showing that immunity to them increases during childhood and stabilises during adulthood.[131,137]Seropositivity for HCoV S1 binding antibody is established in early childhood and by the age of 3 years has been shown to range from 37-81% depending on HCoV type, in a European (Finnish) setting.[138] However, this is the first time that the FcγR binding profile for HCoV spike-specific antibodies has been compared across different age groups including children. Age-dependent FcγR expression profiles have been reported in neutrophils with FcγR 3a (CD16a) more prominent on aged neutrophils.[139] FcγR 3a is an activating receptor in humans and activation of FcγR 3a on NK cells plays a role in mediating antibody-dependent cell-mediated cytotoxicity (ADCC), and generation of ADCC antibodies in response to varicella-zoster virus vaccines has been shown to

decline with age.[140] FcγR polymorphisms are also associated with likelihood of response to treatment with rituximab in B cell lymphoma, and FcγR profiling of patients has shown that FcγR 3a is present in higher quantities in older male patients than younger male or female patients. Although previous studies have primarily reported age differences at the level of cellular receptor expression, it is therefore also plausible that age differences also exist at the level of antibody Fc binding.

"Back-boosting" of antibodies can be defined as an increase in antibody titres against antigens which have previously been encountered by the immune system, where the absolute titre against historical antigens is maintained at a higher level than the absolute titre against a newly seen antigen (such as in the case of SARS-CoV-2 vaccination in naïve individuals) and this phenomenon is thought to arise as a consequence of immune imprinting. Natural infection with SARS-CoV-2 has been linked to back-boosting of serum and nasal mucosal antibodies against betacoronaviruses in both children and adults.[141,142]

A back-boosting pattern was observed for OC43 titres following a first vaccination with ChAdOx1 CoV-19 in children 12-17 years of age, and to a lesser extent with HKU1 titres in younger children. Immunoglobulin heavy chain comparisons of adults and children have shown that pre-pandemic, children possessed clones convergent to SARS-CoV-2 with weak cross-reactivity to HCoVs,[143] whereas such clones were rare in adults in addition to SARS-CoV-2 cross-reactive CD4+ T cells being shown to decline with age. [144]

By contrast, no back-boosting was observed in adults. Instead, baseline OC43 titres were weakly positively correlated with vaccine response (r=0.32) and in children no correlation

was found. Comparable data from the published literature has reported mixed results; Hu et al.[145] found that OC43 S and S1 IgG antibodies at baseline were positively correlated with anti-RBD and neutralising antibodies to SARS-CoV-2 at 12 and 24 weeks after a second whole inactivated BBIBP-CorV vaccination in adults. However, Asamoah et al.[134] found that baseline betacoronavirus spike-specific IgG titres were negatively correlated with immune response to SARS-CoV-2 mRNA vaccination (with either BNT162b2 or mRNA-1273) as measured by SARS-CoV-2 anti-spike IgG, consistent with the phenomenon of immune imprinting.

Real world evidence has shown that vaccines against the ancestral strain demonstrate reduced efficacy against newer variants, [146,147] but neutralising titres against the ancestral strain still correlate with protection against subsequent variants.[148] Although **section 4.3.4** reports that back-boosting is more evident in children than adults, immune imprinting has been reported in both adults and children with regards to SARS-CoV-2 vaccination. Dowell et al.[149] found that primary Omicron SARS-CoV-2 infection in children followed by subsequent vaccination against the ancestral Wuhan strain induced higher anti-spike and neutralising antibody titres against the Omicron variant than the Wuhan strain. However, anti-spike binding and neutralising antibody titres induced against the Wuhan strain were still significantly different from baseline (i.e. those generated by primary Omicron infection).

However, double Omicron exposure has been shown to override ancestral SARS-CoV-2 imprinting[150] and it has been suggested that individuals without prior Omicron exposure should receive two updated boosters. This is consistent with the findings outlined in

Figure 4.8 where backboosting is only measured after a first dose and not a second dose of ChAdOx1 nCoV-19.

Limitations of these data include differences in the timing of sampling for the three age groups. The adult (18-55 years) samples were obtained from a clinical trial which commenced recruitment in April 2020 when UK lockdown measures had only been in place for a month, whereas the older children (12-17 years) were recruited in February 2021 and the younger children (6-11 years) recruited in April 2021 after 12 months of lockdown restrictions. It has been shown that the normal patterns of paediatric hospital admissions for seasonal respiratory tract infections were affected by pandemic lockdown measures (including school closures in effect in the UK from March 2020 to March 2021)[151] and it is possible that levels of immunity to seasonal HCoVs were lower than in equivalent months in pre-pandemic years. Whilst lockdown rules were in effect across all sampling periods, it is still possible that circulation of HCoVs in the community may have occurred between timepoints (vaccination and D28 post vaccination) and could have affected the results.

The favourable reactogenicity profile of the ChAdOx1 nCoV-19 vaccine in adults together with pre-existing data on the reactogenicity of the same viral vector in a single dose schedule in paediatric malaria vaccine trials justified the use of a standard adult dose of the vaccine in the phase II study of ChAdOx1 nCoV-19 in children. However, the effects reported here may differ for SARS-CoV-2 vaccines which were deployed in children in a fractional dose regimen (e.g. BNT1626b2) therefore comparisons of dosing regimens may be needed. Lastly, these results only examine the humoral component of HCoV responses to SARS-CoV-2 vaccination. The conclusions which have been drawn could be further

strengthened by evidence from functional studies such as neutralisation assays, examination of cellular responses and in-depth immunophenotyping such as flow cytometry, to examine in particular S2-specific immune responses. SARS-CoV-2 vaccination has been shown to boost HCoV-specific neutralising antibody in adults[152] and whether this is also true in children and to what extent has not yet been investigated.

Influenza imprinting has been demonstrated in young infants who receive an influenza vaccine before they are exposed to the virus[153] and several countries such as the United States are recommending COVID-19 vaccination of infants 6 months and above. It would be useful to repeat the assays described in this chapter on serum samples from this age group who have received a COVID-19 vaccine and to follow-up their immune responses to their first exposure to SARS-CoV-2, in particular to investigate the antibody response against Omicron variants.

Chapter 5. Antibody avidity, subclass and FcγR binding profile

5.1 Introduction

SARS-CoV-2 specific anti-spike total IgG and SARS-CoV-2 neutralising antibody titres have both been shown to correlate with protection against symptomatic infection conferred by ChAdOx1 nCoV-19 vaccination, as well as other COVID-19 vaccines.[154,155] Evidence suggests that functional qualities of antibodies in addition to binding are important in modulating antibody function and providing protection against COVID-19 disease. Vaccines against the ancestral spike protein continue to protect against severe disease caused by novel variants such as Omicron which possess mutations in the spike and receptor binding domain.[147] Variants may also possess mutations away from the RBD region, so that assays based on the degree of binding alone may not detect differences in antibody function.[156157] Understanding the features of antibody activity in addition to antibody binding may improve our understanding of how humoral immunity generates protection against SARS-CoV-2 infection and the cellular mechanisms responsible.

Generation of humoral immunity in response to an antigenic stimulus, such as a vaccine, depends upon the production of antigen-specific memory B cells and antibody-secreting cells during germinal centre (GC) or extrafollicular (EF) responses. Ablation of the GC in mice vaccinated with ChAdOx1 nCoV-19 has been associated with a ten-fold decrease in RBD-binding antibody compared with controls,[158] illustrating the importance of the GC response in generating a humoral immune response to the vaccine in mice. Within the GC itself, activation-induced cytidine deminase (AID) is required for two processes that determine functional properties of antibodies which are generated.

Affinity can be defined as the strength of the binding between an antibody and a specific epitope on the antigen surface. Avidity can be defined as the overall strength of binding between a multivalent antibody and different epitopes. Affinity maturation results from iterative mutation and selection of B cell clones in the GC, resulting in increased affinity and avidity of antibodies. Affinity of B cell receptors increases with increasing time spent in the GC, and GCs have been detected for up to 6 months in animal models of vaccination.[159] Low avidity SARS-CoV-2 anti-spike antibodies have been associated with repeated infection in adults, [160] and it has been shown that higher avidity antibodies following SARS-CoV-2 infection are associated with greater breadth of neutralising capacity, measured by the ability to neutralise variants in vitro.[161]

Vaccination or natural infection can also trigger class switch recombination. Differences in the IgG subclasses generated affect the binding of the Fc region to Fcγ receptors (FcγRs), which are proteins located on immune effector cells. IgG1 and IgG3 have the highest affinity for FcγRs mediating cytotoxic activity, whereas IgG2 and IgG4 are associated with responses to bacterial capsular polysaccharide antigens and allergic exposure respectively.[162] A class switched response has been measured in convalescent serum following SARS-CoV-2 infection where IgG1 and IgG3 dominate the antibody response[163] and a mature class-switched memory B cell response has been measured in response to SARS-CoV-2 mRNA and ChAdOx1 nCoV-19 vaccination in adults.[164,165] IgG1 has also been shown to correlate more strongly with neutralising antibody titres including for COVID-19 vaccine mRNA-1273.[166] Class switched SARS-CoV-2 anti-spike IgG reactivity has been found in pre-pandemic paediatric sera which is consistent with what is now known about cross-reactivity between SARS-CoV-2 and seasonal coronaviruses epitopes in children.[167]

Differential expression of FcγRs on immune cells and their respective functions will also determine the immune response generated by a vaccine. FcγRII and FcγRIII are low-affinity receptors and considered the main effector receptors on circulating immune cells.[168] An overview of these receptors is outlined in **Table 5.1.**

Table 5.1 Overview of FcγR function (adapted from Vidarsson et al.)[162]

Receptor	Expression	Role	Details
IIa	Monocytes, macrophages, neutrophils, dendritic cells, basophils, mast cells, eosinophils, platelets	Activation	Effector cell function for cytotoxic antibodies. In vivo can be measured by antibody dependent cellular phagocytosis (ADCP)
IIb	Dendritic cell, basophil, B cells	Inhibition	
IIIa	NK cell, monocyte, macrophages	Activation	Dominant pathway for effector function via IgG. In vivo can be measured by antibody dependent cellular cytotoxicity (ADCC)
IIIb	Neutrophils	Inhibition	

FcγR binding may be used as a surrogate marker of antibody-dependent immune cell function, for example FcγR IIa binding with antibody-dependent cellular phagocytosis and FcγR IIIa binding with antibody-dependent cellular cytotoxicity.[70] SARS-CoV-2 mRNA vaccine responses with higher FcγR IIIb binding titres have also been associated with increased protection from breakthrough infection whereas responses with higher FcγR IIb titres (and associated IgA and IgG3 subclass response) are associated with decreased protection from breakthrough infection.[169,170]

The main aim of this chapter is to examine the extent to which children 6-17 years of age generate a mature, class-switched antibody response following ChAdOx1 nCoV-19 vaccination and where possible, make comparisons with adult data.

5.2 Methods

5.2.1 Sample selection

This chapter describes the results of three immunological assays and comparison of results between different age groups (avidity, subclass and isotype and FcγR binding) or interval between doses (avidity).

Total IgG avidity ELISA results were compared between COV006 participants only. Subjects were chosen if they were seronegative at baseline, received two doses of ChAdOx1 nCoV-19 and provided serum samples at, as a minimum, the following two timepoints: one timepoint after receiving the first dose of ChAdOx1 nCoV-19 and one timepoint after receiving the second dose of ChAdOx1 nCoV-19. In-house total IgG titres were also required at matching timepoints to enable standardisation of serum concentration to give an ELISA OD reading of 1.

Isotype and subclass ELISA titres were compared between COV006 participants and adult subjects aged 18-55 years of age who were enrolled in an adult ChAdOx1 nCoV-19 study (COV002). Subjects were chosen if they were seronegative at baseline, received two doses of ChAdOx1 nCoV-19 and provided serum samples at baseline, D112 (day of second dose for paediatric subjects) and D140 (28 days post second dose for paediatric subjects). For

adult subjects, the equivalent day of second dose/28 days post second dose timepoints were D28 and D56 respectively.

FcγR binding assays were conducted on the same COV006 participants as the isotype and subclass ELISAs. However, the results were compared with assay results from COV002 participants who received two doses of vaccine at an interval comparable with COV006 participants (112 days +/- 28 days between first and second vaccine doses). It was necessary to use a comparable interval to account for higher IgG titres which are observed with a longer interval between priming doses. Analyses were not normalised for total IgG titre as D140 total IgG results had been conducted on different platforms for the adult samples versus the paediatric samples.

For all assays, participants were excluded if they reported a SARS-CoV-2 infection before the latest timepoint used to obtain a sample for the assay i.e. 28 days after second dose.

5.2.2 Immunology assays

The results of three immunological assays are reported in this chapter. Please refer to the following sections for each assay:

1. Total IgG avidity ELISA: Chapter 2, section 2.4.3
2. Isotype and subclass ELISA: Chapter 2, section 2.4.2
3. FcγR binding assay (Luminex multipleced immunoassay): Chapter 2, section 2.4.4

5.2.3 Statistical analysis

Two-way ANOVA was used to compare results of serology assays in this chapter, grouping by age group and time point. For further details of the statistical analyses used in this chapter, please see Chapter 2, section 2.5.

5.3 Results

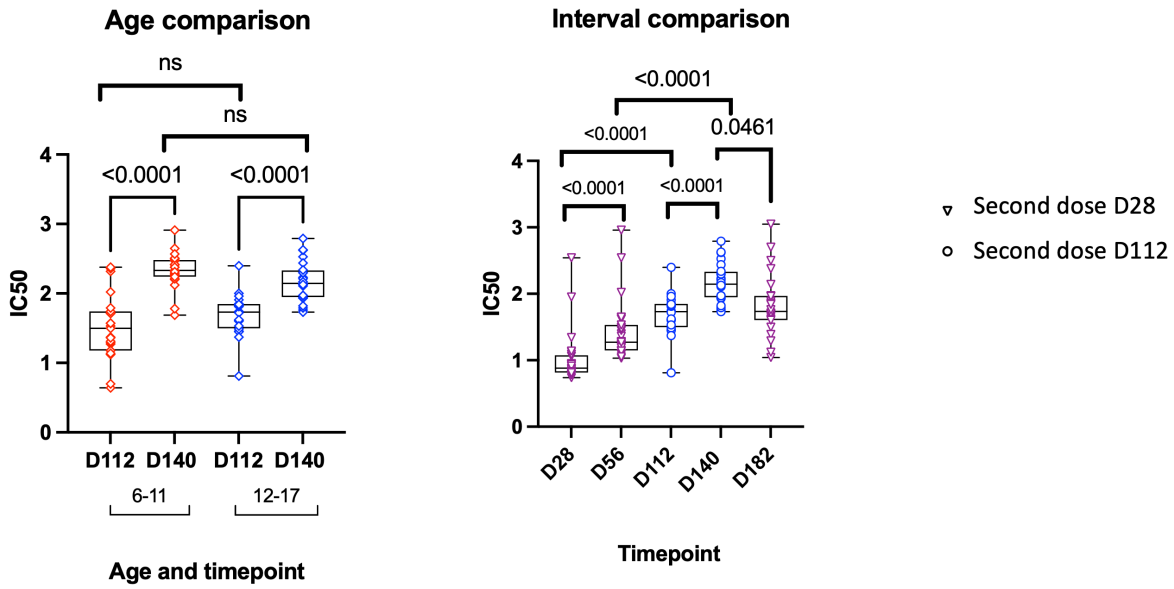
5.3.1 No difference in measured SARS-CoV-2 (Wuhan) anti-spike IgG avidity between children 6-11 and 12-17 years of age after one or two doses of ChAdOx1 nCoV-19 but significantly higher avidity measured when interval between doses is longer

Avidity ELISAs were performed on serum samples from 67 COV006 participants, obtained from at least two timepoints - the day of the second dose and 28 days after the second dose. The breakdown by age group is shown in **Table 5.1.** A third timepoint (D182) was tested in participants 12-17 years of age who received a second dose at D28. This was to facilitate a direct comparison with D28 after the second dose in the group 12-17 years of age who received their second vaccine at D112 (i.e. D140).

Table 5.2 Sample numbers for avidity ELISA

Age range (years)	Interval between vaccines (days)	No. of participants (N)
6 to 11	112	23
12 to 17	112	20
12 to 17	28	24

Figure 5.1 Comparison of standardised avidity ELISA results across age-groups and differing intervals between vaccine doses. *Significance testing conducted by two-way ANOVA. For 6-11 years of age n=23; 12-17 years of age with 112-day interval n=20; 12-17 years of age 28-day interval n=24. IC50 = concentration of sodium thiocyanate (M).*



Receipt of a second dose of ChAdOx1 nCoV-19 was associated with a statistically significant increase in avidity in both age groups **(Figure 5.1, left panel)**. No significant difference in SARS-CoV-2 (Wuhan) anti-spike IgG avidity was measured between children 6-11 years of age and 12-17 years of age who received two ChAdOx1 nCoV-19 vaccines at a 112-day interval. This comparison was made both on the day of the second dose (D112) and 28 days after the second dose was given (D140). However, SARS-CoV-2 (Wuhan) anti-spike IgG avidity was higher at 28 days after a second dose in children 12-17 years of age who received two doses at a 112-day interval (D140) than those who received two doses at a 28-day interval (D56) **(Figure 5.1, right panel).** Even when allowing extra time for affinity maturation in the 28-day interval group, avidity at D182 in this group remained lower than D140 in the 112-day interval group.

5.3.2 Significant increases in SARS-CoV-2 anti-spike IgG1 and IgG3 are measured after two doses of vaccine

Isotype and subclass profiles were performed on 49 COV006 participants at three timepoints (baseline, day of second dose D112 and 28 days after second dose D140) (**Table 5.2**). Assays were also conducted on 39 participants of an adult ChAdOx1 nCOV-19 trial for comparison.

Table 5.3 Sample numbers for isotype and subclass ELISAs

Age range (years)	Interval between vaccines (days)	No. of participants (N)
6 to 11	112	24
12 to 17	112	25
18 to 55	28	39

Statistically significant increases in IgG1, IgG3 and IgM titres but not IgA titre are measured in both the 6-11 and 12-17 age groups after one dose of vaccine **(Figure 5.2)**. No IgG2 or IgG4 responses were detected (data not shown). There was no significant difference between age groups except for IgM titre at D112, which was higher in the younger (6-11 years) age group than the older (12-17 years) age group. However, by D140 this difference was no longer evident (**Figure 5.2**).

5.3.3 Total IgG and IgG1 titres are significantly higher in children 6-11 years of age than adults 18-55 years of age after two doses of vaccine

Comparisons were then made with adult data. **Figure 5.3** shows a comparison of the three age groups (6-11 years, 12-17 years and 18-55 years) at 28 days after the second dose (112-day interval for children, 28-day interval for adults). The first panel shows the

comparison for in-house SARS-CoV-2 anti-spike total IgG ELISA results, followed by isotype and subclass ELISAs. Total SARS-CoV-2 anti-spike IgG and IgG1 titre were significantly higher in participants 6-11 years of age than 18-55 years of age after two doses. No differences in IgG3 or IgA titres were measured between age groups. IgM titre was significantly higher in the 18-55 age group than the 6-11 or 12-17 years of age groups. However, the timepoint used for comparison in adults corresponded to 56 days after the first dose of vaccine was given and therefore closer to the peak IgM response expected after the first vaccination whereas in the paediatric groups the equivalent timepoint for comparison was 140 days after the first dose. Therefore, the peak IgM response may have waned in the COV006 age groups to account for this difference.

Figure 5.2 Isotype and subclass ELISA results comparing responses to ChAdOx1 nCoV-19 in seronegative younger children (6-11 years of age) with older (12-17 years of age) children. Timepoints for comparison are baseline, pre-second vaccine dose and 28 days after second vaccine dose. *Significance testing conducted using one-way ANOVA. 6-11 years n=24, 12-17 years n=25. EU = ELISA units/ml*

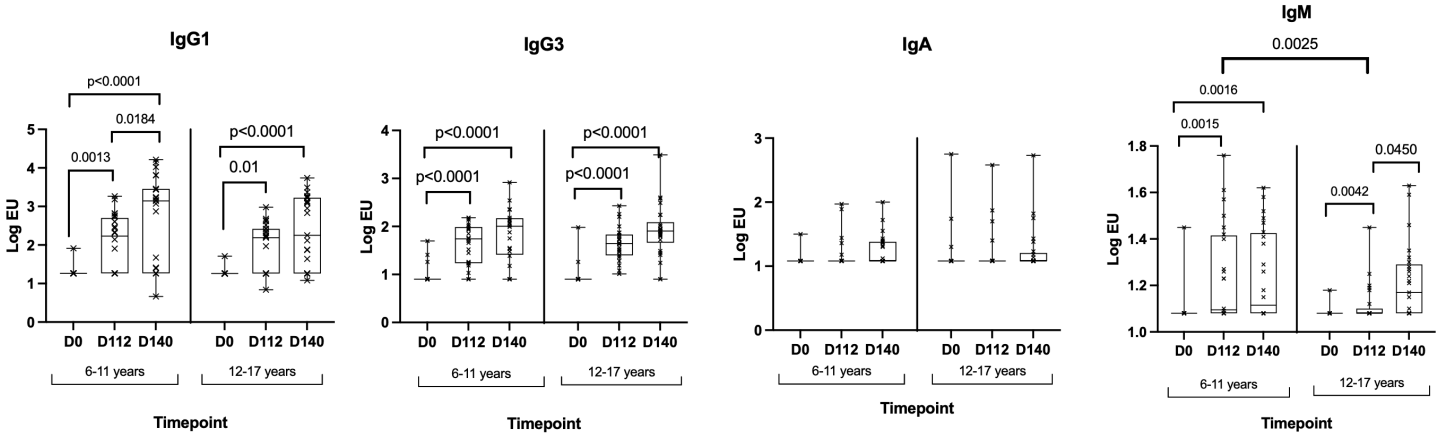
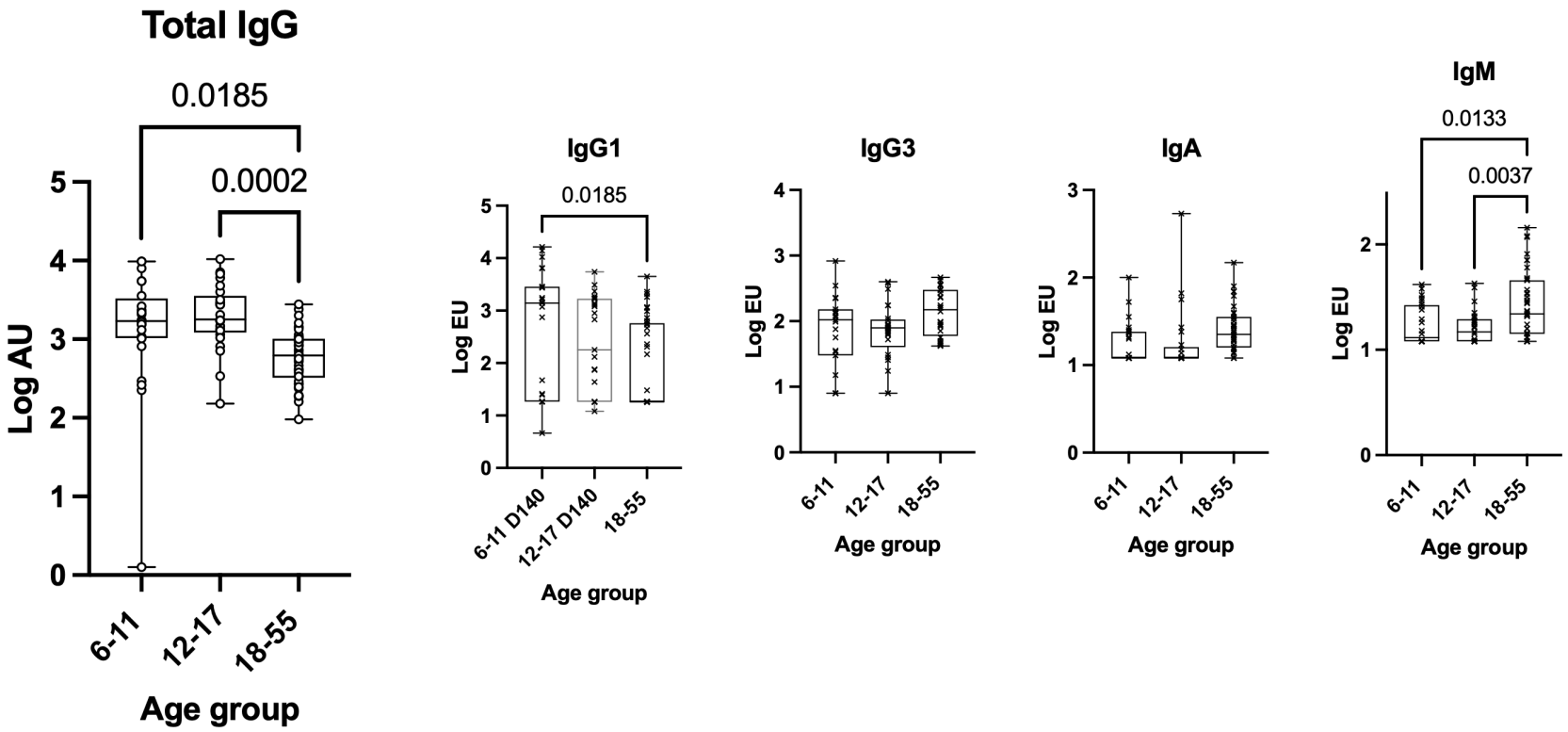


Figure 5.3 SARS-CoV-2 (Wuhan) isotype/subclass ELISA results comparing responses in children (6-11 and 12-17 years of age) with adult (18-55 years of age). *Significance testing conducted using one-way ANOVA. 6-11 years n=24, 12-17 years n=26, 18-55 years n=39. Timepoint for comparison is 28 days after second dose i.e. D140 in children and D56 in adults). AU = arbitrary units. EU = ELISA units/ml*


Total IgG
0.0185
0.0002
Log AU
Age group
6-11
12-17
18-55
IgG1
0.0185
Log EU
6-11 D140
12-17 D140
IgG3
IgA
IgM
0.0133
0.0037

5.3.4 Age-specific SARS-CoV-2 spike-specific FcγR binding profiles mirror the trends measured in total IgG titres

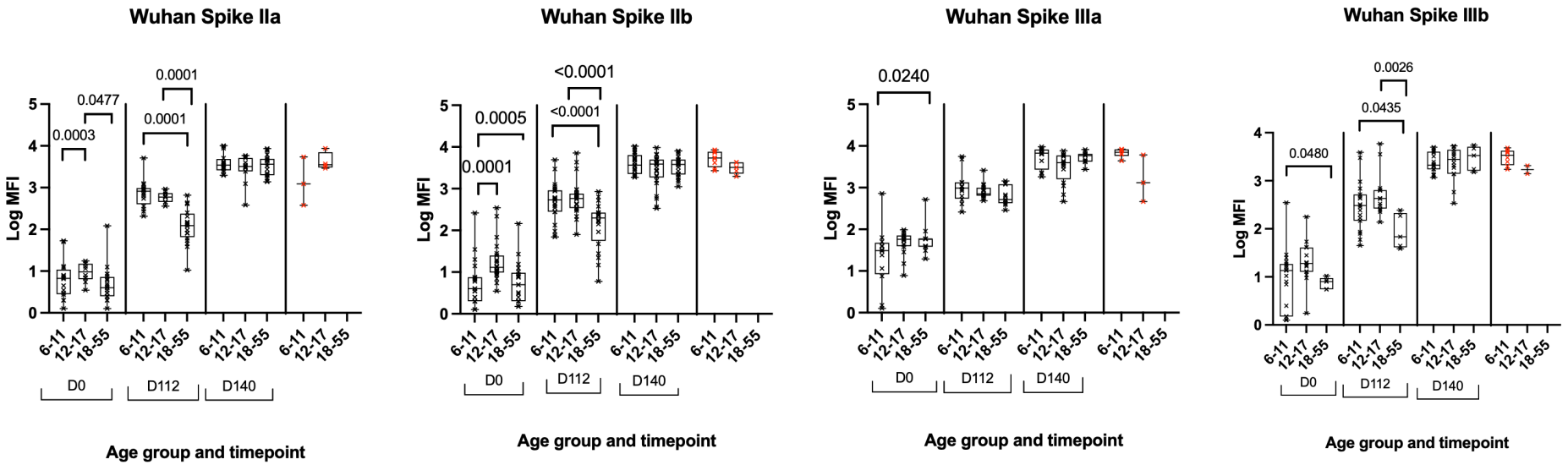
FcγR binding profiles were measured in 49 COV006 participants and 20 adult participants as shown in **Table 5.4**. Assays were conducted at three timepoints (baseline, day of second dose D112 and 28 days after second dose D140). However, due to the high numbers of repeat assays which were required due to differing serum concentrations between adults and children, not all participants contributed to the results for each individual receptor assay.

Table 5.44 Sample numbers for SARS-CoV-2 specific FcγR binding profiles

Age range (years)	Interval between vaccines (days)	No. of participants (N)
6 to 11	112	23
12 to 17	112	26
18 to 55	112	20

Figure 5.4 shows the SARS-CoV-2 spike-specific FcγR binding profile for IIa, IIb, IIIa and IIIb receptors across timepoints and age groups. The results at baseline suggest a degree of cross-reactivity or non-specific binding particularly for receptor IIIa and IIIb. However, significant increases in antibody titre are measured in all four assays after one dose of vaccine. At D112, IIa, IIb and IIIb binding titres are significantly higher in participants 6-11 years of age than 12-17 or 18-55 years of age, mirroring the patterns seen when comparing total IgG results in COV006 and adult participants who were also matched for the interval between doses (**Figure 3.5**). This trend was not observed for FcγR IIIa. At D140, no significant difference in FcγR binding titre was measured between age groups, for any of the receptor types studied. Data from participants reporting symptomatic SARS-CoV-2 infection are plotted in red, sample numbers are too small for formal significance testing but no clear difference can be seen between those who did and did not report symptomatic infection.

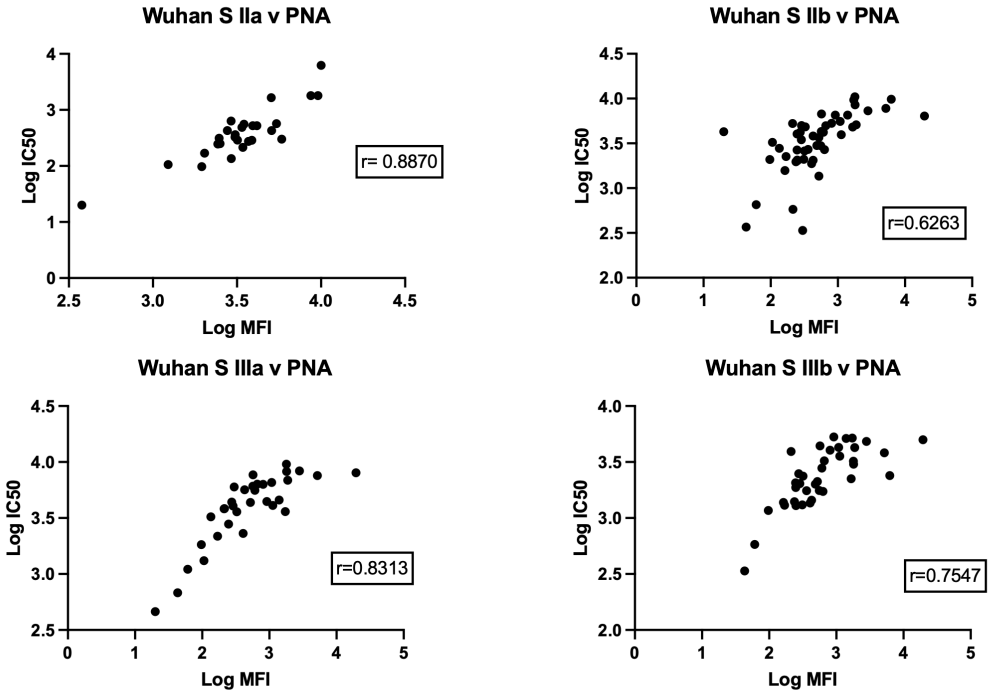
Figure 5.4 SARS-CoV-2 spike-specific FcγR antibody binding profiles across age groups and timepoints following ChAdOx1 nCoV-19 vaccination at D0 and D112. *Error bars denote mean with 95% confidence intervals. Upper panels show log10 mean fluorescence intensity for each receptor subclass, for each age group (6-11 years, 12-17 years, 18-55 years) and timepoint. Significance testing conducted using two-way ANOVA. Red data represent results from participants who reported a symptomatic infection during the follow-up period of the trial.*



5.3.5 SARS-CoV-2 spike-specific FcγR binding antibody titres correlate highly with SARS-CoV-2 neutralising antibody titre, regardless of FcγR type

To assess whether FcγR binding for a specific receptor correlates with SARS-CoV-2 neutralisation titre and therefore protection from infection, correlation between FcγR binding and pseudoneutralisation titre was assessed. Correlation is high and statistically significant for all receptors studied, but highest for FcγR IIa with a correlation co-efficient of 0.8870 (**Figure 5.5**).

Figure 5.5 Correlation between FcγR binding titres and PNA titre. *Pearson correlation co-efficients are shown, all are significant at the level p<0.05. MFI = median fluorescence intensity.*



To assess whether the relationship between PNA and FcγR IIa binding titre varies with age, multiple linear regression was carried out for results from COV006 participants only, due to

the restricted availability of PNA results. **Table 5.5** shows that there is no significant association between age and PNA titre, after controlling for IIa titre.

Table 5.5 Regression of FcγR IIa binding titre and age on PNA titre

Parameter estimate	Variable	Estimate	95% C.I.	p value
$\beta 0$	Intercept	-2.29	-3.49- -1.09	0.0006
$\beta 1$	IIa binding titre	1.46	1.14 – 1.77	<0.0001
$\beta 2$	Age	-0.02	-0.05-0.001	0.12
R2		**0.8073**		

5.4 Discussion

These results show that the time interval elapsed between ChAdOx1 nCoV-19 doses in a two-dose priming series has a greater effect than age in determining SARS-CoV-2 anti-spike IgG avidity in school-aged children. Children generate a class-switched antibody response similar to that observed in adults following two-dose priming, with significant increases in anti-spike IgG1 and IgG3 titres. However, IgG1 titres are significantly higher in younger children 6-11 years of age than adults 18-55 years of age after two doses. FcγR binding profiles show that age-related differences in antibody binding titre emerge after one dose of ChAdOx1 nCOV-19 but are no longer present after two doses and FcγR IIa binding titres correlate most strongly with pseudovirus neutralisation titres and potentially protection from infection. This is the first time that FcγR binding profiles generated after ChAdOx1 nCoV-19 vaccination in children have been described.

The relationship between age, interval between doses and anti-spike IgG avidity in children mirrors the trend between these variables and SARS-CoV-2 anti-spike total IgG (**Chapter 3, Figure 3.5**). Similar results have been observed with other vaccine platforms, for example anti-spike IgG avidity was correlated with total SARS-CoV-2 IgG titre and neutralising ability of sera obtained from adults who received BNT162b2 as a priming series [Singh, 2021].[171] Higher SARS-CoV-2 anti-spike IgG avidity has been measured in adults receiving heterologous priming with ChAdOx1 nCoV-19 and BNT162b2 than those receiving homologous BNT162b2 priming[172] and illustrates one benefit of heterologous immunisation approaches against SARS-CoV-2.

Antibody avidity following a two-dose priming series with BNT162b2 and mRNA-1273 vaccines continues to increase up to 6 months after the second vaccination,[173] however there is variation in timing between priming series doses within the cohorts studied. Avidity has also been shown to increase following a third dose mRNA vaccine booster but not thereafter in older people.[174] ChAdOx1 nCoV-19 was offered to individuals 18 years of age and over during the UK COVID-19 vaccination programme in 2021, with a 12-week interval between doses. In an emergency, pandemic setting, a trade-off may be required between optimising vaccination coverage in the population and the time needed for maturation of the immune response.

The predominance of IgG1 and IgG3 measured in the antibody subclass response to ChAdOx1 nCOV-19 in children is consistent with the antibody subclass profile previously reported in adults in response to a two-dose priming schedule of the vaccine.[165] The type 1 helper T cell response is also consistent with previously published studies which measured immune responses to viral-vectored vaccines in human recipients.[175] IgG1 and IgG3 are

associated with enhanced pathogen clearance and have been shown to exist in higher quantities in the sera of healthy children than healthy adults.[176] Both are strong inducers of Fc effector responses in comparison with IgG2 and IgG4.[177]

Similarly, IgG1 has been shown to dominate the response to mRNA-1273 vaccination in toddlers and children up to 11 years of age.[178,179] In contrast to the antibody subclass profile measured after two doses of ChAdOx1 nCoV-19, the authors also measured a significant increase in serum IgA and IgG4 after a second dose, and class switching to IgG4 has been described in response to other mRNA vaccines in adults.[180,181] Potential benefits of class-switch to IgG4 include its anti-inflammatory role due to its failure to bind complement.[182] However, one study which conducted a direct comparison of mRNA (BNT162b2 and mRNA-1273) versus recombinant protein vaccine recipients (Novovax) found that IgG3 titres were 10-fold higher in recombinant vaccine recipients and were capable of higher FcγR 2a and 3a binding.[183] Therefore evidence suggests that the functional antibody profiles generated in response to differing COVID-19 vaccine platforms diverge, again suggesting that binding antibody titres alone may be insufficient to infer the degree of protection conferred by vaccination.

Age may also be a factor which affects the FcγR binding profile of antibodies generated in response to ChAdOx1 nCoV-19 vaccination. The results from COV006 show that children produce higher titres of antibodies binding to FcγR IIa, IIb and IIIb than adults following the first ChAdOx1 nCoV-19 vaccination and that FcγR IIa binding, a surrogate marker of antibody dependent cellular phagocytosis (ADCP), shows the strongest correlation with neutralising antibody titres of all the Fcγ receptor binding profiles studied. However,

differences were no longer evident after two doses of vaccine. FcγR IIa is also the most widely expressed receptor and the results shown may reflect this.

Spike-specific FcγR-binding antibody profiles generated following ChAdOx1 nCoV-19 vaccination in a South African adult cohort have been associated with risk of developing breakthrough SARS-CoV-2 infection[170] as higher FcγR IIIb binding was associated with protection against infection and conversely lower FcγR IIb binding. However, this contrasts with the results reported here showing that titres of spike-specific antibody binding to all four FcγR types studied positively correlate with neutralising antibody titres. It may be that age differences exist in FcγR IIb function and this could be investigated further.

Several factors including vaccine platform, age and method of antigen encounter (natural infection versus vaccination) all influence the functional antibody profile which is generated in response to ChAdOx1 nCoV-19 vaccination. One limitation of the results presented in this chapter is that they are obtained from three assays which are primarily antibody binding assays, and do not make a formal assessment of antibody functionality which would be possible with a cell-based assay, for example. Antibody fucosylation patterns specific to mRNA vaccination as compared to natural infection with SARS-CoV-2 have been identified and it remains to be seen whether these will correlate with vaccine efficacy.[184] Therefore, any correlations or associations that are described require further investigation into the biological mechanisms underlying them. However, the significant correlation between FcγR IIa (a surrogate marker of ADCP activity) and neutralising antibody activity is consistent with findings from another viral vectored vaccine, Ad26/Ad5 where ADCP was found to be elevated in its recipients versus those who had received an mRNA COVID-19 vaccine.[185]

There are also methodological challenges and significant variations in the approaches used to quantify FcγR binding activity, including binding assays such as ELISA and bead-based assays (such as those reported in this thesis), reporter bioassays and functional cell-based assays. Another limitation of the results presented here is that the assays used recombinant Wuhan spike protein to bind IgG and assess functionality whereas the trial participants may have been exposed to the Delta or Omicron variant during the follow-up period, and correlation between neutralising antibody titres, FcγR binding and clinical outcomes may be inaccurate.

Because COV006 was a phase II trial with 262 randomised participants, there are a limited number of positive SARS-CoV-2 cases with which to correlate immunological findings with clinically meaningful endpoints. Although ChAdOx1 nCoV-19 protects against symptomatic and severe SARS-CoV-2 infection in adults, no cases of COVID-19 disease severe enough to warrant medical attention were reported in COV006. Future work on antibody profiling may have to consider what clinically relevant endpoints should be the focus of future paediatric studies as severe outcomes are so rare. For the 2024-2025 winter season recommendations in the UK are to vaccinate children from the age of six months in clinical risk groups indicated by the Joint Committee on Vaccination and Immunisation. Therefore, greater focus should be placed on immunological profiling of the immune response to vaccination in these age groups for example, in future repeating the assays reported here on serum samples taken from children of pre-school age.

Chapter 6. Conclusion

6.1 Summary of main findings

6.1.1 ChAdOx1 nCOV-19 is safe and immunogenic in children

This is the first study to demonstrate that ChAdOx1 nCoV-19 is safe and immunogenic in a paediatric study population. The local and systemic side-effect profile was acceptable, and the fever rate was comparable with that observed in recipients of the vaccine in adult trials. Fever rates were also similar to those experienced after administration of routine vaccines in the Expanded Programme on Immunisation, and side effects lasted for less than 48 hours. The safety and reactogenicity results suggest that two adult doses of ChAdOx1 nCoV-19 could be used in children as young as 6 years of age without a need for fractional dosing to mitigate against vaccine-related side effects.

Measures of immunogenicity obtained from serology and cell-based assays carried out in seronegative paediatric recipients of ChAdOx1 nCOV-19 show ELISA titres and ELISpot results which are of a similar magnitude to those observed in adults. Although it was not possible to test the efficacy of the vaccine due to the limited number of participants recruited, the levels of total IgG measured are similar to those which prevented infection of the ancestral and alpha variants in clinical trials.

6.1.2 Effect of immunity to seasonal coronaviruses

This is also the first study to examine how pre-existing immunity to circulating seasonal human coronaviruses in children impacts upon ChAdOx1 nCoV-19 immunogenicity and is related to generation of cross-reactive immunity. A direct comparison is made with adults receiving the vaccine at the same doses. The results show that boosting of antibodies specific to HCoVs is significant for betacoronaviruses in children but not adults after a first dose of

vaccine, but not a second dose of vaccine. However, a two-dose priming schedule in children leads to generation of anti-spike total IgG titres of at least a similar, if not higher, magnitude than in adults. These results are consistent with the theory of immune imprinting, however imprinting does not appear to adversely affect vaccine immunogenicity when directed against the ancestral strain. These results complement more recent data, showing that in children, immune imprinting in the context of pre-existing exposure to the Omicron variant followed by vaccination against ancestral SARS-CoV-2 is reduced after two doses of vaccine against the ancestral strain, as opposed to one.[186]

6.1.3 Antibody profiling

The 2021 COVID-19 vaccine rollout was the widest use of a ChAdOx1-vectored vaccine to date, with safety, immunogenicity and efficacy against severe SARS-CoV-2 infection demonstrated at that time in adults both in a trial and real-world setting. Given that SARS-CoV-2 was a recently emerged pathogen, there existed considerable interest in understanding the immunological mechanisms underlying vaccine-induced protection generated by vaccine platforms which were deployed for the first time during the pandemic. The results presented in this thesis compare in-depth antibody profiles of response in adults and paediatric recipients of ChAdOx1 nCoV-19, including results from a novel multiplexed immunoassay to investigate spike-specific antibody FcγR binding profiles after vaccination. The findings describe antibody features beyond binding alone which show that a similar portfolio of immune responses is generated.

The strongest correlation between FcγR binding and neutralising antibody titres was found for FcγR IIa, a known proxy for ADCP, which plays a crucial role in antigen presentation and

lysis of cells infected by virus. ADCP has also been linked to vaccine-mediated protection in other platforms including against HIV.[187]

6.1.4 Overview of results

Table 6.1 summarises the results of SARS-CoV-2 spike-specific assays presented in this thesis, relative to results reported in adults after receiving one and two doses of ChAdOx1 nCoV-19, where comparisons have been made. They show that differences which appear after receiving a first dose are reduced by receiving a second dose. **Table 6.2** illustrates that there may be age differences in Fc receptor binding for circulating coronaviruses at baseline, and that trends in total IgG may not show age-related trends in Fc receptor binding.

Table 6.1 Spike-specific assay results comparing children with adults after one and two doses of ChAdOx1 nCoV-19 *(- denotes no difference, -- = less than, + = greater than, ++ = significantly greater than, *samples for these assays were taken from adults who received two doses at a different time interval to children – 28 days apart instead of 112 days)*

	After 1 dose		After 2 doses	
Assay	6-11 years	12-17 years	6-11 years	12-17 years
Total IgG	++	+	+	-
IgG1*			+	-
IgG3*			-	-
IgA*			-	-
IgM*			--	--
Fc receptor IIa	++	+	-	-
Fc receptor IIb	++	+	-	-
Fc receptor IIIa	-	-	-	-
Fc receptor IIIb	++	+	-	-

Table 6.2 Baseline HCoV MSD assay results comparing children with adults *(- denotes no difference, -- = less than, --- = significantly less than)*

HCoV	Total IgG		2a		2b		3a		3b	
	6-11 years	12-17 years	6-11 years	12-17 years	6-11 years	12-17 years	6-11 years	12-17 years	6-11 years	12-17 years
OC43	-	-	-	-	--	---	--	--	---	--
HKU1	-	-	-	-	--	---	---	---	---	---
NL63	-	-	-	-	-	---	--	---	---	--
229E	--	--	-	-	--	---	---	---	---	--

6.2 Limitations

6.2.1 Efficacy estimation

The COV006 trial was not designed to evaluate vaccine efficacy. The survival analyses outlined in Chapter 3 also illustrated the challenges of inferring efficacy based on results of self-reported SARS-CoV-2 infection in a small sample size. Although anti-nucleocapsid ELISA data were obtained for participants at sampling timepoints, the utility of these results was limited due to the time lag between the participants being infected by SARS-CoV-2 and the anti-nucleocapsid result. The date of the anti-nucleocapsid result could not therefore be used as an accurate endpoint for time-to-event analysis. The results may underrepresent the true burden of infection as anti-N antibody responses are known to wane and infections in children are often asymptomatic.[188]

Routine weekly nasopharyngeal swabbing was incorporated into the design of phase II/III adult ChAdOx1 nCoV-19 trials together with additional blood testing when a participant tested positive for SARS-CoV-2. However, this approach was not used in COV006 due to its design being phase II study only (with no plans for expansion into phase III) and concerns that additional blood tests would not be appropriate or acceptable in this age group.

6.2.2 Participant population

The population from which participants for the COV006 study were selected was healthy volunteers 6-17 years of age in the South-West of England, UK. Current JCVI guidance recommends vaccination of children from 6 months of age if they are immunosuppressed. Therefore, the results from this study do not comprehensively cover the paediatric age range which is currently targeted for immunisation. Therefore, further studies of SARS-CoV-2 vaccines in children may require studies in this specific population.

The self-disclosed ethnicity of participants in COV006 in each trial arm ranged from 85-100% Caucasian with the remaining participants describing themselves as being of Asian or mixed descent. Therefore, further evidence would be needed to extrapolate the results to populations of greater diversity. However, clinical trials in adults where participants were of greater ethnic diversity[189] have shown that immunogenicity in South Asian, South African populations appears to be similar or greater than that seen in Caucasian populations.

6.2.3 Variant selection for vaccination and timing of trial

The ChAdOx1 nCoV-19 vaccine used in this study was designed against the ancestral strain of SARS-CoV-2 which was first sequenced at the beginning of the pandemic. Although this may have had a limited effect on the safety and immunogenicity readout of the clinical trial, this meant that any interpretation of vaccine efficacy (measuring protection against infection only) may have been affected. Real-word data has shown that protection against infection by emerging variants would be expected to be low, however the ancestral vaccine would still be expected to provide good protection against severe disease. Serum from individuals vaccinated with ancestral ChAdOx1 nCoV-19 has demonstrated reduced neutralisation against the Omicron lineage.[190]

6.2.4 *Sampling and laboratory assay limitations*

Due to the way in which the clinical trial evolved, sampling timepoints were not symmetrical across different trial arms. The trial also faced a significant number of withdrawals as participants were able to receive COVID-19 vaccinations in the national immunisation programme. Many of the younger participants (6-11 years of age) became more hesitant about blood draws towards the end of the study and opted out of them altogether. Future study designs might explore ways in which blood draws could be spread across different trial arms (however increased study recruitment numbers would be required) or use of newer, less invasive methods of obtaining blood such as transdermal sampling.[191]

There are several ways in which the approach to the assays used to obtain the results for this thesis could have been improved. As shown in Chapter 4, it is possible that cross-reactivity between antibodies directed against SARS-CoV-2 spike and the spike domains specific to circulating HCoVs affected the results and antibody depletion prior to serum testing could have allowed the generation of results which more accurately reflected the generation of antibodies specifically in response to vaccination. Similarly, now that more is understood about the mechanisms underlying immune imprinting, differentiating between S1 and S2 across the range of assays used might be informative.

6.2.5 *Limitations of statistical analyses*

ANOVA was the main statistical test which was used for comparative analysis of results between groups and across timepoints. Although the data were approximately normally distributed, because sample sizes particularly for exploratory assays such as the Luminex bead-based assay were particularly small, the goodness of fit of a normal distribution varied between groups.

The analyses in this thesis are mostly descriptive. However, given the exploratory nature of the analyses in Chapters 4 and 5 and the use of primarily antibody-binding assays, it was felt misleading to conduct analyses beyond correlation and univariate regression, to explore relationships with other clinical endpoints. However, this would not preclude the use of the data presented here as part of a wider comparison across age groups where sample sizes could be expanded upon for increased reliability, for example.

6.3 Contribution to vaccine and COVID-19 vaccine research

6.3.1 Contribution to field of ChAdOx1 research

The results of this study are the first to examine the safety and immunogenicity of a ChAdOx1-vectored vaccine in a two-dose priming regiment in children, demonstrating it to be safe and immunogenic. These findings are further supported by detailed characterisation of the antibody response with regards to antibody avidity, isotyping and FcγR binding. Although safety concerns have been raised about an association between the ChAdOx1 vector and vaccine-induced thrombocytopenia and thrombosis, and this is an area of active investigation by the scientific community, the vector continues to undergo pre-clinical and early clinical development as a vaccine designed against outbreak pathogens for which mortality is high (such as Ebolavirus) or for which alternatives are not yet available (Crimean Congo Haemorrhagic Fever). Although there are currently no plans to deploy these vaccines in children, the results presented in this thesis would provide an evidence based to support the use of these vaccines in children should the need arise and should the safety concerns be resolved.

6.3.2. Contribution to field of paediatric COVID-19 vaccine research

The nature of the ChAdOx1 nCoV-19 clinical trials conducted by the University of Oxford allowed a direct comparison across the age groups of participants who had received two adult doses of the vaccine. Serum and PBMC processing were standardised across all age groups. The results from COV006 together with those from adult trials of ChAdOx1 nCoV-19 will enable further comparisons to be made with clinical trial results of other COVID-19 vaccines, to delineate which features of vaccine-mediated protection are platform-specific and which features may be age-related and of relevance to future vaccination trial readouts. The results also provide an example of the challenges that paediatric COVID-19 vaccine trials face in terms of the high proportion of asymptomatic infection which make it challenging to accurately and reliably assess efficacy without increased testing (e.g. nasopharyngeal swabbing) of children.

6.4 Future directions

ChAdOx1 nCoV-19 was withdrawn from use by AstraZeneca in May 2024, with the manufacturer citing the rise of newer variants and switch in demand away from ancestral vaccines to updated variant vaccines. As the vaccine was never formally approved for use in children, future research questions in paediatric COVID-19 vaccinology arising from the work of this thesis might be considered with regards to other COVID-19 vaccines which are available to children, such as mRNA vaccines. Future questions leading on from this thesis can be divided in those at the laboratory, clinical and public health level.

Given the evidence that immune imprinting is of greater significance in children than in adults, specific studies may be needed in children to assess whether regular administration of booster vaccines may affect the immunogenicity of variant vaccines. The finding that FcγR

IIa binding shows the strongest correlation with PNA titres could be investigated further with verification by cell-based assays (such as ADCP) as well as wider analyses conducted to assess whether this relationship is also true for clinical endpoints in studies of a larger scale.

As current JCVI policy advises the administration of COVID-19 vaccines in only immunosuppressed children as young as 6 months of age, clinical studies demonstrating vaccine immunogenicity in this specific target population will be needed. As SARS-CoV-2 has become endemic and vulnerable populations are likely to require regular booster vaccinations, the potential to combine COVID-19 vaccination with other seasonal vaccinations is being explored. For example, combined vaccines for influenza and SARS-CoV-2 are now being developed by Moderna.[192] Safety and immunogenicity studies in children will be required for any novel vaccines to determine appropriate dosing and scheduling.

As influenza and COVID-19 vaccination are recommended for pregnant women, future studies of transplacental transfer of immunity to infants and young children may be useful to understand how long protection persists and whether there are any additional benefits in terms of reduction in transmission from children to older adults in addition to the protection of very young infants.

6.5 Conclusion

The primary goal of this thesis was to evaluate the safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine in children, through a clinical trial which was conceived at the height of the SARS-CoV-2 pandemic in 2020. Whilst clinical trial results from the adult trials were used to inform licensing of the vaccine in the UK and ChAdOx1 nCoV-19 was instrumental in bringing the pandemic under control in the adult population, licensing in children was never pursued. The reason for this is likely multifaceted. By summer 2021, vulnerable children had already been vaccinated. The weight of the differing arguments for and against the need for vaccination against SARS-CoV-2 in children had changed by the end of the trial follow-up period, with a decline in the number of observed PIMS-TS cases in children in subsequent waves of SARS-CoV-2 infection. The risk to healthy children was very low and the rationale for vaccination was questioned by parents and experts. In addition, vaccine uptake by children was low even after vaccination became available. The safety concerns surrounding VITT which arose (and affected recruitment into COV006) are also likely to have played a role.

Vaccination of vulnerable individuals continues to remain an important tool with which COVID-19 disease can be controlled. Safe, cost-effective vaccines will be needed for vulnerable children and adults. A greater understanding of how these vaccines provide protection at a cellular level may enable future clinical trials to be designed with immunological rather than clinical endpoints which could potentially reduce the scale of trials which are required to assess vaccine efficacy. Whilst the COV006 trial may not have contributed to vaccine rollout of ChAdOx1 nCoV-19 in children, the trial has nonetheless contributed towards our understanding of age differences, or similarities, in immune responses to vaccination as well as enabling a detailed characterisation of the humoral

antibody response across age groups. By comparing these results with similar data from alternative COVID-19 vaccines or vaccines directed against other viral or intracellular pathogens, we may be able to increase our understanding of how vaccines mediate protection more broadly.

COVID-19 vaccine research in children is still needed for several reasons. Optimal dosing and scheduling will need to be ascertained, particularly as fractional dosing of mRNA vaccines was used in phase 3 paediatric trials and a seasonal pattern of COVID-19 infection is not yet established. The longer-term effects of paediatric vaccination on protection of children against severe consequences of the disease in addition to effects on community transmission will take some time to assess. This is because serious disease is fortunately rare in children and observation of successive COVID-19 waves together with emergence of novel variants will be required for an accurate analysis of their impact. Ensuring that such research continues to have a positive impact on child health will require translation of research results into policy. Policy will only be effective if parents and children are able to trust that clinical trials are conducted safely and transparently, necessitating experienced clinical oversight to weigh up the potential benefits and risks of future studies.

References

1. Piret J, Boivin G. Pandemics Throughout History. Vol. 11, Frontiers in Microbiology. 2021.15;11:631736.
2. World Health Organisation. Plague fact sheet. 2022. [Accessed 2024 Jul 19]. Available from: https://www.who.int/news-room/fact-sheets/detail/plague
3. Bramanti B, Dean KR, Walløe L, Stenseth NC. The third plague pandemic in Europe. Proceedings of the Royal Society B: Biological Sciences. 2019. 286:1901.
4. Bertherat E. Plague around the world in 2019. Weekly Epidemiological Record, Number 25, 289-292.
5. Rakotosamimanana S, Kassie D, Taglioni F, Ramamonjisoa J, Rakotomanana F, Rajerison M. A decade of plague in Madagascar: a description of two hotspot districts. BMC Public Health. 2021. 21:112.
6. Randremanana R, Andrianaivoarimanana V, Nikolay B, Ramasindrazana B, Paireau J, ten Bosch QA, et al. Epidemiological characteristics of an urban plague epidemic in Madagascar, August–November 2017: an outbreak report. Lancet Infect Dis. 2019. 19:537–45.
7. World Health Organisation. Cholera fact sheet. 2023 [Accessed 2024 Jul 19]. Available from: https://www.who.int/news-room/fact-sheets/detail/cholera
8. Lam C, Octavia S, Reeves P, Wang L, Lan R. Evolution of seventh cholera pandemic and origin of 1991 epidemic, Latin America. Emerg Infect Dis. 2010.16:1130–2.
9. Ilic I, Ilic M. Global Patterns of Trends in Cholera Mortality. Trop Med Infect Dis. 2023.8:169.
10. Frerichs RR, Keim PS, Barrais R, Piarroux R. Nepalese origin of cholera epidemic in Haiti. Clinical Microbiology and Infection. 2012. 18:E158-163.
11. World Health Organisation. Cholera - Haiti. 2022 [Accessed 2024 Jul 19]. Available from: https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON427
12. Kai Wong M, Brooks DJ, Ikejezie J, Gacic-Dobo M, Dumolard L, Nedelec Y, et al. COVID-19 Mortality and Progress Toward Vaccinating Older Adults — World Health Organization, Worldwide, 2020–2022. [Accessed 2024 Jul 19]. Available from: https://www.cdc.gov/mmwr/mmwr_continuingEducation.html
13. Bigdelou B, Sepand MR, Najafikhoshnoo S, Negrete JAT, Sharaf M, Ho JQ, et al. COVID-19 and Preexisting Comorbidities: Risks, Synergies, and Clinical Outcomes. Frontiers in Immunology. 2022. 13:890517.
14. van Wijhe M, Ingholt MM, Andreasen V, Simonsen L. Loose ends in the epidemiology of the 1918 pandemic: Explaining the extreme mortality risk in young adults. Am J Epidemiol. 2018.187:2503–10.
15. Tumpey TM, Basler CF, Aguilar P V, Zeng H, Solórzano A, Swayne DE, et al. Characterization of the Reconstructed 1918 Spanish Influenza Pandemic Virus. Science. 2005.310:77-80.
16. Taubenberger JK. The Origin and Virulence of the 1918 ‘Spanish’ Influenza Virus 1. Proc Am Philos Soc. 2006. 150:86-112.
17. Stern AM, Reilly MB, Cetron MS, Markel H. ‘Better Off in School’: School Medical Inspection as a Public Health Strategy During the 1918-1919 Influenza Pandemic in the United States. Public Health Rep. 2010. 125 Suppl 3:63-70.
18. Li AM, Ng PC. Severe acute respiratory syndrome (SARS) in neonates and children. Arch Dis Child Fetal Neonatal Ed. 2005. 90;6: F461-5.
19. Ng PC, Lam CWK, Li AM, Wong CK, Leung TF, Cheng FWT, et al. Chemokine response in children with SARS. Arch Dis Child. 2005. 4:422–3.

20. World Health Organisation. Evolution of a pandemic A(H1N1) 2009. April 2009 – August 2010. 2nd Edition.
21. Donaldson LJ, Sachedina N. Articles Paediatric mortality related to pandemic influenza A H1N1 infection in England: an observational population-based study. *Lancet*. 2010. 376:1846–52.
22. European Medicines Agency. European Medicines Agency recommends authorisation for additional vaccine for influenza pandemic (H1N1) 2009. [Accessed 2024 July 19]. https://www.ema.europa.eu/en/documents/press-release/european-medicines-agency-recommends-authorisation-additional-vaccine-influenza-pandemic-h1n1-2009_en.pdf.
23. Neuzil KM, Jackson LA, Nelson J, Klimov A, Cox N, Bridges CB, et al. Immunogenicity and Reactogenicity of 1 versus 2 Doses of Trivalent Inactivated Influenz Vaccine in Vaccine-Naive 5-8-Year-Old Children. J Infect Dis. 2006. 194:1032-9
24. Borse RH, Shrestha SS, Fiore AE, Atkins CY, Singleton JA, Furlow C, et al. Effects of vaccine program against pandemic influenza A(H1N1) virus, United States, 2009-2010. Emerg Infect Dis. 2013. 19:439–48.
25. Nohynek H, Jokinen J, Partinen M, Vaarala O, Kirjavainen T, Sundman J, et al. AS03 adjuvanted AH1N1 vaccine associated with an abrupt increase in the incidence of childhood narcolepsy in Finland. PLoS One. 2012. 7(3).
26. Assiri A, Al-Tawfiq JA, Al-Rabeeah AA, Al-Rabiah FA, Al-Hajjar S, Al-Barrak A, et al. Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: A descriptive study. Lancet Infect Dis. 2013. 13:752–61.
27. MacIntyre CR, Chen X, Adam DC, Chughtai AA. Epidemiology of paediatric Middle East respiratory syndrome coronavirus and implications for the control of coronavirus virus disease 2019. J Paediatr Child Health. 2020. 56:1561–4.
28. Al-Tawfiq JA, Auwaerter PG. Healthcare-associated infections: the hallmark of Middle East respiratory syndrome coronavirus with review of the literature. J Hosp Infec. 2019. 101:20-29.
29. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. New England Journal of Medicine. 2020. 382:727–33.
30. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG. A new coronavirus associated with respiratory disease in China. Nature 2020. 579:265-269.
31. World Health Organisation. Statement on the second meeting of the International Health Regulations (2005) Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV). 2020 [cited 2024 Jul 19]. Available from: https://www.who.int/news/item/30-01-2020-statement-on-the-second-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-(2019-ncov)
32. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. The Lancet. 2020. 395:565–74.
33. Brian DA, Baric RS. Coronavirus Genome Structure and Replication. Curr Top Microbiol Immunol. 2005. 287:1-30.
34. Du L, He Y, Zhou Y, Liu S, Zheng BJ, Jiang S. The spike protein of SARS-CoV - A target for vaccine and therapeutic development. Nat Rev Microbiol. 2009. 7:226-236.
35. Greenhalgh T, Jimenez JL, Prather KA, Tufekci Z, Fisman D, Schooley R. Ten scientific reasons in support of airborne transmission of SARS-CoV-2. Lancet. 2021. 397:1603–5.
36. World Health Organisation. Transmission of SARS-CoV-2: implications for infection prevention precautions. 202.0 [Accessed 2024 Jul 20]. Available from: https://www.who.int/news-room/commentaries/detail/transmission-of-sars-cov-2-implications-for-infection-prevention-precautions

37. Milton DK. A rosetta stone for understanding infectious drops and aerosols. Journal of the Pediatric Infectious Diseases Society. J Pediatric Infect Dis Soc. 2020. 9:413-415.
38. World Health Organisation. Checklist to support schools re-opening and preparation for COVID-19 resurgences or similar public health crises. 2020. [Accessed 2024 July 19]. Available from: https://www.who.int/publications/i/item/9789240017467
39. Wang K, Zhao S, Li H, Song Y, Wang L, Wang MH, et al. Real-time estimation of the reproduction number of the novel coronavirus disease (COVID-19) in China in 2020 based on incidence data. Ann Transl Med. 2020. 11:689–689.
40. Flaxman S, Mishra S, Gandy A, Unwin HJT, Mellan TA, Coupland H, et al. Estimating the effects of non-pharmaceutical interventions on COVID-19 in Europe. Nature. 2020. 584:257–61.
41. Birrell PJ, Pebody RG, Charlett A, Zhang XS, De Angelis D. Real-time modelling of a pandemic influenza outbreak. Health Technol Assess. 2017. 21:1–118.
42. Galmiche S, Cortier T, Charmet T, Schaeffer L, Chény O, von Platen C, et al. SARS-CoV-2 incubation period across variants of concern, individual factors, and circumstances of infection in France: a case series analysis from the ComCor study. Lancet Microbe. 2023. 4: e409–17.
43. Marshall JC, Murthy S, Diaz J, Adhikari N, Angus DC, Arabi YM, et al. A minimal common outcome measure set for COVID-19 clinical research. Lancet Infect Dis. 2020. 20: e192-197.
44. NHS England. COVID-19 Hospital Activity. 2020 [cited 2024 Jul 19]. Available from: https://www.england.nhs.uk/statistics/statistical-work-areas/covid-19-hospital-activity/
45. Richards-Belle A, Orzechowska I, Doidge J, Thomas K, Harrison DA, Koelewyn A, et al. Critical care outcomes, for the first 200 patients with confirmed COVID-19, in England, Wales and Northern Ireland: A report from the ICNARC Case Mix Programme. J Intensive Care Soc. 2021 Nov 1;22(4):270–9.
46. Wingert A, Pillay J, Gates M, Guitard S, Rahman S, Beck A, et al. Risk factors for severity of COVID-19: A rapid review to inform vaccine prioritisation in Canada.BMJ Open. 2020. 11:e044684.
47. Mizumoto K, Chowell G. Estimating risk for death from coronavirus disease, China, January-February 2020. Emerg Infect Dis. 2020. 26:1251–6.
48. Hasan MN, Haider N, Stigler FL, Khan RA, McCoy D, Zumla A, et al. The global case-fatality rate of COVID-19 has been declining since May 2020. Am J Trop Med Hyg. 2021. 104: 2176–84.
49. Villani L, McKee M, Cascini F, Ricciardi W, Boccia S. Comparison of deaths rates for COVID-19 across Europe during the first wave of the COVID-19 pandemic. Front Public Health. 2020. 8:620416.
50. Sorci G, Faivre B, Morand S. Explaining among-country variation in COVID-19 case fatality rate. Sci Rep. 2020.10:1.
51. UK Health Security Agency. COVID-19 confirmed deaths in England (to 31 December 2022). 2023. [Accessed 2024 Jul 21]. Available from: https://www.gov.uk/government/publications/covid-19-reported-sars-cov-2-deaths-in-england/covid-19-confirmed-deaths-in-england-to-31-december-2022-report
52. Ladhani SN, Amin-Chowdhury Z, Davies HG, Aiano F, Hayden I, Lacy J, et al. COVID-19 in children: analysis of the first pandemic peak in England. Arch Dis Child. 2020. 105:1180–5.
53. World Health Organisation. COVID-19 epidemiological update 15th July 2024. [Accessed 2024 Jul 21]. Available from: https://www.who.int/publications/m/item/covid-19-epidemiological-update-edition-169

54. Ahmed IS, Tapponi SL, Widatallah ME, Alakkad YM, Haider M. Unmasking the enigma: An in-depth analysis of COVID-19 impact on the pediatric population. J Infect Public Health. 2023. 16:1346-1360.
55. Gallardo J, Pérez-Illana M, Martín-González N, San Martín C. Adenovirus Structure: What Is New? Int J Mol Sci. 2021 May 15;22(10):5240.
56. Ewer K, Sebastian S, Spencer AJ, Gilbert S, Hill AVS, Lambe T. Chimpanzee adenoviral vectors as vaccines for outbreak pathogens. Hum Vaccin Immunother. 2017 Dec 2;13(12):3020–32.
57. Coughlan L. Factors Which Contribute to the Immunogenicity of Non-replicating Adenoviral Vectored Vaccines. Front Immunol. 2020 May 19;11.
58. Watanabe Y, Mendonça L, Allen ER, Howe A, Lee M, Allen JD, et al. Native-like SARS-CoV-2 Spike Glycoprotein Expressed by ChAdOx1 nCoV-19/AZD1222 Vaccine. ACS Cent Sci. 2021. 71:594–602.
59. Graham SP, McLean RK, Spencer AJ, Belij-Rammerstorfer S, Wright D, Ulaszewska M, et al. Evaluation of the immunogenicity of prime-boost vaccination with the replication-deficient viral vectored COVID-19 vaccine candidate ChAdOx1 nCoV-19. NPJ Vaccines. 2020. 5(1).
60. van Doremalen N, Lambe T, Spencer A, Belij-Rammerstorfer S, Purushotham JN, Port JR, et al. ChAdOx1 nCoV-19 vaccine prevents SARS-CoV-2 pneumonia in rhesus macaques. Nature. 2020. 586:578–82.
61. Delves. Essential Immunology. 13th edition. 2017.
62. Douzi B. Protein–Protein Interactions: Surface Plasmon Resonance. Methods Mol Biol. 2017. p. 257–75.
63. Erasmus MF, Dovner M, Ferrara F, D'Angelo S, Teixeira AA, Leal-Lopes C, et al. Determining the affinities of high-affinity antibodies using KinExA and surface plasmon resonance. MAbs. 2023. 15(1).
64. O'Mahoney C, Watt I, Fiedler S, Devenish S, Srikanth S, Justice E, et al. Microfluidic Diffusional Sizing (MDS) Measurements of Secretory Neutralizing Antibody Affinity Against SARS-CoV-2. Ann Biomed Eng. 2024. 52(6):1653–64.
65. Pullen GR, Fitzgerald MG, Hosking CS. Antibody avidity determination by ELISA using thiocyanate elution. J Immunol Methods. 1986. 86(1):83–7.
66. Correa VA, Rodrigues TS, Portilho AI, Trzewikoswki de Lima G, De Gaspari E. Modified ELISA for antibody avidity evaluation: The need for standardization. Biomed J. 2021. 44(4):433–8.
67. Zhang A, Stacey HD, D'Agostino MR, Tugg Y, Marzok A, Miller MS. Beyond neutralization: Fc-dependent antibody effector functions in SARS-CoV-2 infection. Nat Rev Immunol. 2023. 23(6):381–96.
68. Junker F, Gordon J, Qureshi O. Fc Gamma Receptors and Their Role in Antigen Uptake, Presentation, and T Cell Activation. Front Immunol. 2020.11.
69. Bournazos S, Gupta A, Ravetch J V. The role of IgG Fc receptors in antibody-dependent enhancement. Nat Rev Immunol. 2020, 20(10):633–43.
70. Lemke MM, McLean MR, Lee CY, Lopez E, Bozich ER, Rerks-Ngarm S, et al. A systems approach to elucidate personalized mechanistic complexities of antibody-Fc receptor activation post-vaccination. Cell Rep Med. 2021. 2(9).
71. Folegatti PM, Ewer KJ, Aley PK, Angus B, Becker S, Belij-rammerstorfer S, et al. Articles Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. Lancet. 2020. 396:467-478.

72. Marchevsky NG, Li G, Aley P, Costa Clemens SA, Barrett JR, Belij-Rammerstorfer S, et al. An exploratory analysis of the response to ChAdOx1 nCoV-19 (AZD1222) vaccine in males and females. EBioMedicine. 2022. 81:1–12.
73. Koen AL, Izu A, Baillie V, Kwatra G, Cutland CL, Fairlie L, et al. Efficacy of primary series AZD1222 (ChAdOx1 nCoV-19) vaccination against SARS-CoV-2 variants of concern: Final analysis of a randomized, placebo-controlled, phase 1b/2 study in South African adults (COV005). Vaccine. 2023. 41:3486–92.
74. Hamaluba M, Sang S, Orindi B, Njau I, Karanja H, Kamau N, et al. Safety and immunogenicity of ChAdOx1 nCoV-19 (AZD1222) vaccine in adults in Kenya: a phase 1/2 single-blind, randomised controlled trial. Wellcome Open Res. 2023. 8:182.
75. AstraZeneca. Investigator's Brochure. AZD1222 - A recombinant chimpanzee adenovirus expressing the severe respiratory syndrome-coronavirus-2 Spike (S) surface (SARS-CoV-2) glycoprotein. 2021. Version 3.1.
76. Li G, Cappuccini F, Marchevsky NG, Aley PK, Aley R, Anslow R, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 (AZD1222) vaccine in children aged 6–17 years: a preliminary report of COV006, a phase 2 single-blind, randomised, controlled trial. Lancet. 2022. 399:2212–25.
77. Smith C, Odd D, Harwood R, Ward J, Linney M, Clark M, et al. Deaths in Children and Young People in England following SARS-CoV-2 infection during the first pandemic year: a national study using linked mandatory child death reporting data. Nat Med. 2022. 28:185-192.
78. Ioannidis JPA. Infection fatality rate of COVID-19 inferred from seroprevalence data. Bull World Health Organ. 2021. 99:19-33F.
79. Kitano T, Kitano M, Krueger C, Jamal H, Al Rawahi H, Lee-Krueger R, et al. The differential impact of pediatric COVID-19 between high-income countries and low- And middle-income countries: A systematic review of fatality and ICU admission in children worldwide. PLoS One. 2021.16:1–12.
80. Swann O V., Holden KA, Turtle L, Pollock L, Fairfield CJ, Drake TM, et al. Clinical characteristics of children and young people admitted to hospital with covid-19 in United Kingdom: Prospective multicentre observational cohort study. BMJ. 2020. 370:m3249.
81. United Nations Inter-agency Group for Child Mortality Estimation (UN IGME). Levels and Trends in Child Mortality. 2022.
82. Polisena J, Ospina M, Sanni O, Matenchuk B, Livergant R, Amjad S, et al. Public health measures to reduce the risk of SARS-CoV-2 transmission in Canada during the early days of the COVID-19 pandemic: A scoping review. BMJ Open. 2021. 11:e046177.
83. Ismail SA, Saliba V, Lopez Bernal J, Ramsay ME, Ladhani SN. SARS-CoV-2 infection and transmission in educational settings: a prospective, cross-sectional analysis of infection clusters and outbreaks in England. Lancet Infect Dis. 2021. 21:344–53.
84. Viner R, Waddington C, Mytton O, Booy R, Cruz J, Ward J, et al. Transmission of SARS-CoV-2 by children and young people in households and schools: A meta-analysis of population-based and contact-tracing studies. Journal of Infection. 84(3):361–82.
85. Centers for Disease Control and Prevention. Health Department-Reported Cases of Multisystem Inflammatory Syndrome in Children (MIS-C) in the United States. [Accessed 2024 July 219]. Available from: https://covid.cdc.gov/covid-data-tracker/#mis-national-surveillance
86. Masonbrink AR, Hurley E. Advocating for children during the COVID-19 school closures. Pediatrics. 2020. 146:e20201440.
87. Afolabi MO, Tiono AB, Adetifa UJ, Yaro JB, Drammeh A, Nébié I, et al. Safety and Immunogenicity of ChAd63 and MVA ME-TRAP in West African Children and Infants. Molecular Therapy. 2016. 24:1470–7.

88. Tapia MD, Sow SO, Mbaye KD, Thiongane A, Ndiaye BP, Ndour CT, et al. Safety, reactogenicity, and immunogenicity of a chimpanzee adenovirus vectored Ebola vaccine in children in Africa: a randomised, observer-blind, placebo-controlled, phase 2 trial. Lancet Infect Dis. 2020. 20:719–30.
89. Tiono AB, Nébié I, Anagnostou N, Coulibaly AS, Bowyer G, Lam E, et al. First field efficacy trial of the ChAd63 MVA ME-TRAP vectored malaria vaccine candidate in 5-17 months old infants and children. PLoS One. 2018. 13:12.
90. Mensah VA, Roetynck S, Kanteh EK, Bowyer G, Ndaw A, Oko F, et al. Safety and immunogenicity of malaria vectored vaccines given with routine expanded program on immunization vaccines in gambian infants and neonates: A randomized controlled trial. Front Immunol. 2017. 8:1551.
91. Afolabi MO, Tiono AB, Adetifa UJ, Yaro JB, Drammeh A, Nébié I, et al. Safety and Immunogenicity of ChAd63 and MVA ME-TRAP in West African Children and Infants. Molecular Therapy. 2016. 24:1470–7.
92. Ngai AL, Staehle BO, Kuter BJ, Cyanovich NM, Cho I, Matthews H et al. Safety and immunogenicity of one vs, two injections of Oka.Merck varicella vaccine in healthy children. Paediatr Infect Dis J. 1996. 15:49-54.
93. London School of Hygiene and Tropical Medicine. COVID-19 vaccine tracker . 2024. [Accessed 2024 Jul 24]. Available from: https://vac-lshtm.shinyapps.io/ncov_vaccine_landscape/
94. Lopez Bernal J, Andrews N, Gower C, Gallagher E, Simmons R, Thelwall S, et al. Effectiveness of Covid-19 Vaccines against the B.1.617.2 (Delta) Variant. New England Journal of Medicine. 2021. 385:585–94.
95. Public Health England. Effectiveness of COVID-19 vaccines against hospital admission with the Delta (B.1.617.2) variant. 2021.
96. WHO. COVID-19 advice for the public: Getting vaccinated. 2021. [Accessed 2024 Jul 19]. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/covid-19-vaccines/advice
97. Han B, Song Y, Li C, Yang W, Ma Q, Jiang Z, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy children and adolescents: a double-blind, randomised, controlled, phase 1/2 clinical trial. Lancet Infect Dis. 2021. 3099:1–9.
98. Xia SL, Zhang YT, Wang YX, Wang H, Yang YK, Gao GF, et al. Safety and immunogenicity of an inactivated COVID-19 vaccine, BBIBP-CorV, in people younger than 18 years: a randomised, double-blind, controlled, phase 1/2 trial. Lancet Infect Dis. 2022. 22:196–208.
99. Frenck RW, Klein NP, Kitchin N, Gurtman A, Absalon J, Lockhart S, et al. Safety, Immunogenicity, and Efficacy of the BNT162b2 Covid-19 Vaccine in Adolescents. New England Journal of Medicine. 2021. 385:239–50.
100. Ali K, Berman G, Zhou H, Deng W, Faughnan V, Coronado-Voges M, et al. Evaluation of mRNA-1273 SARS-CoV-2 Vaccine in Adolescents. New England Journal of Medicine. 2021. 385:2241–51.
101. Walter EB, Talaat KR, Sabharwal C, Gurtman A, Lockhart S, Paulsen GC, et al. Evaluation of the BNT162b2 Covid-19 Vaccine in Children 5 to 11 Years of Age. New England Journal of Medicine. 2022. 386:35–46.
102. Frenck RW, Klein NP, Kitchin N, Gurtman A, Absalon J, Lockhart S, et al. Safety, Immunogenicity, and Efficacy of the BNT162b2 Covid-19 Vaccine in Adolescents. New England Journal of Medicine. 2021. 385:239–50.
103. European Medicines Agency. Comirnaty COVID-19 vaccine: EMA recommends approval for children aged 5 to 11. 2021 [Accessed 2024 Jul 24]. Available from:

https://www.ema.europa.eu/en/news/comirnaty-covid-19-vaccine-ema-recommends-approval-children-aged-5-11
104. Folegatti PM, Ewer KJ, Aley PK, Angus B, Becker S, Belij-Rammerstorfer S, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. Lancet. 2020.396:467–78.
105. Falsey AR, Sobieszczyk ME, Hirsch I, Sproule S, Robb ML, Corey L, et al. Phase 3 Safety and Efficacy of AZD1222 (ChAdOx1 nCoV-19) Covid-19 Vaccine. N Eng J Med. 2021. 385:2348-2360.
106. Xia S, Zhang Y, Wang Y, Wang H, Yang Y, Gao GF, et al. Safety and immunogenicity of an inactivated COVID-19 vaccine, BBIBP-CorV, in people younger than 18 years: a randomised, double-blind, controlled, phase 1/2 trial. Lancet Infect Dis. 2022. 22:196–208.
107. Walter EB, Talaat KR, Sabharwal C, Gurtman A, Lockhart S, Paulsen GC, et al. Evaluation of the BNT162b2 Covid-19 Vaccine in Children 5 to 11 Years of Age. N Engl J Med. 2021. 386:35-46.
108. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety, Immunogenicity and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine in Adolescents. N Eng J Med. 2020. 383:2603-2615.
109. Dorabawila V, Hoefer D, Bauer UE, Bassett MT, Lutterloh E, Rosenberg ES. Risk of Infection and Hospitalization Among Vaccinated and Unvaccinated Children and Adolescents in New York After the Emergence of the Omicron Variant. JAMA. 2022. 327:2242.
110. Tarke A, Coelho CH, Zhang Z, Dan JM, Yu ED, Methot N, et al. SARS-CoV-2 vaccination induces immunological T cell memory able to cross-recognize variants from Alpha to Omicron. Cell. 2022. 185:847-859.
111. UK Health Security Agency (UKHSA). SARS-CoV-2 variants of concern and variants under investigation in England- Technical briefing 31. 2021.
112. Voysey M, Costa Clemens SA, Madhi SA, Weckx LY, Folegatti PM, Aley PK, et al. 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. Lancet. 2021. 397:881–91.
113. Voysey M, Costa Clemens SA, Madhi SA, Weckx LY, Folegatti PM, Aley PK, et al. 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. Lancet. 2021. 397:881–91.
114. Soboleva K, Shankar NK, Al. MY et. Geographical distribution of TTS cases following AZD1222 (ChAdOx1 nCoV-19) vaccination. Lancet Glob Health. 2022. 10:e33-34.
115. Achey MA, Nag UP, Robinson VL, Reed CR, Arepally GM, Levy JH, et al. The Developing Balance of Thrombosis and Hemorrhage in Pediatric Surgery: Clinical Implications of Age-Related Changes in Hemostasis. Clin Appl Thromb Hemost. 2020. 26.
116. See I, Su JR, Lale A, Woo EJ, Guh AY, Shimabukuro TT, et al. US Case Reports of Cerebral Venous Sinus Thrombosis with Thrombocytopenia after Ad26.COV2.S Vaccination, March 2 to April 21, 2021. JAMA. 2021. 325:2448–56.
117. Hippisley-Cox J, Patone M, Mei XW, Saatci D, Dixon S, Khunti K, et al. Risk of thrombocytopenia and thromboembolism after covid-19 vaccination and SARS-CoV-2 positive testing: Self-controlled case series study. BMJ. 2021;374.
118. Dionne A, Sperotto F, Chamberlain S, Baker AL, Powell AJ, Prakash A, et al. Association of Myocarditis with BNT162b2 Messenger RNA COVID-19 Vaccine in a Case Series of Children. JAMA Cardiol. 2021. 6:1446–50.

119. Marshall M, Ferguson ID, Lewis P, Jaggi P, Gagliardo C, Collins JS, et al. Symptomatic acute myocarditis in 7 adolescents after pfizer-biontech covid-19 vaccination. Pediatrics. 2021. 148:3.
120. Countries vaccinating children against COVID-19. Reuters. 2021.
121. Payne AB, Gilani Z, Godfred-Cato S, Belay ED, Feldstein LR, Patel MM, et al. Incidence of Multisystem Inflammatory Syndrome in Children among US Persons Infected with SARS-CoV-2. JAMA Netw Open. 2021. 4:e2116420.
122. Walker AS, Vihta KD, Gethings O, Pritchard E, Jones J, House T, et al. Tracking the Emergence of SARS-CoV-2 Alpha Variant in the United Kingdom. New England Journal of Medicine. 2021. 385:2582–5.
123. Meng B, Kemp SA, Papa G, Datir R, Ferreira IATM, Marelli S, et al. Recurrent emergence of SARS-CoV-2 spike deletion H69/V70 and its role in the Alpha variant B.1.1.7. Cell Rep. 2021. 35:13.
124. Liu Y, Liu J, Plante KS, Plante JA, Xie X, Zhang X, et al. The N501Y spike substitution enhances SARS-CoV-2 infection and transmission. Nature. 2022. 602:294–9.
125. Uwamino Y, Yokoyama T, Shimura T, Nishimura T, Sato Y, Wakui M, et al. The effect of the E484K mutation of SARS-CoV-2 on the neutralizing activity of antibodies from BNT162b2 vaccinated individuals. Vaccine. 2022. 40:1928–31.
126. Dhawan M, Sharma A, Priyanka, Thakur N, Rajkhowa TK, Choudhary OP. Delta variant (B.1.617.2) of SARS-CoV-2: Mutations, impact, challenges and possible solutions. Hum Vaccin Immunother. 2022. 18:2068883.
127. Centre for Genomic Pathogen Surveillance. Phylogenetic Assignment of Named Global Outbreak Lineages. 2024. 2024 [Accessed 2024 Jul 21]. Available from: https://cov-lineages.org/resources/pangolin.html
128. Cicaloni V, Costanti F, Pasqui A, Bianchini M, Niccolai N, Bongini P. A Bioinformatics Approach to Investigate Structural and Non-Structural Proteins in Human Coronaviruses. Front Genet. 2022. 13:891418.
129. Hicks J, Klumpp-Thomas C, Kalish H, Shunmugavel A, Mehalko J, Denson JP, et al. Serologic Cross-Reactivity of SARS-CoV-2 with Endemic and Seasonal Betacoronaviruses. J Clin Immunol. 2021. 41:906-913.
130. Mateus J, Grifoni A, Tarke A, Sidney J, Ramirez SI, Dan JM, et al. Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans. Science. 2020. 370:89-94.
131. Galipeau Y, Siragam V, Laroche G, Marion E, Greig M, McGuinty M, et al. Relative Ratios of Human Seasonal Coronavirus Antibodies Predict the Efficiency of Cross-Neutralization of SARS-CoV-2 Spike Binding to ACE2. EBioMedicine. 2021. 74:103700.
132. Shrwani K, Sharma R, Krishnan M, Jones T, Mayora-Neto M, Cantoni D, et al. Detection of Serum Cross-Reactive Antibodies and Memory Response to SARS-CoV-2 in Prepandemic and Post-COVID-19 Convalescent Samples. Journal of Infectious Diseases. 2021. 224:1305–15.
133. Garrido JL, Medina MA, Bravo F, McGee S, Fuentes-Villalobos F, Calvo M, et al. IgG targeting distinct seasonal coronavirus- conserved SARS-CoV-2 spike subdomains correlates with differential COVID-19 disease outcomes. Cell Rep. 2022. 39:110904.
134. Asamoah-Boaheng M, Grunau B, Karim ME, Jassem AN, Bolster J, Marquez AC, et al. Are higher antibody levels against seasonal human coronaviruses associated with a more robust humoral immune response after SARS-CoV-2 vaccination? Front Immunol. 2022. 13:964093.
135. Ratcliffe H, Tiley KS, Andrews N, Amirthalingam G, Vichos I, Morey E, et al. Community seroprevalence of SARS-CoV-2 in children and adolescents in England, 2019-2021. Arch Dis Child. 2022.108;2:123–30.

136. Lin CY, Wolf J, Brice DC, Sun Y, Locke M, Cherry S, et al. Pre-existing humoral immunity to human common cold coronaviruses negatively impacts the protective SARS-CoV-2 antibody response. Cell Host Microbe. 2022. 30:83-96.
137. De Thoisy A, Woudenberg T, Pelleau S, Donnadieu F, Garcia L, Pinaud L, et al. Seroepidemiology of the Seasonal Human Coronaviruses NL63, 229E, OC43 and HKU1 in France. Open Forum Infect Dis. 2023. 10:ofad340.
138. Kolehmainen P, Huttunen M, Iakubovskaia A, Maljanen S, Tauriainen S, Yatkin E, et al. Coronavirus spike protein-specific antibodies indicate frequent infections and reinfections in infancy and among BNT162b2-vaccinated healthcare workers. Sci Rep. 2023. 12:8416.
139. Gasparoto TH, Dalboni TM, Amôr NG, Abe AE, Perri G, Lara VS, et al. Fcγ receptors on aging neutrophils. Journal of Applied Oral Science. 2021. 29:e20200770.
140. Park SY, Levin MJ, Canniff J, Johnson M, Schmid DS, Weinberg A. Development of antibody-dependent cellular cytotoxicity in response to recombinant and live-attenuated herpes zoster vaccines. NPJ Vaccines. 2022. 7:123..
141. Aydillo T, Rombauts A, Stadlbauer D, Aslam S, Abelenda-Alonso G, Escalera A, et al. Immunological imprinting of the antibody response in COVID-19 patients. Nat Commun. 2021. 12:3781.
142. Dowell AC, Butler MS, Jinks E, Tut G, Lancaster T, Sylla P, et al. Children develop robust and sustained cross-reactive spike-specific immune responses to SARS-CoV-2 infection. Nat Immunol. 2022. 23:40–9.
143. Yang F, Nielsen SCA, Hoh RA, Röltgen K, Fabian Wirz O, Haraguchi E, et al. Shared B cell memory to coronaviruses and other pathogens varies in human age groups and tissues. Science. 372:738-741.
144. Humbert M, Olofsson A, Wullimann D, Niessl J, Hodcroft EB, Cai C, et al. Functional SARS-CoV-2 cross-reactive CD4+ T cells established in early childhood decline with age. Proc Natl Acad Sci U S A. 2023. 120;12:e2220320120.
145. Hu C, Wang Z, Ren L, Hao Y, Zhu M, Jiang H, et al. Pre-existing anti-HCoV-OC43 immunity influences the durability and cross-reactivity of humoral response to SARS-CoV-2 vaccination. Front Cell Infect Microbiol. 2022. 12:978440.
146. Nielsen KF, Moustsen-Helms IR, Schelde AB, Gram MA, Emborg HD, Nielsen J, et al. Vaccine effectiveness against SARS-CoV-2 reinfection during periods of Alpha, Delta, or Omicron dominance: A Danish nationwide study. PLoS Med. 2022. 19:e1004037.
147. Kirsebom FCM, Andrews N, Sachdeva R, Stowe J, Ramsay M, Lopez Bernal J. Effectiveness of ChAdOx1-S COVID-19 booster vaccination against the Omicron and Delta variants in England. Nat Commun. 2022. 13: 7688.
148. Tran TT, Vaage EB, Mehta A, Chopra A, Tietze L, Kolderup A, et al. Titers of antibodies against ancestral SARS-CoV-2 correlate with levels of neutralizing antibodies to multiple variants. NPJ Vaccines. 2022. 7:174.
149. Dowell AC, Lancaster T, Bruton R, Ireland G, Bentley C, Sylla P, et al. Immunological imprinting of humoral immunity to SARS-CoV-2 in children. Nat Commun. 2023. 14:3845.
150. Yisimayi A, Song W, Wang J, Jian F, Yu Y, Chen X, et al. Repeated Omicron exposures override ancestral SARS-CoV-2 immune imprinting. Nature. 2024. 625:148–56.
151. Lumley SF, Richens N, Lees E, Cregan J, Kalimeris E, Oakley S, et al. Changes in paediatric respiratory infections at a UK teaching hospital 2016–2021; impact of the SARS-CoV-2 pandemic. Journal of Infection. 2022. 84:40–7.
152. Lawrenz J, Xie Q, Zech F, Weil T, Seidel A, Krnavek D, et al. Severe Acute Respiratory Syndrome Coronavirus 2 Vaccination Boosts Neutralizing Activity Against Seasonal Human Coronaviruses. Clinical Infectious Diseases. 2022.75: E653–61.

153. Kuraoka M, Curtis NC, Watanabe A, Tanno H, Shin S, Ye K, et al. Infant Antibody Repertoires during the First Two Years of Influenza Vaccination. mBio. 2022. 13:e0254622.
154. Feng S, Phillips DJ, White T, Sayal H, Aley PK, Bibi S, et al. Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. Nature Medicine 2021. 27:2032-2040.
155. Goldblatt D, Alter G, Crotty S, Plotkin SA. Correlates of protection against SARS-CoV-2 infection and COVID-19 disease. Immunol Rev. 310:6-26.
156. Harvey WT, Carabelli AM, Jackson B, Gupta RK, Thomson EC, Harrison EM, et al. SARS-CoV-2 variants, spike mutations and immune escape. Nat Rev Microbiol. 2021. 19:409-424.
157. Liu Z, Li J, Pei S, Lu Y, Li C, Zhu J, et al. An updated review of epidemiological characteristics, immune escape, and therapeutic advances of SARS-CoV-2 Omicron XBB.1.5 and other mutants. Front Cell Infect Microbiol. 2023. 13:1297078.
158. Foster WS, Lee JL, Thakur N, Newman J, Spencer AJ, Davies S. Tfh cells and the germinal centre are required for memory B cell formation & humoral immunity after ChAdOx1 nCoV-19 vaccination. Cell Rep Med. 2022. 3:100845.
159. Lee JH, Sutton HJ, Cottrell CA, Phung I, Ozorowski G, Sewall LM, et al. Long-primed germinal centres with enduring affinity maturation and clonal migration. Nature. 2022. 609:998–1004.
160. Prelog M, Jeske SD, Asam C, Fuchs A, Wieser A, Gall C, et al. Clinical and immunological benefits of full primary COVID-19 vaccination in individuals with SARS-CoV-2 breakthrough infections: A prospective cohort study in non-hospitalized adults. J Clin Virol. 2024. 170:105622.
161. Nakagama Y, Candray K, Kaku N, Komase Y, Rodriguez-Funes MV, Dominguez R, et al. Antibody Avidity Maturation Following Recovery From Infection or the Booster Vaccination Grants Breadth of SARS-CoV-2 Neutralizing Capacity. J Infect Dis. 2023. 227:780–7.
162. Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: From structure to effector functions. Front Immunol. 2014. 5:250.
163. Yates JL, Ehrbar DJ, Hunt DT, Girardin RC, Dupuis AP, Payne AF, et al. Serological analysis reveals an imbalanced IgG subclass composition associated with COVID-19 disease severity. Cell Rep Med. 2021. 2:100329.
164. Goel RR, Apostolidis SA, Painter MM, Mathew D, Pattekar A, Kuthuru O, et al. Distinct antibody and memory B cell responses in SARS-CoV-2 naïve and recovered individuals after mRNA vaccination. Sci Immunol. 2021. 6:eabi6950.
165. Barrett JR, Folegatti PM, Gilbride C, Halkerston R, Hill J, Jenkin D, et al. Phase 1/2 trial of SARS-CoV-2 vaccine ChAdOx1 nCoV-19 with a booster dose induces multifunctional antibody responses. Nat Med. 2021. 27:279–88.
166. Tejedor Vaquero S, de Campos-Mata L, Ramada JM, Díaz P, Navarro-Barriuso J, Ribas-Llaurado C, et al. The mRNA-1273 Vaccine Induces Cross-Variant Antibody Responses to SARS-CoV-2 With Distinct Profiles in Individuals With or Without Pre-Existing Immunity. Front Immunol. 2021. 12:737083.
167. Dowell AC, Butler MS, Jinks E, Tut G, Lancaster T, Sylla P, et al. Children develop robust and sustained cross-reactive spike-specific immune responses to SARS-CoV-2 infection. Nat Immunol. 2022. 23(1):40–9.
168. Junker F, Gordon J, Qureshi O. Fc Gamma Receptors and Their Role in Antigen Uptake, Presentation, and T Cell Activation. Front Immunol. 2020. 11:1393.
169. Bartsch YC, Tong X, Kang J, Avendaño MJ, Serrano EF, García-Salum T, et al. Omicron variant Spike-specific antibody binding and Fc activity are preserved in recipients of mRNA or inactivated COVID-19 vaccines. Sci. Transl. Med. 2022. 14:eabn9243.

170. Kaplonek P, Cizmeci D, Kwatra G, Izu A, Lee JSL, Bertera HL, et al. ChAdOx1 nCoV-19 (AZD1222) vaccine-induced Fc receptor binding tracks with differential susceptibility to COVID-19. Nat Immunol. 2023. 24:1161–72.
171. Singh G, Abbad A, Tcheou J, Mendu DR, Firpo-Betancourt A, Gleason C, et al. Binding and Avidity Signatures of Polyclonal Sera From Individuals With Different Exposure Histories to Severe Acute Respiratory Syndrome Coronavirus 2 Infection, Vaccination, and Omicron Breakthrough Infections. Journal of Infectious Diseases. 2023. 228:564–75.
172. Vogel E, Kocher K, Priller A, Cheng CC, Steininger P, Liao BH, et al. Dynamics of humoral and cellular immune responses after homologous and heterologous SARS-CoV-2 vaccination with ChAdOx1 nCoV-19 and BNT162b2. EBioMedicine. 2022. 85:104294.
173. Bullock JL, Hickey TE, Kemp TJ, Metz J, Loftus S, Haynesworth K, et al. Longitudinal Assessment of BNT162b2- and mRNA-1273-Induced Anti-SARS-CoV-2 Spike IgG Levels and Avidity Following Three Doses of Vaccination. Vaccines. 2024. 12:516.
174. Oyebanji OA, Sundheimer N, Ragavapuram V, Wilson BM, Abul Y, Gravenstein S, et al. Avidity maturation of humoral response following primary and booster doses of BNT162b2 mRNA vaccine among nursing home residents and healthcare workers. Geroscience. 2024. May 25.
175. Perdiguero B, Gómez CE, García-Arriaza J, Sánchez-Corzo C, Sorzano CÓS, Wilmschen S, et al. Heterologous Combination of VSV-GP and NYVAC Vectors Expressing HIV-1 Trimeric gp145 Env as Vaccination Strategy to Induce Balanced B and T Cell Immune Responses. Front Immunol. 2019. 10:2941.
176. Fickentscher C, Magorivska I, Janko C, Biermann M, Bilyy R, Nalli C, et al. The Pathogenicity of Anti-β 2GP1-IgG Autoantibodies Depends on Fc Glycosylation. J Immunol Res. 2015. 2015:638129.
177. de Taeye SW, Rispens T, Vidarsson G. The ligands for human igG and their effector functions. Antibodies. 2019. 8:30.
178. Nziza N, Institute R, Deng Y. Humoral prooles of toddlers and young children following SARS-CoV-2 mRNA vaccination. Nat Commun. 2024.15:905.
179. Bartsch YC, St. Denis KJ, Kaplonek P, Kang J, Lam EC, Burns MD, et al. SARS-CoV-2 mRNA vaccination elicits robust antibody responses in children. Sci Transl Med. 2022. 14:eabn9237.
180. Irrgang P, Gerling J, Kocher K, Lapuente D, Steininger P, Habenicht K, et al. Class switch toward noninflammatory, spike-specific IgG4 antibodies after repeated SARS-CoV-2 mRNA vaccination. Sci Immunol. 2023. 8:eade2798.
181. Espino AM, Armina-Rodriguez A, Alvarez L, Ocasio-Malavé C, Ramos-Nieves R, Rodriguez Martinó EI, et al. The Anti-SARS-CoV-2 IgG1 and IgG3 Antibody Isotypes with Limited Neutralizing Capacity against Omicron Elicited in a Latin Population a Switch toward IgG4 after Multiple Doses with the mRNA Pfizer–BioNTech Vaccine. Viruses. 2024. 16:187.
182. Damelang T, de Taeye SW, Rentenaar R, Roya-Kouchaki K, de Boer E, Derksen NIL, et al. The influence of Human IgG subclass and Allotype on Complement Activation. J Immunol. 2023. 211:1725-1735.
183. Kalkeri R, Zhu M, Cloney-Clark S, Plested JS, Parekh A, Gorinson D, et al. Altered IgG4 antibody response to repeated mRNA versus recombinant protein SARS-CoV-2 vaccines. J Infect. 2024. 88:106119.
184. Farkash I, Feferman T, Cohen-Saban N, Avraham Y, Morgenstern D, Mayuni G, et al. Anti-SARS-CoV-2 antibodies elicited by COVID-19 mRNA vaccine exhibit a unique glycosylation pattern. Cell Rep. 2021.37:110114.

185. Klingler J, Kowdle S, Bandres JC, Emami-Gorizi R, Alvarez RA, Rao PG, et al. Heterologous Ad26/Ad5 adenovirus-vectored vaccines elicited SARS-CoV-2-specific antibody responses with potent Fc activities. Front Immunol. 2024. 15:1382619.
186. Dowell AC, Lancaster T, Bruton R, Ireland G, Bentley C, Sylla P, et al. Immunological imprinting of humoral immunity to SARS-CoV-2 in children. Nat Commun. 2023. 14:3845.
187. Balasubramanian P, Williams C, Shapiro MB, Sinangil F, Higgins K, Nádas A, et al. Functional Antibody Response Against V1V2 and V3 of HIV gp120 in the VAX003 and VAX004 Vaccine Trials. Sci Rep. 2018. 8:542.
188. Navaratnam AMD, Shrotri M, Nguyen V, Braithwaite I, Beale S, Byrne TE, et al. Nucleocapsid and spike antibody responses following virologically confirmed SARS-CoV-2 infection: an observational analysis in the Virus Watch community cohort. International Journal of Infectious Diseases. 2022. 123:104–11.
189. Koen AL, Izu A, Baillie V, Kwatra G, Cutland CL, Fairlie L, et al. Efficacy of primary series AZD1222 (ChAdOx1 nCoV-19) vaccination against SARS-CoV-2 variants of concern: Final analysis of a randomized, placebo-controlled, phase 1b/2 study in South African adults (COV005). Vaccine. 2023.41:3486–92.
190. Tuekprakhon A, Nutalai R, Dijokaite-Guraliuc A, Zhou D, Ginn HM, Selvaraj M, et al. Antibody escape of SARS-CoV-2 Omicron BA.4 and BA.5 from vaccine and BA.1 serum. Cell. 2022. 185:2422-2433.
191. Besser REJ, Long AE, Owen KR, Law R, Birks JS, Pearce O, et al. Transdermal Blood Sampling for C-Peptide Is a Minimally Invasive, Reliable Alternative to Venous Sampling in Children and Adults With Type 1 Diabetes. Diabetes Care. 2024. Feb 1;47:239–45.
192. Moderna. Moderna Announces Positive Phase 3 Data for Combination Vaccine Against Influenza and COVID-19. 2024 [Accessed 2024 Jul 19]. Available from: https://investors.modernatx.com/news/news-details/2024/Moderna-Announces-Positive-Phase-3-Data-for-Combination-Vaccine-Against-Influenza-and-COVID-19-/default.aspx