

Use of serological and molecular techniques to determine prior infection status and guide vaccine strategies in at-risk groups

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1. Acknowledgements

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I'd like to thank Shabana Bi and Dr Sanjiv Rughooputh from UK NEQAS for Microbiology, who kindly shared their 'Molecular detection of SARS-CoV-2 external quality assessment scheme' data with me for this thesis.

My clinical colleagues deserve acknowledgement of their patience and excellent listening skills whilst I have been undertaking this work. I am particularly grateful to Dr Anne Tunbridge and Dr Cariad Evans who have been incredibly flexible, supportive and ensured I have had the bandwidth to write this thesis whilst back in clinical training.

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Finally, I am thankful to my cats for keeping me company in the early mornings when I was writing, and to my husband, friends and family for absolutely everything they do for me (particularly making sure I was getting outside enough).

2. Abstract

Aims and objectives: Healthcare workers and recipients of haematopoietic stem cell transplant (HSCT) or chimeric antigen receptor T-cells (CAR-T) are at increased risk from vaccine preventable diseases. My thesis incorporates four published papers presenting molecular and serological data, aimed at studying infection status and antibody responses in these groups to guide vaccine strategies.

Methods and Results: In **Paper 1** and **Paper 2**, I investigated COVID-19 infection status. **Paper 1** presented early findings from in-house molecular testing when capacity was limited, and **Paper 2** explored healthcare worker serostatus. Seroprevalence data revealed approximately one quarter were infected during the first pandemic wave. Healthcare workers on the Acute Medical Unit and physio/occupational therapists were most likely to be seropositive. Antibody titre correlated positively with age, suggesting seroprevalence studies may underestimate infection status in younger populations.

Paper 3 and **Paper 4** focussed on humoral immunity in HSCT/CAR-T recipients. In **Paper 3**, I applied a pseudoneutralisation assay to evaluate SARS-CoV-2 neutralising antibody responses following COVID-19 vaccination. Compared to healthcare workers, HSCT/CAR-T recipients had significantly poorer responses, particularly after one dose. Those who received viral vector vaccines were more likely to develop neutralising antibodies than messenger ribonucleic acid (mRNA) vaccinees. Older age, rituximab and prior HSCT were associated with lower titres. In **Paper 4**, I investigated binding antibody responses against a range of vaccine preventable diseases in autologous HSCT recipients who missed their re-vaccination schedule. This cohort had significant humoral defects, with the vast majority susceptible to pneumococcal disease and diphtheria. Following re-vaccination, increases in antibody titres were observed.

Conclusions: Improved awareness of factors associated with exposure among healthcare workers presents an opportunity to reduce risk through targeted interventions. Bespoke vaccination programmes post-HSCT/CAR-T are needed. Further exploration into the immunogenicity of different vaccine platforms would be valuable in this unique cohort.

3. List of abbreviations

ACE2	angiotensin-converting enzyme 2
AMU	Acute Medical Unit
BSBMTCT	British Society of Blood and Marrow Transplantation and Cellular Therapy
CAR-T	chimeric antigen receptor T-cell
COG-UK	COVID-19 Genomics UK
COVID-19	coronavirus disease 2019
CT	cycle threshold
DNA	deoxyribonucleic acid
E	envelope
ECIL	European Conference on Infections in Leukaemia
ELISA	enzyme-linked immunosorbent assay
GP	general practitioner
GvHD	graft versus host disease
HSCT	haematopoietic stem cell transplant
IDSA	Infectious Diseases Society of America
JCVI	Joint Committee on Vaccination and Immunisation
IgG	immunoglobulin G
M	membrane
MMR	measles, mumps and rubella
mRNA	messenger RNA
N	nucleocapsid
NAAT	nucleic acid amplification test
NHS	National Health Service
PCR	polymerase chain reaction
PHE	Public Health England
PITCH	Protective Immunity from T cells to COVID-19 in Health workers
PPE	personal protective equipment
PCV13	13-valent pneumococcal conjugate vaccine
PCV20	20-valent pneumococcal conjugate vaccine
PPV23	pneumococcal polysaccharide vaccine
RBD	receptor binding domain
RdRP	RNA-dependent RNA polymerase
RNA	ribonucleic acid

S	spike
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
STH	Sheffield Teaching Hospitals NHS Foundation Trust
TMPRSS2	transmembrane serine protease 2
UK	United Kingdom
US	United States
VZV	varicella zoster virus
WGS	whole genome sequencing

4. List of papers together with contribution leading to MD thesis

This MD thesis contains four published peer reviewed papers for which I was the first (or co-first) author. As these papers were collaborative, my personal contributions are as listed below underneath each paper listed.

Paper 1: Colton H, Ankcorn M, Yavuz M, Tovey L, Cope A, Raza M, Keeley AJ, State A, Poller B, Parker M, de Silva TI, Evans C. **Improved sensitivity using a dual target, E and RdRp assay for the diagnosis of SARS-CoV-2 infection: Experience at a large NHS Foundation Trust in the UK.** J Infect. 2021 Jan;82(1):159-198. doi: 10.1016/j.jinf.2020.05.061. Epub 2020 May 28. PMID: 32474037; PMCID: PMC7255707.

This was my first published paper, for which I was the co-first author. I conceptualised the idea of the paper following observations during my clinical work. I undertook the data collection, statistical analyses and created the manuscript figures. I led the writing of the original draft of the manuscript with support from my co-first author Dr Mike Ankcorn, who revised and submitted the paper.

Summary of my contributor roles: Conceptualisation, Data curation, Formal analysis, Investigation, Methodology, Visualisation, Writing – original draft

Paper 2: Colton H, Hodgson D, Hornsby H, Brown R, McKenzie J, Bradley KL, James C, Lindsey BB, Birch S, Marsh L, Wood S, Bayley M, Dickson G, James DC, Nicklin MJ, Sayers JR, Zafred D, Rowland-Jones SL, Kudesia G, Kucharski A; CMMID COVID-19 Working Group; Darton TC, de Silva TI, Collini PJ. **Risk factors for SARS-CoV-2 seroprevalence following the first pandemic wave in UK healthcare workers in a large NHS Foundation Trust.** Wellcome Open Res. 2022 Jun 10;6:220. doi: 10.12688/wellcomeopenres.17143.3. PMID: 35600250; PMCID: PMC9091808.

I was the co-first author for this paper with Dr David Hodgson from the London School of Hygiene and Tropical Medicine and Ms Hailey Hornsby from the University of Sheffield. I curated the clinical data and undertook data analysis

(frequentist statistical analysis). I wrote the abstract, introduction and discussion sections in full, in addition to some of the method ("Background and setting", "Recruitment and consent", "Regulatory review") and results sections ("Registration and study visits", "Demographics, job role, work locations, and environment", "Unadjusted seroprevalence"). I led on coordinating the writing, submission and revision of the paper, and I was responsible for responding to reviewer questions. Dr David Hodgson performed the Bayesian data analyses, wrote their respective method ("Sample size" and "Statistical modelling") and result sections ("Adjusted seroprevalence estimates", "Antibody kinetics model", "Heterogeneous sensitivity model"), and led on graphical analysis. Ms Hailey Hornsby led on the ELISA validation and laboratory data curation, and drafted the respective method sections ("Serology assay development" and "SARS-CoV-2 serology").

Summary of my contributor roles: Data Curation, Formal Analysis, Project administration, Visualisation, Writing – original draft, Writing - review & editing

Paper 3: Colton H, Barratt N, Temperton N, Hornsby H, Angyal A, Grouneva I, Lindsey BB, Kearns P, Barnes E, Goodyear CS, Richter A, Thomas D, Cook G, McInnes IB, Willicombe M, Siebert S, Orchard K, Selby R, Bowden S, Collini PJ, Pope A, Kirkham A, Kronsteiner B, Dunachie SJ, Miller P, Clay J, Hurst E, Malladi R, Kesavan M, Kinsella F, Sanderson R, Yong KL, Rea D, Parry H; PITCH Consortium, OCTAVE Collaborative Group, OCTAVE-DUO Investigators, PROSECO Investigators; Lim SH, Snowden JA, de Silva TI. **Greater preservation of SARS-CoV-2 neutralising antibody responses following the ChAdOx1-S (AZD1222) vaccine compared with mRNA vaccines in haematopoietic cell transplant recipients.** Br J Haematol. 2024 Dec;205(6):2206-2218. doi: 10.1111/bjh.19874. Epub 2024 Nov 17. PMID: 39551718; PMCID: PMC11637739.

I was the first author for this paper, which was an output using data and samples from several large UK multicentre studies (OCTAVE, OCTAVE-DUO, PROSECO and PITCH). I wrote the data sharing agreement for access to data and samples for OCTAVE/OCTAVE-DUO. I selected and requested which sera to test from available samples from all studies. I performed all laboratory work and analyses relating to the pseudotype neutralisation assay, with the laboratory data from anti-RBD and

cellular extracted from the original study data. I curated the metadata across all studies, and performed all of the statistical and graphical analyses. I also wrote the original draft of the manuscript, and revised it with feedback from co-authors. I submitted the paper and responded to reviewer questions.

Summary of my contributor roles: Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Visualisation, Writing – original draft, Writing – review & editing

Paper 4: Colton H. Greenfield DM, Snowden JA, Miller PDE, Morley NJ, Wright J, Darton TC, Evans CM, de Silva TI. **Long-term survivors following autologous haematopoietic stem cell transplantation have significant defects in their humoral immunity against vaccine preventable diseases, years on from transplant.** Vaccine. 2021 Aug 9;39(34):4778-4783. doi: 10.1016/j.vaccine.2021.07.022. Epub 2021 Jul 20. PMID: 34294480.

I was the first author for this paper. The data were collected during a cellular therapy long term effects clinic by Professor Diana Greenfield. I cleaned the data, and undertook statistical and graphical analyses. I wrote the original draft of the manuscript, and revised it with feedback from co-authors. I submitted the paper and responded to reviewer questions.

Summary of my contributor roles: Data curation, Formal analysis, Project administration, Visualisation, Writing – original draft, Writing – review & editing

5. Commentary

5.1. Introduction and background

The emergence and spread of a novel coronavirus in late 2019, subsequently named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), triggered the rapid development and deployment of testing and vaccination programmes in the United Kingdom (UK) and beyond. Molecular testing to allow viral detection was initially prioritised in order to identify cases, prevent onward transmission and better understand disease epidemiology and pathogenesis^{1,2}. However, access to molecular testing was restricted in the early stages of the coronavirus disease 2019 (COVID-19) pandemic due to capacity limitations¹. Only individuals fulfilling specific symptomatic case definitions within certain settings were tested (e.g. hospitalised cases)¹. Given their ability to determine the past infection status of asymptomatic or untested symptomatic cases, serological assays subsequently played a key role in establishing the true prevalence of COVID-19 during this period³. They were also later used to evaluate humoral responses following the rollout of the SARS-CoV-2 vaccination programme to at-risk adults in December 2020⁴⁻⁹.

Historically, children have been the focus for national vaccination programmes. In the UK, the first national programme began in 1853 with the introduction of compulsory childhood smallpox vaccination¹⁰. Subsequently, vaccines were offered free of charge to protect children against other endemic and potentially fatal infections such as diphtheria in 1940, and tuberculosis in 1953¹⁰. The remarkable fall in cases and deaths following the introduction of these programmes encouraged the development of vaccines against other diseases^{10,11}. During the 1970-80s, the vaccine repertoire expanded following the introduction of platforms presenting specific antigen(s) to the immune system, such as subunit and protein conjugated vaccines¹¹. During the COVID-19 pandemic, viral vector and messenger ribonucleic acid (mRNA) vaccines were employed for mass vaccination programmes¹². These novel technologies using nucleic acids

were found to be well tolerated, and were highly effective at preventing severe disease by eliciting robust humoral and cellular responses^{11–16}.

There is now a comprehensive UK routine immunisation schedule to protect children against 15 infections, in addition to selective programmes for at-risk groups¹⁷. Examples include programmes offered to pregnant women, older adults, and those with specific risk factors such as immunosuppression or exposure through certain occupations¹⁷. One of the most vulnerable groups within the immunosuppressed cohort are recipients of haematopoietic stem cell transplant (HSCT) and chimeric antigen receptor T-cell (CAR-T) therapy, as pre-existing immune memory is lost during treatment^{18–22}. HSCT and CAR-T recipients are therefore considered ‘never-vaccinated’ by expert groups^{18,22}.

This thesis focuses on using molecular and serological testing in two specific populations of interest: 1) healthcare workers during the COVID-19 pandemic and 2) HSCT/CAR-T recipients, to determine infection status, and to evaluate vaccination strategies.

5.2. Healthcare workers

Healthcare workers were identified as a high-risk group early on in the COVID-19 pandemic^{23,24}. During the first wave, a limited number of studies suggested that healthcare workers were almost twelve times more likely to acquire COVID-19 than the general population, and seven times more likely to develop severe disease^{23,24}. In addition, infected healthcare workers risked onward transmission to vulnerable patients and other staff members, leading to nosocomial spread and outbreaks²⁵.

5.2.1. Early testing within the COVID-19 pandemic

5.2.1.1. Molecular testing

Nucleic acid amplification tests (NAATs) such as polymerase chain reaction (PCR) assays were widely used to diagnose people with COVID-19, who were then advised to self-isolate to avoid infecting others¹. The public health agency operating at the time, Public Health

England (PHE), developed and introduced a COVID-19 PCR test shortly after the genetic sequence of ancestral SARS-CoV-2 (B.1) was shared on GenBank in early January 2020^{1,26,27}. PCR testing was offered to people who met the continually updated clinical case definition, which included specific criteria for symptoms and relevant epidemiological exposure(s) i.e. travel to an affected area, or contact with a confirmed case².

As cases rose and demand for testing outstripped capacity, molecular testing was restricted to hospitalised patients on 12 March 2020 when the UK moved from a 'contain' phase into a 'delay'^{28,29}. Testing recommendations were replaced with fixed duration self-isolation guidelines for people in the community with compatible symptoms, along with their asymptomatic household contacts to limit onward transmission²⁸. This resulted in high rates of healthcare worker staff absences across the National Health Service (NHS), and concerns that some staff members may feel pressured to continue working if they only had mild symptoms²⁸. Capacity for testing healthcare workers and their household members was ultimately restored in mid-late April 2020¹.

Paper 1³⁰ of this thesis presents the early experience using an in-house real time reverse-transcriptase PCR assay at a regional clinical virology laboratory, when molecular testing capacity elsewhere was limited. This in-house assay was offered to healthcare workers and their household contacts with symptoms of COVID-19 following the discontinuation of community testing²⁸, as well as patients presenting to STH with respiratory symptoms.

The PCR method involves extraction of clinical samples to purify nucleic acids, and if necessary, complementary deoxyribonucleic acid (DNA) is synthesised during a reverse transcription step if the target is a ribonucleic acid (RNA) virus^{31,32}. Typically 40 thermal cycles of PCR are performed, with each cycle consisting of three steps: 1) heating to denature DNA into single strands, 2) cooling for primer binding to the

target sequence and 3) extension of the primers by DNA polymerase to synthesise double stranded DNA^{31,32}. Real time PCR is frequently used in diagnostic laboratories as it is semiquantitative and faster than classical PCR^{31,32}. Real time PCR uses labelled probes which release a fluorescent signal when target amplification occurs, removing the requirement for any additional wet laboratory work such as gel electrophoresis³¹. The number of cycles taken for fluorescence to rise above the background level is deemed the cycle threshold (CT) value, thus a lower CT is indicative of a higher viral load in the sample³¹.

Like other coronaviruses, the SARS-CoV-2 genome is comprised of genes encoding four structural proteins: Spike (S), Nucleocapsid (N), Membrane (M) and Envelope (E); and several non-structural proteins including RNA-dependent RNA polymerase (RdRp)²⁷. Careful selection of stable target genes can help avoid primer-probe mismatches, and ensure consistent detection over time despite viral evolution^{33,34}. For SARS-CoV-2, the RdRp, E and N genes were found to be similar to SARS-CoV-1 and bat SARS-related coronaviruses, therefore NAATs were developed to detect these conserved gene regions²⁶.

The primers for the in-house assay were ordered on 10 February 2020 based on sequences for RdRp and E gene targets published by Corman et al²⁶. This differed from other molecular assays used in the UK at the time which targeted RdRp and/or N gene(s), i.e. the assays from PHE and those adapted from the United States (US) Centers for Disease Control and Prevention^{30,35}. Despite early work showing that the E gene was likely a conserved region, there were some expert concerns regarding non-specificity and reagent contamination causing isolated E gene amplification, which I investigated in this paper.

As part of the High Consequence Infectious Diseases network, Sheffield Teaching Hospitals NHS Foundation Trust (STH) received their first confirmed COVID-19 patients from the Diamond Princess cruise ship on 23 February 2020³⁶. Having access to positive samples so

early in the pandemic allowed rapid validation of the assay which subsequently entered clinical use from 02 March 2020.

Between 02 March and 05 April 2020, a total of 2593 nose and throat swabs testing positive for one or both genes on the in-house assay were evaluated in the paper. Dates of symptom onset were obtained from the request forms.

The CT for E gene was found to be on average 5.7 cycles earlier than RdRp in samples which tested positive on both targets (n=2273, 87.7%). This difference between RdRp and E gene CT was consistent at each day post symptom onset, and samples taken later in the illness showed a persistence of E gene detection after RdRp was no longer detectable in serially sampled patients.

For the samples with E gene amplification in isolation (n=319, 12.3%), most were considered true positives as they had an early CT of less than 35. A selection of these were sequenced to confirm that isolated E gene detection was not due to an RdRp primer-probe mismatch (n=11, E gene CT 31.2-34.7, RdRp not detected). This sequencing was performed as part of the COVID-19 Genomics UK (COG-UK) consortium, a network of laboratories performing rapid next generation SARS-CoV-2 genomic sequencing³⁷. The platform used was Oxford Nanopore Technologies with the ARTIC network protocol. Genomic coverage of over 90% was seen in 10 samples. The results confirmed there was no variation within the RdRp gene that would result in a primer-probe mismatch.

The samples with isolated low-level E-gene amplification (i.e. CT $\geq$ 35) were explored further (n=69). Most were subsequently deemed true positives, either because the result was confirmed on a second assay using different targets (n=48), or they were taken from a patient who had another sample with both genes detected (n=11). The remainder were unconfirmed as they either did not undergo subsequent testing due to high pre-test probability (n=4), or did not confirm on the second

assay (n=6). The reason behind E gene persistence and its earlier CT values may be because coronaviruses have a unique discontinuous transcription process, which could result in higher copies of genes towards the 3' end of the genome³⁸.

In the years following the publication of **Paper 1**, a multitude of high throughput commercial NAATs have been approved for SARS-CoV-2 routine diagnostic molecular testing in the UK³⁹. Despite initial expert concerns around E gene, it has consistently remained a popular target for COVID-19 molecular testing in clinical laboratories reporting to the UK National External Quality Assessment Scheme for Microbiology⁴⁰.

Substitutions in E, M and N proteins have been shown to contribute to increased infectivity of SARS-CoV-2³³. However, as the spike receptor binding domain (RBD) binds angiotensin-converting enzyme 2 (ACE2) receptors to infect cells, mutations in the RBD can result in immune escape if antibodies can no longer neutralise it³³. Such mutants resulting in enhanced transmission were deemed variants of concern, and include Alpha, Beta, Gamma, Delta and Omicron sublineages³³.

Following the emergence of these variants, molecular testing strategies were adapted for both surveillance and clinical purposes. For example, genotyping was deployed within routine clinical testing between April 2021 and March 2022 for large scale variant monitoring, and to identify escape mutations such as E484K which rendered some COVID-19 monoclonal antibody therapies ineffective³⁴. When used to identify variants in real time, genotyping was found to be a median of 6 days quicker than whole genome sequencing (WGS), however in some of my other work, we found turnaround time for rapid sequencing varied significantly between laboratories (range 3-29 days), due to a variety of factors^{34,37}.

WGS was used extensively in the UK throughout the pandemic, predominantly for genomic surveillance through COG-UK, but also to identify transmission events in hospitals and influence infection

control decision making^{25,37}. Analysis of WGS data from NAAT positive STH healthcare workers and patients during the first wave of the pandemic demonstrated most healthcare worker COVID-19 infections were acquired from other healthcare workers, rather than from patients²⁵.

In addition to the symptomatic PCR programme, healthcare workers also performed routine twice-weekly lateral flow antigen testing from December 2020 after it became evident that there were asymptomatic cases with onward transmission of SARS-CoV-2^{25,41}. At STH, these testing programmes ultimately concluded in August 2022 for twice-weekly asymptomatic lateral flow testing, and in March 2023 for symptomatic PCR testing. Data modelled on re-introducing asymptomatic testing in the Omicron era have shown that twice-weekly staff testing could potentially reduce COVID-19 infections between healthcare workers, but it did not significantly impact transmission from healthcare workers to patients⁴¹. In comparison, testing all patients on admission to hospital was predicted to reduce hospital-acquired COVID-19 by up to 21.5%⁴¹.

5.2.1.2. Utility of Serology

The true scale of how many healthcare workers contracted COVID-19 early in the pandemic was unclear until the results of serosurveys became available. Although serological testing is not helpful for diagnosing acute SARS-CoV-2, these assays were invaluable for retrospectively identifying those who were not diagnosed due to subclinical infection, or unable to access molecular testing^{3,42}. Unlike NAAT which has a brief detection window, antibodies against SARS-CoV-2 have been shown to remain detectable for months, or even years, after infection (Figure 1)^{42,43}. Serological assays were developed to detect antibodies against S and N antigens from SARS-CoV-2 as these were found to be most immunogenic^{3,42}.

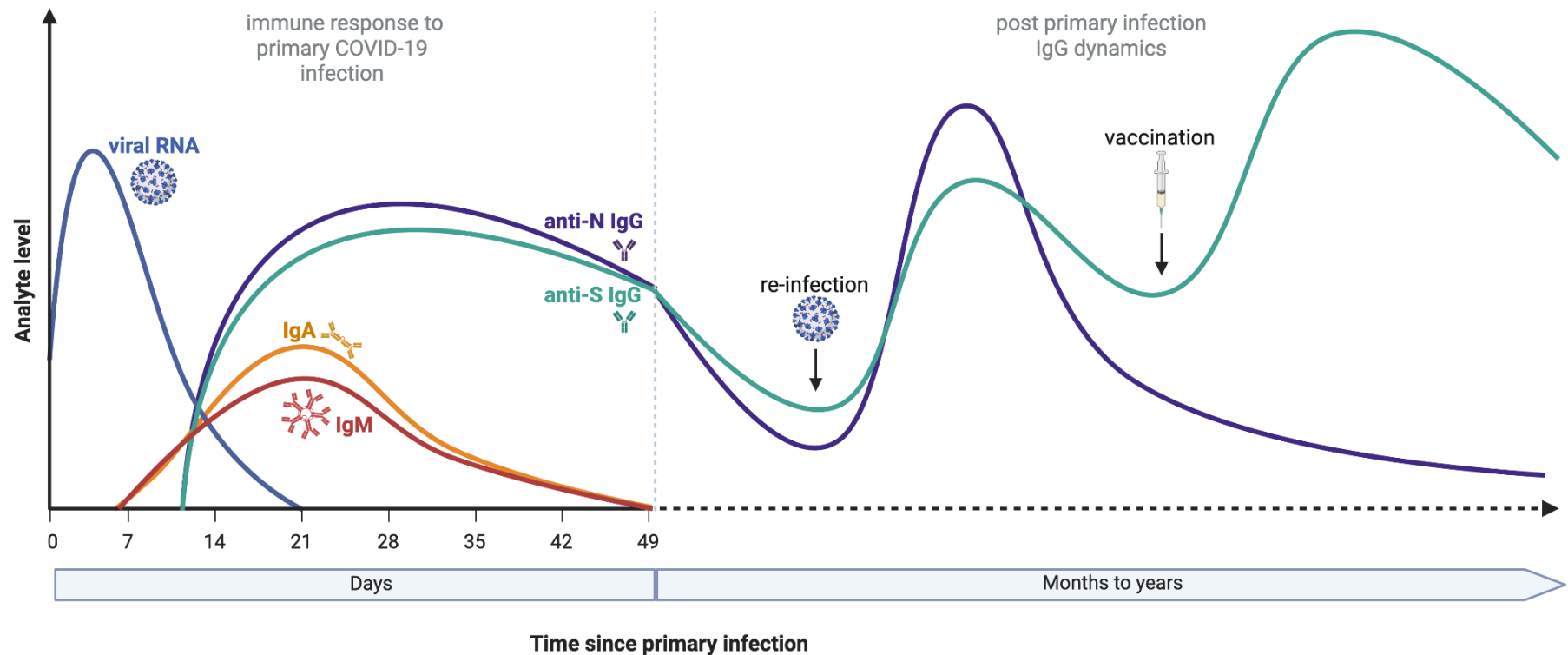


Figure 1: Schematic to represent antibody dynamics post primary COVID-19 infection^{36,42–45}. Created with BioRender.com.

Viral RNA	1 to 21 days	(peaks within first week)
IgM	4 days to 7 weeks	(peak 3 weeks)
IgA	4 days to 7 weeks	(peak 3 weeks)
IgG	10 days to months/years	(peak 4 weeks)

Abbreviations: COVID-19 - coronavirus disease 2019, Ig - immunoglobulin, N - nucleocapsid, RNA - ribonucleic acid, S - spike

Methods for antibody testing range from simple binary detection using lateral flow assays at the bedside to complex assessment of function against live virus in a containment level 3-4 laboratory. Enzyme-linked immunosorbent assays (ELISAs) are the most widely used immunoassay as they are quick to perform, inexpensive, and are able to be developed in-house and/or automated for high throughput testing^{32,46}. ELISAs can be designed to be qualitative, semi-quantitative or quantitative depending on their application⁴⁶. The method involves adding sera to pre-prepared microtitre wells which have specific antigen bound to the base^{32,46}. If complementary antibodies are present, an antigen-antibody complex forms, binds further substrates, and results in an objectively measured colour change^{32,46}.

Paper 2³⁶ of this thesis describes a seroprevalence study using an in-house ELISA to determine STH healthcare workers' serostatus following the first SARS-CoV-2 wave and prior to the introduction of COVID-19 vaccines. Given its timing, the study provided a unique opportunity to evaluate the extent of UK healthcare worker exposure to a novel respiratory pathogen.

Sera were collected between 15 May 2020 and 12 June 2020. As the study was carried out prior to vaccine roll out, participants were expected to be serologically 'naive', i.e. have neither S or N antibodies. Demographic data collected included age, sex, ethnicity, place of work and job role. We were particularly interested in evaluating specific occupational risk factors associated with exposure. Details of any prior illness suggestive of COVID-19 and previous positive molecular tests was also sought - this included the STH staff testing programme which commenced 17 March 2020 using either the method described in **Paper 1**, or a commercial platform using two RdRp gene targets.

The in-house ELISA tested for immunoglobulin G (IgG) reactivity against S and N antigens. Specificity was optimised by reporting the assay as positive only if IgG were detected against both proteins. In

addition, pre-pandemic sera were included in the validation dataset to ensure there was minimal cross-reactivity with pre-existing antibodies against similar pathogens such as seasonal coronaviruses. To enhance detection of pauci- and asymptomatic infections, the validation dataset also included convalescent sera from outpatients, in addition to hospitalised cases. The assay had a high sensitivity and specificity of 99.47% and 99.56% respectively. These approximate to the Infectious Diseases Society of America (IDSA) recommendations of $\geq 99.5\%$, however they note that a high specificity was more important for seroprevalence studies early on in the pandemic to avoid overinflating seropositivity³.

In the seroprevalence model, approximately one quarter of STH healthcare workers were seropositive, and a large proportion of these individuals had not reported a prior positive NAAT or illness consistent with COVID-19 (40%). This demonstrated the huge burden of subclinical SARS-CoV-2 infection in UK healthcare workers in the first few months of the pandemic. Modelling estimated that seroreversion would take 100 weeks for anti-S IgG, and 52 weeks for anti-N IgG. Real world data have subsequently confirmed that IgG against S and N proteins can persist for at least 18 months after infection⁴³, thus seroprevalence estimates reported in **Paper 2** and from other published UK healthcare worker studies are likely an accurate representation of healthcare workers infected in the early stages of the pandemic (seropositivity ranging 10-44%)⁴⁷⁻⁵⁰.

Older healthcare workers had higher antibody titres than their younger colleagues. Further modelling from **Paper 2** suggested that as overall assay sensitivity declined, seroprevalence in younger age groups would be disproportionately underestimated, compared to older groups. Commercial assays in use at the time may have further underestimated seroprevalence as they were often validated with sera from hospitalised cases who would have likely had higher antibody titres due to severe disease and increased age, than milder non-hospitalised cases.

Despite universal guidance for use of personal protective equipment (PPE) and infection control precautions, the adjusted seroprevalence varied between work areas and occupation. Healthcare workers working on the Acute Medical Unit (AMU) were at significantly higher risk of acquiring COVID-19 than other acute admission areas. A possible explanation could be that prior to universal PPE recommendations, AMU would have had longer stays of paucisymptomatic patients compared to the Emergency Department, but higher turnover and larger bays than medical wards. In addition, the nature of AMU working includes multiple staff handovers per day and increased interdisciplinary working compared to other areas.

Physiotherapists and occupational therapists were the occupation most likely to be seropositive. This group works across multiple clinical areas with prolonged close patient contact, resulting in increased cumulative exposure. Many physiotherapists also perform aerosol generating procedures such as induction of sputum and suctioning of the lower respiratory tract, however data on the additional risk posed by these procedures are lacking⁵¹. In other studies, physiotherapists have been found to have similar self-perceived and tested knowledge of PPE to doctors⁵², however compared to other healthcare workers they were less likely to have access to PPE during the first wave⁵³. Healthcare workers with reduced access to PPE have been found to not only be more likely to contract COVID-19, but also more likely to develop severe disease^{50,54}.

In the literature, other difficulties reported by healthcare workers affecting adherence included inadequate training outside of 'high risk units', and challenges of wearing PPE for long periods^{55,56}. In addition to the physical effects of prolonged PPE use, social difficulties were also reported, with some removing masks in order to communicate with patients^{55,56}. Incomplete adherence to PPE was also associated with regular contact with patients with confirmed COVID-19 and "fatalism"⁵⁶. However, objective data on healthcare worker adherence

to PPE guidelines during the early pandemic are scarce. These published studies largely evaluate self-reported PPE compliance and proficiency^{52,56,57}, which does not necessarily reflect true adherence or correct use^{56,58}. Even when PPE guidelines were reported to be followed for patient care, close contact between healthcare workers without PPE was commonplace^{56,57}. When planning for future outbreaks of respiratory viruses with pandemic potential, offering additional support, training and education around PPE and vaccination to the high seroprevalence areas and occupations described in **Paper 2** and other studies, could help reduce occupational exposure risk to healthcare workers⁴⁷⁻⁵⁰.

Healthcare worker COVID-19 cases fell during the second wave, in part due to a lower risk of re-infection in those with prior COVID-19⁵⁹. However, previously infected healthcare workers have been reported to have reduced confidence in PPE, and increased vaccine hesitancy once a vaccine was available^{52,60}. Initial vaccine uptake in England for frontline healthcare workers was reported as 76.8% for influenza, and 75.2% for at least two doses of COVID-19 vaccine by August 2021^{61,62}. Uptake in seasonal programmes has been falling since, with only 37.5% and 21.0% of frontline healthcare workers vaccinated against influenza and COVID-19 respectively during the 2024-25 season⁶³. Surveys have shown that the barriers to vaccination in healthcare workers are complex, including perceived level of risk, ethnicity, region, level of education, profession, gender and age^{60,64-67}.

Whilst healthcare workers ceased to be eligible for seasonal COVID-19 vaccination after the autumn 2024-2025 campaign due to high population immunity, extremely clinically vulnerable groups continue to be offered seasonal vaccination as they remain at higher risk of severe disease and death from COVID-19^{6,12,68-70}.

5.3. Vaccination strategies

The UK became the first country to roll out a COVID-19 vaccination programme in early December 2020¹². Following the emergency use authorisation of mRNA (BNT162b2, mRNA-1273) and viral vector (ChAdOx1-S, Ad26.COV2.S) COVID-19 vaccines, the focus for serological evaluation shifted to investigate real world post-vaccination immunogenicity in various higher-risk groups.

Healthcare workers were one of the first priority groups to be offered vaccination, in order to protect both the staff and their patients from healthcare-associated COVID-19^{4,12}. Longitudinal serological testing of UK healthcare workers was already underway through large studies such as the SARS-CoV-2 Immunity and Reinfection Evaluation (SIREN) and the Protective Immunity from T cells to COVID-19 in Health workers (PITCH) studies, which subsequently provided real world post-vaccination humoral and cellular response data^{5,44,59,71,72}.

In late December 2020, UK policy changed to extend the dosing interval between the first two doses from 3-4 weeks to 12 weeks, in order to maximise the number of at-risk people receiving at least one dose of vaccine⁵. As people deemed extremely clinically vulnerable were offered vaccination from 18 January 2020, they likely received doses with longer intervals⁷³. Prior to vaccine roll out, limited data were available on vaccine efficacy in this cohort, as people who were immunosuppressed were excluded from participating in phase II/III vaccine studies^{6,13-16}.

5.3.1. Post-vaccination immunogenicity

The post-vaccination immune response is most commonly and easily measured through testing serum for antibodies produced by B-lymphocytes (humoral response)¹¹. Peripheral blood T-lymphocyte function can also be assessed by measuring interferon production in response to antigen (cellular response), however these are performed

less frequently due to expense, logistics and difficulties with standardisation^{11,74}.

Several vaccine preventable diseases have a well established correlate of protection, defined by Plotkin as “*an immune function that correlates with and may be biologically responsible for vaccine-induced efficacy*”⁷⁵. Measles is an example, with measles IgG ≥ 120 mIU/ml protective against disease, however correlates of protection for many pathogens are still not known^{75,76}. Defining a correlate of protection is dependent on how the pathogen is neutralised, host factors and what specific protection is offered, i.e. from infection, severe disease or death. Whilst multiple correlates of protection have been proposed for SARS-CoV-2, detection of neutralising antibodies against S protein have been described as the most reliable^{75,77}. The detection of neutralising antibodies has been widely reported to be protective against severe disease and death from COVID-19, with titres inversely correlated with breakthrough infections⁷⁸⁻⁸¹.

Standard ELISAs do not differentiate between neutralising and non-neutralising (also known as binding) antibodies³, so neutralising function cannot be directly ascertained from these results. The gold standard for assessing the neutralising function of antibodies against viruses are plaque reduction neutralisation tests, however these are time consuming and require specific training in order to work with live viruses^{82,83}. SARS-CoV-2 is currently classified as a category 3 pathogen by the Advisory Committee on Dangerous Pathogens, therefore viral culture must be conducted in a laboratory satisfying containment level 3 requirements to reduce the risk of laboratory acquired infection for laboratory staff^{82,84}. To circumvent some of these challenges, alternative methods to measure neutralising antibodies have been developed^{82,83,85,86}.

ELISA-based surrogate virus neutralisation tests for SARS-CoV-2 measure inhibition of RBD-ACE2 binding, and have a moderate to

strong positive correlation with plaque reduction neutralisation assays^{82,85,86}. Whilst they are fast, safe and inexpensive to perform, they only detect antibodies that neutralise the RBD, thus are less sensitive than cell based neutralisation assays which can measure antibodies that neutralise other regions on the S protein^{82,83,85,86}. Surrogate virus neutralisation tests have also been shown to be less specific, cross-reacting with other antibodies more frequently than plaque reduction neutralisation tests⁸⁵.

Pseudotype neutralisation assays use a somewhat similar method to the plaque reduction neutralisation test, and a strong correlation is reported between their results for neutralising antibody titres against SARS-CoV-2 and other viruses^{83,86}. The use of a replication deficient pseudotyped virus in lieu of live virus means they can be performed at containment level 2^{87,88}. Pseudotyped virus particles are constructed from plasmids encoding a) the genome for a replication-deficient viral vector, b) the envelope protein(s) of the virus of interest and c) a luciferase reporter gene^{87,88}. The burden of infected cells is measured through luminescence following cellular uptake and expression of the reporter gene, rather than assessing for cytopathic effects⁸⁷. Pseudotype neutralisation assays therefore offer a safer and faster method of accurately measuring functional neutralising antibodies than plaque neutralisation assays.

5.4. Clinically vulnerable groups

Following vaccine roll out, several studies were set up to investigate vaccine immunogenicity and real world outcomes in clinically vulnerable groups, such as people living with cancer, solid organ transplant, haemodialysis, human immunodeficiency virus and various autoimmune conditions^{6-9,89-97}.

Perhaps unsurprisingly, clinically vulnerable groups were more likely to have suboptimal humoral responses compared to healthy controls following two doses of vaccine^{6,8,9,89,92,93,97}. Patients with haematological

malignancies stood out as a group who were significantly less likely to have detectable anti-RBD or neutralising antibodies following their first two doses of COVID-19 vaccination, with particularly poor responses seen in individuals who had received B-cell targeted therapies, HSCT/CAR-T or had a diagnosis of lymphoid malignancy^{6-9,89}. Based on available data, including some early studies demonstrating an improvement in anti-RBD titres following a third dose in solid organ transplant recipients⁹⁴⁻⁹⁶, the Joint Committee on Vaccination and Immunisation (JCVI) recommended a third primary vaccine dose from September 2021 for people who were severely immunosuppressed⁹⁸.

Most post-vaccination humoral data were derived from anti-RBD antibody titres using ELISAs, however some studies included data on neutralising antibodies using the methods described earlier^{7,8,89,93,96}. Statistically significant positive correlations were seen between anti-RBD and neutralising antibody titres against ancestral SARS-CoV-2, and variants of concern Alpha, Beta and Delta^{8,89}. However, it was noted that some patients had undetectable neutralising antibodies against these variants despite having detectable anti-RBD IgG⁸.

Following the emergence of SARS-CoV-2 variant of concern Omicron in late 2021, a cohort study of millions of people in England demonstrated that the severely immunocompromised continued to be over four times more likely to be hospitalised or die from COVID-19 compared to the general population⁶⁸. People who had received HSCT in the preceding two years were at highest risk of hospitalisation (n=1435, adjusted incidence rate ratio 14.24, 95% confidence interval 10.15–19.97), however the authors reported limited interpretability of the data on admission to critical care units or death due to the small sample sizes⁶⁸.

5.4.1. HSCT and CAR-T recipients

HSCT and CAR-T are therapies primarily used to treat haematological malignancies, but are also used for other illnesses such as solid

tumours and autoimmune diseases (Figure 2)^{99–101}. Prior to HSCT, recipients undergo a conditioning regime which varies in severity, from lymphodepletion through to toxic myeloablative conditioning¹⁰². The bone marrow is subsequently repopulated through infusion of either their own (autologous) or donor (allogeneic) stem cells¹⁰².

CAR-T is a more novel therapy that has revolutionised the treatment for relapsed or refractory haematological B-cell malignancies, with higher rates of remission compared to more traditional treatments such as chemoradiotherapy or even HSCT^{101,103,104}. CAR-T therapies currently in use involve a lymphodepletive conditioning regime with subsequent infusion of engineered T-cells which proliferate and target CD19 cells, killing malignant B cells but also the recipient's memory B-lymphocytes (Figure 2)^{22,100,101,103}.

The intensity of both of these therapies results in a loss of immune memory from prior vaccination and infection. This 'immune re-birth' makes them unique when compared to other immunosuppressed groups, where pre-existing immune memory may be maintained.

In addition, HSCT/CAR-T recipients' risk from infection in both the short and long term is further compounded by potential side effects of treatment. These include prolonged cytopenias, impaired lymphocyte recovery, hypogammaglobulinaemia and additional immunosuppressive treatments to treat complications such as graft versus host disease (GvHD) and cytokine release syndrome^{20,22,101,105}. All of these factors contribute to why infection is the leading cause of non-relapse mortality following both HSCT and CAR-T^{104,105}. Their increased risk of morbidity and mortality from infection can persist for years following transplant, thus a full primary re-vaccination schedule against several vaccine preventable diseases, including COVID-19, is recommended^{12,17–22,106–108}.

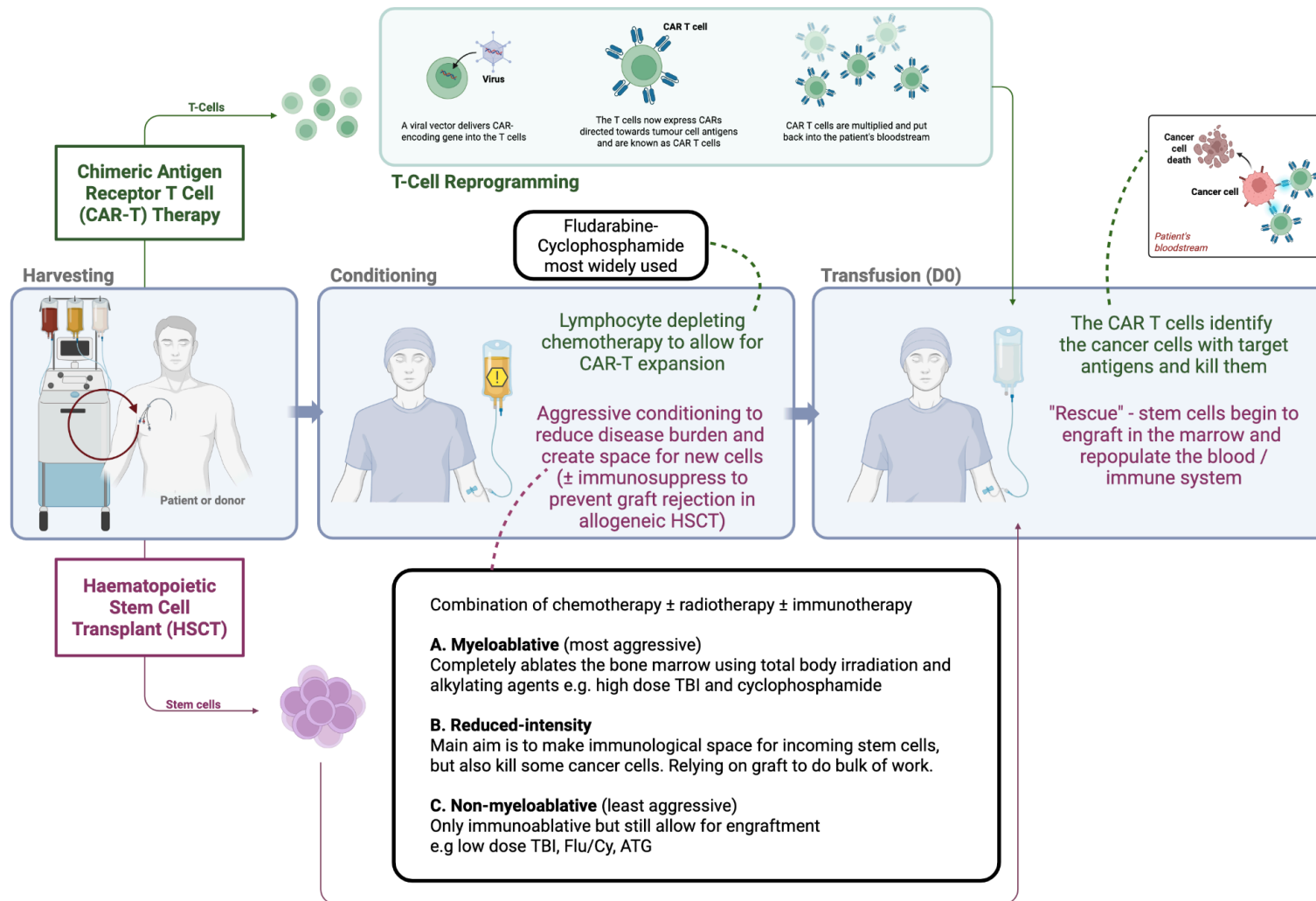


Figure 2: Overview of HSCT and CAR-T. Created with BioRender.com.

Abbreviations: ATG - anti-thymocyte globulin, CAR-T - chimeric antigen receptor T cell, Flu/Cy - fludarabine-cyclophosphamide, HSCT - haematopoietic stem cell transplant, TBI - total body irradiation

5.4.1.1. Humoral immunity following COVID-19 vaccination

Whilst published data were available on post-vaccination anti-RBD antibodies in people who were immunosuppressed, neutralising antibody responses and data specific to HSCT/CAR-T were limited^{6,8,89–91,109–111}. One study of 63 HSCT recipients vaccinated with BNT162b2 demonstrated that 50% and 88% developed 'highly protective' neutralising antibody levels after the first and second dose respectively, using a surrogate viral neutralisation assay¹¹¹. However, only 9% of the allogeneic HSCT recipients on immunosuppression were found to have these levels after one dose, increasing to 36% after a second¹¹¹. Neutralising capacity against Omicron appeared to be lower. In another study using live-virus micro-neutralisation, few HSCT (n=8/35) and CAR-T (n=0/3) recipients had detectable neutralising antibodies against Omicron following three doses of vaccine (two doses of either BNT162b2 or ChAdOx1-S, with a third dose of BNT162b2)¹¹⁰.

Paper 3¹¹² of this thesis aims to address these data gaps by studying both anti-RBD and neutralising antibody responses following the first three COVID-19 immunisations in a large cohort of HSCT/CAR-T recipients. Patient and treatment related factors specific to HSCT/CAR-T were also explored to look for significant associations with vaccine response.

The study cohort was composed of 198 HSCT/CAR-T recipients recruited to three prospective multicentre studies of clinically vulnerable adults in the UK (OCTAVE⁶, OCTAVE-DUO⁹⁰ and PROSECO^{9,89}) whose sera were available for testing. These were tested in parallel with samples collected from 96 healthcare workers recruited to PITCH⁷². All participants received mRNA (BNT162b2 or mRNA-1273) or viral vector (ChAdOx1-S) vaccines encoding ancestral SARS-CoV-2 spike in the initial NHS roll out.

Sera were tested for neutralising antibody function against ancestral (B.1) and Omicron sublineages (BA.1, BA.5, BQ.1.1, XBB) of SARS-CoV-2 using a pseudotype neutralising assay. Sera were diluted and mixed with a pre-titrated concentration of pseudotyped viruses. Following one hour incubation, cells expressing transmembrane serine protease 2 (TMPRSS2) and ACE2 were added prior to a further 48 hour incubation. The proportion of infected cells were determined by comparing mean luminescence values to the pseudotype virus only control, and samples with luminescence $\leq 50\%$ of the control were repeated at serial dilutions to estimate a half-maximal neutralising titre.

One of the most striking findings from the paper was how poor post-vaccination neutralising antibody responses were in HSCT/CAR-T recipients against the vaccine target B.1. Following a single dose, only 25.0% had detectable neutralising antibodies, compared to 94.1% of healthcare workers ($p < 0.001$), with neutralising antibody titres 167-fold lower in the HSCT/CAR-T group ($p < 0.001$). A second dose increased the proportion with detectable neutralising antibodies to 71.0% versus 100% of healthcare workers, however titres were still 7-fold lower ($p < 0.001$).

Whilst a larger proportion of HSCT/CAR-T recipients had detectable anti-RBD antibodies following their first and second doses (55.5% and 85.7% respectively), a smaller proportion had a titre above the lowest value measured in healthcare workers (4.9% and 56.4% respectively). For HSCT/CAR-T recipients who had lower anti-RBD responses following their first two doses, 69.7% developed detectable neutralising antibodies after a third dose, however their titres were still 11-fold lower than healthcare workers ($p < 0.001$).

Compared to B.1, a reduced neutralisation capacity was seen in both HSCT/CAR-T recipients and healthcare workers against the Omicron sublineages tested. This is likely due to mutations in the S protein

resulting in these variants evading neutralisation following ancestral SARS-CoV-2 vaccines.

Post hoc analysis of these data (Figure 3) has shown a strong correlation between anti-RBD IgG and B.1 neutralising antibody titres ($R = 0.83$, $p < 0.001$), with few mismatches seen (McNemar's χ^2 8.47, $p < 0.001$). Although correlations were relatively strong for most of the Omicron sublineages (R : BA.1 = 0.56, BA.5 = 0.67, BQ.1.1 = 0.62, XBB = 0.36; all $p < 0.0001$), a normal anti-RBD antibody titre did not reliably predict the presence of neutralising antibodies (McNemar's χ^2 : BA.1 = 24.0, BA.5 = 5.06, BQ.1.1. = 24.0, XBB = 44.0; $p < 0.001$ for all).

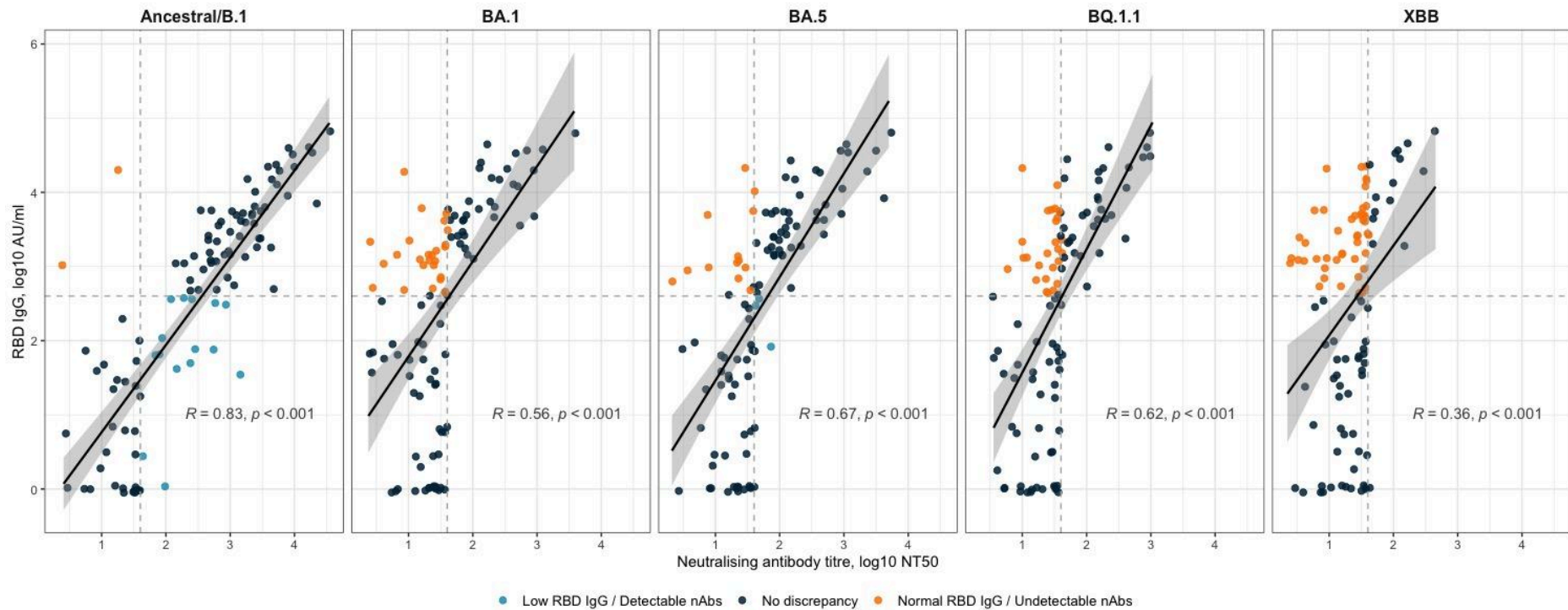


Figure 3: Relationship between neutralising and anti-RBD antibody titres, by SARS-CoV-2 pseudotype tested (Ancestral = B.1, Omicron sublineages = BA.1, BA.5, BQ.1.1 and XBB).

Dashed lines on each axis indicate the thresholds used in **Paper 3** for a normal anti-RBD IgG (400 AU/ml) and for detection of nAbs (NT50 40). Where there were discrepancies between anti-RBD IgG and nAb results, points are coloured orange if nAbs were not detected despite a normal anti-RBD IgG, and light blue where nAbs were detected despite a low anti-RBD IgG. The shaded areas around the lines of best fit represent 95% confidence intervals. R values represent the Pearson correlation coefficient.

Abbreviations: AU - arbitrary units, IgG - immunoglobulin G, nAb - neutralising antibody, NT50 - half-maximal neutralising titre, RBD - receptor binding domain

Treatment-related factors significantly associated with poorer neutralisation of the vaccine target B.1 on multivariate analyses were previous HSCT, rituximab and lymphocyte depletion with anti-thymocyte globulin. Prior COVID-19, receipt of ChAdOx1-S and younger age were patient-related factors that were significantly associated with developing neutralising antibodies.

In healthy individuals and other immunosuppressed groups, it is well established that a greater humoral response is seen following immunisation with an mRNA vaccine compared to other platforms^{6,12,71,113–118}. This was not seen in this HSCT/CAR-T recipient cohort who were eight times more likely to develop neutralising antibodies if they received two doses of ChAdOx1-S, than two doses of mRNA vaccine. In addition, neutralising antibody titres were comparable between HSCT/CAR-T recipients and healthcare workers following two doses of ChAdOx1-S, suggesting neutralising antibody responses are preserved following ChAdOx1-S. Neutralising antibody titres taken after a third dose were higher for low-responder HSCT/CAR-T recipients if they had received ChAdOx1-S for their first two doses, compared to mRNA vaccines.

The reason for the differences seen between vaccine platforms and neutralising antibody responses in this cohort remains unclear, however ChAdOx1-S and mRNA vaccines are known to induce immunity by different methods. For example, ChAdOx1-S has been shown to induce plasmablasts and circulating T-lymphocytes including mucosal-associated invariant T cells, which is not seen with mRNA vaccines^{119,120}.

Following the pandemic, access to COVID-19 vaccines other than mRNA platforms has become limited, with many products no longer available in the UK¹². The Green Book recommends two doses of an mRNA vaccine for newly transplanted HSCT/CAR-T recipients, to be given 8-12 weeks apart in order to extend protection, based on data

from healthy individuals^{5,12}. The recommendations in the Green Book were updated in February 2024 to include *“this interval may be reduced to three weeks on specialist clinical advice to maximise short term protection”*¹² following a joint letter from The British Society of Blood and Marrow Transplantation and Cellular Therapy (BSBMTCT) vaccines subcommittee and Anthony Nolan. This letter to JCVI and UK Health Security Agency presented data on the benefits of a reduced vaccine interval for HSCT/CAR-T recipients, and included preliminary data on poor post first vaccine neutralising antibody responses from **Paper 3** to lobby for this revised recommendation.

Rates for both allogeneic-HSCT and CAR-T are increasing annually across Europe, with the most recent UK data reporting that 4645 people underwent HSCT in 2023, and since its approval in 2018 over 1000 received CAR-T^{121–123}. These newly transplanted individuals will continue to require primary vaccination schedules for COVID-19, thus new data on vaccine efficacy in this group remain useful and relevant. Further work on studying viral vector vaccines in HSCT/CAR-T recipients would be challenging given the two viral vector vaccines used during the pandemic (ChAdOx1-S and Ad26.COV2.S) are no longer produced or authorised for use in the UK, US or European Union^{124–126}. In the UK, mRNA vaccines are now almost universally recommended for SARS-CoV-2 immunisation due to the high neutralising antibody responses generated¹². However, the data from **Paper 3** support the investigation of novel vaccine platforms in HSCT/CAR-T recipients to optimise primary vaccination courses further in this vulnerable cohort.

5.4.1.2. Humoral immunity to other vaccine preventable diseases

As previously discussed in this thesis, infection is the leading cause of non-relapse death in HSCT recipients¹⁰⁵. They also experience morbidity from infections following transplant, which could be attenuated by vaccination e.g. influenza-like illnesses, shingles, cervical smear abnormalities, pneumococcal disease and chickenpox¹²⁷. As these risks can persist for years post transplant, the Green Book now

aligns with expert published guidelines and recommends HSCT/CAR-T recipients “*should be considered for a full course of revaccination for all vaccines used in the routine programme*” due to the loss of their pre-existing immunity, however also recommends seeking expert advice^{12,18–22,106–108}.

In addition to selecting which pathogens and vaccine platforms to include, the construction of a routine immunisation schedule for HSCT/CAR-T recipients must also consider when to commence the programme post transplant. The optimal timing balances enough time to reconstitute the immune system in order to respond adequately to the vaccine, whilst limiting the time spent vulnerable to infection¹⁸. Additional consideration must be given to allogeneic HSCT recipients who may develop GvHD. The prolonged heavy immunosuppression used to treat GvHD increases the likelihood of an inadequate vaccine response, and risks vaccine-derived disease from live attenuated vaccines^{19,22,128}.

There are several published guidelines for re-vaccinating adults post HSCT/CAR-T (most recent are included in Table 1)^{18–21,107,108}. The BSBMTCT guidelines are the most recently published, and recommend re-vaccination against nearly all of the pathogens included in the UK childhood vaccination programme (*Haemophilus influenzae* type B, hepatitis B, human papillomavirus, influenza, measles, meningococcal disease, mumps, pertussis, pneumococcal disease, polio, rubella, tetanus and varicella zoster virus[VZV])¹⁷, with the exception of rotavirus, where the risk of vaccination was felt to outweigh its benefit¹⁸. The programme is recommended to start 3-6 months post transplant, with live vaccines to be considered when the following criteria are met “*i) 24 months post HSCT; (ii) No active GvHD; (iii) Remain in remission from underlying disease; (iv) No systemic immunosuppressive therapy for 12 months including monoclonal antibodies; (v) No intravenous immunoglobulin (IVIg) in last 3 months.*”¹⁸.

	BSBMTCT ¹⁸ Miller et al 2023	ECIL 7 ²¹ Cordonnier et al 2019	IDSA ¹⁰⁷ Rubin et al 2014	International Consensus Conference on Clinical Practice in chronic GvHD ¹⁰⁸ Hilgendorf et al 2011
Non-live vaccines				
Diphtheria Tetanus Pertussis	Three doses DTaP/IPV/Hib/HepB at 1-month intervals from 6 months post-transplant	Three doses at 1-2 month intervals from 6 months post-transplant	Three doses from 6 months post-transplant	Three doses DTaP/Hib/IPV at 1-month intervals from 6 months post-transplant
Polio		Three doses at 1-2 month intervals from 6-12 months post-transplant	Three doses from 6-12 months post-transplant	
Hib		Three doses at 1-month intervals from 3 months post transplant	Three doses from 6-12 months post-transplant	
Hepatitis B		Three doses at 0, 1 and 6 months, starting 6-12 months post-transplant	Three doses from 6 months post-transplant with further doses guided by surface antibody titres	If seronegative, three doses at 1-monthly intervals from 6 months post-transplant
Meningococcal	Two doses MenB with a 2-month interval from 6 months post-transplant; plus two doses MenACWY at 8 months and 18 months post-transplant	Two doses of a MenB and MenC vaccine from 6 months post-transplant “in accordance with country recommendations”	No recommendation for adults	Optional: three doses of Men C at 1-month intervals starting at 6-12 months post-transplant

Pneumococcal	Three doses PCV13 from 6 months post-transplant <u>and</u> One dose PPV23 at 18 months post-transplant (PCV13 instead if active GvHD)	Three doses PCV13 at 1-month intervals from 3 months post-transplant (additional fourth dose 6 months after the third dose if chronic GvHD) <u>and</u> One dose PPV23 at 12 months post-transplant	Three doses PCV13 from 3-6 months post-transplant <u>and</u> One dose PPV23 at 12 months post-transplant	Three doses PCV at 1-month intervals from 6 months post-transplant <u>and</u> a booster at 18 months post-transplant
Human papillomavirus	Three doses at 18, 19 and 24 months post-transplant	Schedule as per country recommendations for general population, starting at 6-12 months post-transplant	In adults up to and including 26 years old, three doses from 6 months post-transplant	Optional: three doses from 6-12 months post-transplant
Influenza inactivated	One dose from 3-6 months post-transplant	One dose from 3-6 months post-transplant at the beginning of the season	One dose from 4-6 months post-transplant	One dose from 4-6 months post-transplant
SARS-CoV-2	Three doses from 3-6 months post-transplant	No recommendation as published prior to COVID-19 pandemic		
Inactivated zoster vaccine	Two doses with a 2-month interval from 6 months post-transplant	No recommendation as published prior to availability of the inactivated shingles vaccine		
Hepatitis A	Not mentioned	Not mentioned	Two doses from 6 months post-transplant	Optional: three doses at 1-month intervals from 6 months post-transplant

Live attenuated vaccines				
Measles, mumps and rubella (MMR)	If measles seronegative, two doses with a 6-month interval from 24 months post-transplant*	If measles seronegative (and/or rubella seronegative in women of childbearing potential), one dose from 24 months post-transplant*	If measles seronegative, two doses from 24 months post-transplant*	Optional: one or two doses from 24 months post-transplant*
Live attenuated varicella vaccine	If VZV seronegative, two doses with a 2-month interval from 24 months post-transplant*	If VZV seronegative, one dose from 24 months post-transplant*, with a second dose considered if seronegative pre-HSCT or no prior history of VZV infection	If VZV seronegative, two doses from 24 months post-transplant*	Optional, however no dosing recommendation given for adults
Live attenuated zoster vaccine	Not recommended	Not recommended	Not recommended	Not recommended
Rotavirus	Not recommended	Not recommended	Not recommended	Not recommended
Bacillus Calmette Guérin (BCG)	Not recommended	Not recommended	Not recommended	Not recommended
Live attenuated influenza vaccine	Not recommended	Not recommended	Not recommended	Not recommended

Table 1: Published recommendations for primary revaccination of adults following haematopoietic stem cell transplant from the last 15 years

*additional conditions listed that must be met (e.g. no ongoing immunosuppression, no active GvHD)

Abbreviations: BSBMTCT - British Society of Blood and Marrow Transplantation and Cellular Therapy, ECIL - European

Conference on Infections in Leukaemia, GvHD - graft versus host disease, IDSA - Infectious Diseases Society of America, PCV - pneumococcal conjugate vaccine, PCV13 - 13-valent pneumococcal conjugate vaccine, PPV23 - pneumococcal polysaccharide vaccine, VZV - varicella zoster virus

Published guidelines are relatively consistent in their advice for inactivated vaccines, however there are some discrepancies on live vaccine recommendations. All of the guidelines are in agreement that live vaccines should only be considered from at least 24 months post HSCT providing there is no ongoing severe immunosuppression e.g. GvHD. Whilst all agree that Bacillus Calmette-Guérin (BCG), live attenuated influenza, oral polio and rotavirus are contra-indicated, the guidance for measles, mumps and rubella (MMR) and VZV vaccine is more nuanced.

Where MMR is recommended, guidance for adults is typically for two doses if measles seronegative^{18,20,107}. Some advise one dose^{19,21} and/or include indications for women of childbearing potential who are rubella seronegative¹⁹⁻²¹, or don't give a recommendation for checking serostatus at all prior to MMR¹⁰⁸. The more recent guidelines from IDSA, European Conference on Infections in Leukaemia (ECIL) and BSBMTCT recommend either one or two doses of live attenuated varicella vaccine for those who are seronegative^{18,21,107}, with the older guidelines citing it as optional due to lack of data on safety and efficacy^{19,20,108}. A recombinant shingles vaccine has only become available after the publication of most of these guidelines, so it is currently only recommended by the BSBMTCT for all adults at six months post transplant¹⁸.

Despite the abundance of available guidelines on re-vaccinating this cohort, various surveys have shown that re-vaccination schedules post transplant are poorly undertaken around the world^{18,127,129-131}. Practical reasons such as poor communication and care coordination are the most frequently reported barriers to immunisation of HSCT survivors¹³², however factors including vaccine hesitancy and the patient's own perceived level of risk may play a role¹²⁹.

Re-vaccination recommendations do not differ between autologous or allogeneic HSCT, however autologous recipients have been reported to

have poorer uptake of re-vaccination schedules^{132,133}. Possible reasons include differences in maintenance therapy and follow up, beliefs around reconstitution of immunity, and higher rates of relapse^{19,132}.

Paper 4¹³⁴ is an observational study of autologous HSCT recipients who did not undergo a routine vaccination programme post transplant. The objective of the paper was to investigate whether there were longstanding humoral defects towards vaccine preventable diseases in the absence of re-vaccination.

Participants were recruited when attending a late effects clinic, run by specialist nurses to monitor for long term physical and psychological issues following HSCT. Data from 56 autologous HSCT recipients were included in the study, who had undergone transplant within the previous three decades with a median of 7 years since HSCT. Sera were tested using available serological assays for antibodies against vaccine preventable diseases (diphtheria, *Haemophilus influenzae* type B, measles, mumps, rubella, *Streptococcus pneumoniae*, tetanus and VZV) at the local clinical virology laboratory at STH.

The methods used were predominantly ELISAs, however commercial chemiluminescence immunoassays, a similar form of immunoassay to ELISAs measuring luminescence rather than absorbance, were used for some pathogens. For *Streptococcus pneumoniae*, sera were forwarded to a reference laboratory in Manchester to test for antibodies against the Danish pneumococcal serotypes (1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23), which correspond to 12 of the 13 serotypes in 13-valent pneumococcal conjugate vaccine (PCV13), introduced into the UK childhood programme in 2010¹³⁵.

Correlates of protection are defined for most of the pathogens studied. The threshold for protection against pneumococcal disease in adults was less clear^{136,137}, and was therefore based on expert opinion. As a control group was not studied, comparable data from both UK general

population and other immunosuppressed groups were used where available.

The main findings of the paper were that most autologous HSCT recipients studied would be considered susceptible to pneumococcal disease (100%) and diphtheria (76.8%). Rates of measles, mumps and rubella seronegativity were lower (34.5%, 50.9% and 29.1% respectively), however our cohort appeared more likely to be susceptible than the general population and other immunosuppressed groups.

The late effects clinic wrote to general practitioners (GPs) to advise on a bespoke re-vaccination schedule for those considered non-immune, and most GPs were willing to facilitate this. Sera for post-vaccination antibody testing were collected either by the GP or at a subsequent clinic attendance. Increases in antibody titres were seen against Danish pneumococcal serotypes and diphtheria on follow up testing, reducing the proportion of those considered susceptible. The majority of participants susceptible to measles seroconverted on follow up testing (61.5%, n=8/13). Repeat mumps and rubella serology were performed less frequently given MMR was only recommended for those who were measles seronegative. Where mumps and rubella serology were performed, 60.0% (n=3/5) and 100% (n=3/3) seroconverted respectively, although numbers were small. Only one participant had repeat VZV antibody testing, perhaps due to difficulties with access or funding for chickenpox vaccination in primary care.

Statistical associations were limited given the small sample size, however some significant associations were identified using multivariate linear and logistic regression. Older participants were more likely to be measles seropositive, with those receiving BEAM conditioning (combination chemotherapy with Carmustine, Etoposide, Cytarabine and Melphalan) or total body irradiation less likely. Recipients of BEAM conditioning were also less likely to be mumps seropositive.

Morbidity and mortality secondary to *Streptococcus pneumoniae* is relatively common in the general population, with recent data showing that the serotypes present in PCV13 continue to cause a high burden of pneumococcal disease in the UK¹³⁸. Pneumococcal correlates of protection have been shown to be serotype specific in children, however the protective titre for adults remains unclear^{139–141}. Even using the putative threshold defined by the World Health Organisation, only two HSCT recipients had antibody titres above ≥ 0.35 $\mu\text{g/ml}$ for all serotypes tested prior to re-vaccination. Comparable data on all of the Danish serotypes were not available in the published literature, however a serosurvey in the UK general population prior to the introduction of pneumococcal vaccine to the routine childhood schedule reported that the vast majority of adults had antibody titres ≥ 0.35 $\mu\text{g/ml}$ against serotypes 1, 4, 5, 9V, 14, 18C, 19F and 23F, with most (60-70%) against 6B¹³⁷. Pneumococcal conjugate vaccines have been shown to be effective in adults^{142,143}, and the 20-valent pneumococcal conjugate vaccine (PCV20) is expected to replace the pneumococcal polysaccharide vaccine (PPV23) for at-risk people in the UK schedule in early 2026¹³⁵. Immunogenicity following immunisation against pneumococcal disease in the immunocompromised remains understudied, and there is a need to standardise reporting of these data¹⁴⁴.

The most recent measles data from the UK have shown that incidence is at its highest levels since pre-2000, likely due to a combination of reduced vaccine uptake and waning of antibodies post childhood vaccination in adults^{145,146}. A study performed since **Paper 4** was published found that 30% of laboratory diagnosed cases of measles did not have a rash¹⁴⁷. Underrecognition of measles cases reduces the opportunity for public health interventions to prevent onward transmission and offer post-exposure prophylaxis to those at-risk. Recent serostudies in the UK including healthcare workers have shown that a significant proportion of the younger population have suboptimal antibody levels^{146,148}. The triad of increasing measles

incidence, potential for underrecognition of cases and increasing population seronegativity is concerning for future outbreaks and uncontrolled spread of measles. Ensuring seronegative HSCT recipients have been vaccinated, and educating those unable to receive MMR on post-exposure prophylaxis is prudent.

Fortunately, autochthonous infection with diphtheria in the UK is rare due to the success of the childhood vaccination programme. However, HSCT survivors could be exposed if they travel to countries at risk of outbreaks secondary to low vaccine uptake, or if diphtheria vaccine uptake in the UK begins to fall. The proportion of susceptible autologous HSCT recipients was higher than the general population and people with solid cancers, but similar to non-transplanted haematological malignancies. The risk of additional morbidity and mortality from diphtheria following HSCT is unknown given the rarity of the disease in higher income countries which perform these transplants. However, it would be reasonable to assume on basic principles that HSCT recipients would be at increased risk compared to the general population.

Where re-vaccination programmes are missed and the patient is willing, a pragmatic approach as suggested in **Paper 4** could be implemented. This would be universal pneumococcal and diphtheria vaccination given the high proportions of seronegativity in the study cohort, with serological testing to guide in whom it would be beneficial to offer immunisation against MMR and VZV. In addition, given the findings from **Paper 3**, exploration into the immunogenicity of different vaccine platforms in HSCT/CAR-T recipients would be beneficial given the rising use of these therapies and the development of novel platforms.

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7. Published papers

Paper 1

Colton H, Ankcorn M, Yavuz M, Tovey L, Cope A, Raza M, Keeley AJ, State A, Poller B, Parker M, de Silva TI, Evans C. **Improved sensitivity using a dual target, E and RdRp assay for the diagnosis of SARS-CoV-2 infection: Experience at a large NHS Foundation Trust in the UK.** J Infect. 2021 Jan;82(1):159-198. doi: 10.1016/j.jinf.2020.05.061. Epub 2020 May 28. PMID: 32474037; PMCID: PMC7255707.

Dear Editor,

We read with interest the letter from Hao et al highlighting the issues regarding the sensitivity of real time reverse-transcriptase polymerase chain reaction (RT-PCR) testing of upper respiratory tract samples for COVID19 disease [1]. Extensive RT-PCR testing by has been key to clinical decision-making, epidemiological analysis and policy development during the current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. The majority of RT-PCR assays target the RNA-dependent RNA polymerase (RdRp), envelope protein (E) or nucleocapsid protein (N) genes [2]. However, initial testing algorithms and expert opinion from the European Centre for Disease Prevention and Control (ECDC) advised that E gene amplification in isolation should be treated cautiously, due to concerns of non-specificity and issues related to contamination of reagents [3]. Early experience at Sheffield Teaching Hospitals NHS Foundation Trust (UK) on serially sampled patients with confirmed SARS-CoV-2 infection suggested that E gene detection persists beyond RdRp detection, and may offer enhanced diagnostic sensitivity. Therefore we explored the significance of E gene detection in relation to RdRp, and in the absence of RdRp detection in a retrospective evaluation of SARS-CoV-2 RT-PCR testing.

A total of 12,015 clinical samples (combined nose/throat swabs or lower respiratory tract samples) were tested for SARS-CoV-2 as part of routine clinical diagnostics between 2nd March 2020 and 5th April 2020 at Sheffield Teaching Hospitals NHS Foundation Trust. Samples were extracted on the MagnaPure96 platform (Roche Diagnostics Ltd, Burgess Hill, UK). SARS-CoV-2 RNA was detected on 6µl of extract

using a dual target (E gene and the RdRp gene) in-house PCR on ABI Thermal Cycler (Applied Biosystems, Foster City, United States) (supplementary material) [4]. The assay was modified to a multiplex single-well assay with the addition of PCR primers to detect a housekeeping gene, Ribonuclease P (RNase P), which acts as an internal control and to assess sample quality.

Of the samples tested, 2,593 samples (21.6%) were positive with amplification curves for one or both target genes. Amongst positive results, we found E gene amplification alone to be common (n= 319, 12.3%), although the majority were positive for both RdRp and E gene targets (n = 2273, 87.7 %) and only 1 sample (<0.1 %) had RdRp gene amplification alone.

From the E-only positive group (n=319), 69 (21.6%) samples had low level amplification in the E gene (cycle threshold (CT) ≥ 35) and were investigated further. Within this subset, the majority (n=59, 85.5%) were considered to be true positives because they were either a) confirmed by an alternative assay (n=48) or b) a preceding or subsequent sample was positive for both E and RdRp (n=11) (Table 1). The alternative assay employed was a modified version of the Centers for Disease Control and Prevention (CDC) assay targeting the N gene (Micropathology Ltd, Coventry, UK) in most cases (n=47) or an alternative RdRp assay (n=1) [7]. Six samples (8.7 %) could not be confirmed in an alternative assay which had either high CT values for the E gene (n=4, CT values ≥ 39.0) or had good amplification curves not reaching the threshold (n=2). To further confirm the presence of SARS-CoV-2 RNA in samples with E gene only amplification, 11 samples were selected and successfully underwent whole genome sequencing (supplementary material). Analysis of the RdRp primer or probe binding sites in these samples did not reveal any mismatches to explain the lack of RdRp RT-PCR positivity (supplementary material).

We further explored the relationship between E gene detection and RdRp gene detection. Amongst samples with both RdRp gene and E gene amplification (n= 2273), we found that CT values for the E gene target were significantly lower than the CT values for RdRp, with a mean difference of 5.8 (Paired t test, p-value < 2.2×10^{-16} , 95% CI 5.79-5.92) (supplementary material). In a subset of samples where symptom onset was available (145 samples from 128 patients), it was clear that the CT values for both RdRp and E gene were lowest around 48 – 72 hours following

symptom onset (Fig. 1). At each stage of infection, the median CT values for RdRp were higher than those for the E gene.

By using the E gene target in addition to the RdRp gene target we observed a significantly increased diagnostic pick up (11.9%). In one patient, E gene amplification was detected for three days beyond RdRp amplification, indicating a possible widening of the diagnostic window. Our findings confirm that clinical samples with E only amplification should not be dismissed as non-specific results. Not only were we able to obtain whole genome sequences for SARS-CoV-2 from a subset of this group, we also found that 85% of E only samples with high CT values were confirmed by a second assay targeting the N gene or an alternative RdRp only assay.

The enhanced sensitivity seen for the E gene in our dual target E-RdRP assay is yet to be explained. We observed a mean difference of over five CT values when comparing E gene to RdRp values, which may suggest the possibility of higher copy numbers of E gene being present in the primary or extracted sample. Due to the unique transcription strategy of coronaviruses, genes towards the 3' end of the genome would be present in higher copy numbers during active viral replication, which could explain these findings [5]. It is also possible that PCR optimised conditions in a multiplex system favours E gene amplification, however we found no significant loss of RdRp detection when comparing single and multiplex systems during validation, with observed CT rises averaging 1-2 cycles (data not shown). In addition, we found no evidence of primer or probe mismatches in the RdRp region.

We believe dual target testing, using the E gene as a second target, will help improve both diagnostic sensitivity and the appropriate clinical response to this pandemic. We urge testing laboratories to carefully consider the use of the E gene as a target in order to optimise SARS-CoV-2 diagnostics, including strategies to confirm samples with E gene only amplification as we have described.

	n	%
Sent for confirmation at reference laboratory ^ϕ	54	78.26
- Confirmed by alternative assay	48	(88.89)
- Not confirmed	6	(11.11)
Repeat clinical sample positive	5	7.25
Previous clinical samples positive ^ψ	6	8.70
Resulted without further testing [‡]	4	5.80
Total	69	

Table 1.

Summary of samples with low level E gene amplification alone (CT ≥ 35). CT, cycle threshold; E, envelope gene.

ϕMost samples (n=53) were tested at Micropathology Ltd (Coventry) using a SARS-CoV-2 N gene assay using a modified CDC assay [6]. The other sample confirmed positive at PHE Colindale using an alternative SARS-CoV-2 RdRp assay.

ψAs part of the High Consequences Infectious Diseases network, Sheffield received some of the first positive patients in the United Kingdom, who had daily swabs taken. E gene amplification appeared to persist in this cohort after the RdRp became negative.

‡Four results were authorised without further testing due to high pre-test probability e.g. compatible symptoms with a confirmed household exposure to SARS-CoV-2.

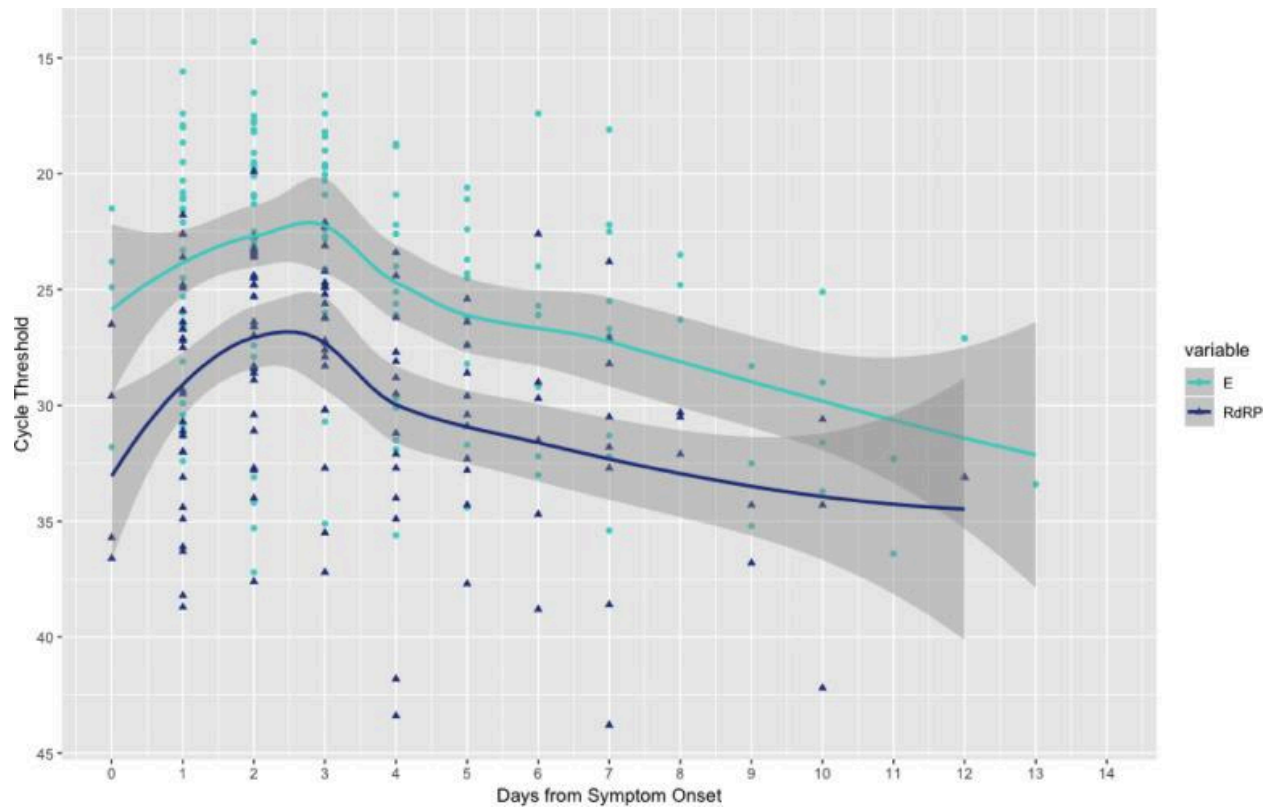


Figure 1

E and RdRp gene cycle threshold results in relation to symptom onset. E and RdRp amplification results plotted against days of symptom onset in 145 samples from 128 patients. Lowest CT values were seen around day 3 of symptoms, with mean RdRp CT higher at a given day compared to E gene CT value. The lines represent the smoothed conditional mean with 95% confidence intervals in the grey bars. E, envelope gene; RdRp, RNA-dependent RNA-polymerase.

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Footnotes

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2020.05.061.

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Paper 2

Colton H, Hodgson D, Hornsby H, Brown R, McKenzie J, Bradley KL, James C, Lindsey BB, Birch S, Marsh L, Wood S, Bayley M, Dickson G, James DC, Nicklin MJ, Sayers JR, Zafred D, Rowland-Jones SL, Kudesia G, Kucharski A; CMMID COVID-19 Working Group; Darton TC, de Silva TI, Collini PJ. **Risk factors for SARS-CoV-2 seroprevalence following the first pandemic wave in UK healthcare workers in a large NHS Foundation Trust.** Wellcome Open Res. 2022 Jun 10;6:220. doi: 10.12688/wellcomeopenres.17143.3. PMID: 35600250; PMCID: PMC9091808.

Abstract

Background: We aimed to measure SARS-CoV-2 seroprevalence in a cohort of healthcare workers (HCWs) during the first UK wave of the COVID-19 pandemic, explore risk factors associated with infection, and investigate the impact of antibody titres on assay sensitivity.

Methods: HCWs at Sheffield Teaching Hospitals NHS Foundation Trust were prospectively enrolled and sampled at two time points. We developed an in-house ELISA for testing participant serum for SARS-CoV-2 IgG and IgA reactivity against Spike and Nucleoprotein. Data were analysed using three statistical models: a seroprevalence model, an antibody kinetics model, and a heterogeneous sensitivity model.

Results: Our in-house assay had a sensitivity of 99.47% and specificity of 99.56%. We found that 24.4% (n=311/1275) of HCWs were seropositive as of 12th June 2020. Of these, 39.2% (n=122/311) were asymptomatic. The highest adjusted seroprevalence was measured in HCWs on the Acute Medical Unit (41.1%, 95% CrI 30.0–52.9) and in Physiotherapists and Occupational Therapists (39.2%, 95% CrI 24.4–56.5). Older age groups showed overall higher median antibody titres. Further modelling suggests that, for a serological assay with an overall sensitivity of 80%, antibody titres may be markedly affected by differences in age, with sensitivity estimates of 89% in those over 60 years but 61% in those ≤ 30 years.

Conclusions: HCWs in acute medical units and those working closely with COVID-19 patients were at highest risk of infection, though whether these are infections acquired from patients or other staff is unknown. Current serological

assays may underestimate seroprevalence in younger age groups if validated using sera from older and/or more severe COVID-19 cases.

Keywords: Seroprevalence; antibody; Healthcare Worker; SARS-CoV-2; COVID; modelling; age; risk

Introduction

Healthcare workers (HCWs) are at increased risk of COVID-19^{1,2}. The true number of HCWs infected with SARS-CoV-2 to-date is unknown, particularly during the early stages of the pandemic. Initial methods to estimate HCW COVID-19 cases included extrapolation from work absenteeism rates, and are unlikely to be reliable³. Confirmation by molecular testing increased the accuracy of case detection, although access to nucleic acid amplification testing (NAAT) was limited during the early stages of the pandemic in the UK⁴. Serological testing can be performed at large scale, and is less affected by symptom-activated testing pathways, so may provide a more accurate estimate of previously infected HCWs and could be used in conjunction with other data to determine their risk factors for exposure⁵⁻⁸.

To enable the accurate interpretation of seroprevalence readouts, detailed characterisation of antibody evolution relative to the sampling time-frame, immunoglobulin isotype, antigenic target and assay performance is required⁹⁻¹². Several commercial SARS-CoV-2 antibody assays have been validated using samples from patients with more severe COVID-19, and some studies have suggested those with milder or asymptomatic COVID-19 are less likely to develop detectable antibodies¹³⁻¹⁷. Furthermore, antibody levels to some coronaviruses are known to be higher in older individuals¹⁸⁻²². We theorised this may lead to age-specific differences in antibody assay sensitivity, which could be a significant confounder in population seroprevalence studies.

In this study we aimed to investigate SARS-CoV-2 seroprevalence in HCWs at Sheffield Teaching Hospitals (STH), a National Health Service (NHS) Foundation Trust in the United Kingdom (UK), following the first wave of the pandemic in the UK. To achieve this, we sought to measure SARS-CoV-2 antibody titres by

developing and using an in-house assay, prior to using statistical modelling to explore risk factors associated with seropositivity, the evolving antibody response, and the impact of age on assay sensitivity.

Methods

Background and setting

STH is an NHS trust offering secondary- and tertiary-level care across four sites in South Yorkshire, UK, with 1,669 inpatient beds and 18,500 employees²³. Patients with a medical reason for admission are typically admitted to STH either through attending the Emergency Department (ED), or through referral to the Acute Medical Unit (AMU) by their General Practitioner (GP). On AMU, patients are given an initial management plan before being triaged to the most appropriate medical specialty ward e.g. respiratory medicine. The first patient with confirmed COVID-19 was admitted to STH on 23 February 2020; the first wave of the UK pandemic occurred between March 2020 and June 2020. Patients with suspected or confirmed COVID-19 were referred directly to the infectious diseases ward by GPs and other STH admission areas as capacity allowed. When capacity was reached, suspected COVID-19 patients would be either placed in side rooms or cohort bays on AMU or other wards, whilst confirmed COVID-19 patients could be moved to cohort wards.

Testing of symptomatic staff for SARS-CoV-2 by NAAT was introduced on 17 March 2020. On the same day, Public Health England (PHE) de-escalated the recommendations for the personal protective equipment (PPE) required by HCWs caring for inpatients with suspected or confirmed COVID-19 from 'Level 3 Airborne' to 'Level 2 Droplet' for routine care²⁴. Subsequently, the requirement for universal 'Level 2 Droplet' PPE for all inpatient and outpatient care began on 08 April 2020. Local STH policy was changed on 15 June 2020 to mandate staff use surgical face masks while on hospital premises.

Recruitment and consent

From 13–18 May 2020, all contactable STH staff (n=17,757) were invited to take part in the COVID-19 Humoral ImmunE RespOnses in front-line HCWs (HERO) study by email and intranet alert. To engage staff in areas with limited communications

access, additional recruitment posters and face-to-face enrolment sessions were used.

Following an electronic informed consent process, participants provided self-reported data on-line on age, gender, ethnicity, job role, and pandemic working environment ('COVID-19 zones')²⁴. Details of any possible or confirmed prior COVID-19 illnesses occurring since 01 February 2020 was also collected. These were categorised as: i), diagnosed with COVID-19 and confirmed by NAAT, ii), clinically diagnosed with COVID-19 but NAAT not performed, and iii), self-reported symptoms only²⁴. Together, we defined these three groups as "symptomatic", as asymptomatic testing was only introduced after the study recruitment period. Those reporting no illness between 01 February 2020 to the date of recruitment were defined as "asymptomatic". All that had enrolled were emailed times of phlebotomy appointments, and were invited to attend on a first come first served basis for the first visit, and then invited by email to book a specific appointment slot to attend for their second visit after four weeks +/- 7 days. An 8.5ml serum sample was taken at each visit to outpatient phlebotomy services for serological testing.

Serology assay development

To develop the in house ELISA, we created an assay validation dataset²⁵ consisting of serum from 190 SARS-CoV-2 NAAT-confirmed cases (52 hospitalised patients and 138 healthcare workers with mild infections sampled between 14 and 120 days from NAAT positivity), and 675 patients sampled prior to 2017 (Extended data: Table S1). Thresholds based on the absorbance value at 450nm (A 450) for defining reactivity to spike (A 450 0.1750) or NCP (A 450 0.1905) were set to optimise the sensitivity of each assay. Given the IDSA guidance for ensuring a specificity of $\geq 99.5\%$ in assays used for SARS-CoV-2 seroprevalence studies, specificity was enhanced by defining a SARS-CoV-2 seropositive sample as one where both spike and NCP were reactive²⁶.

SARS-CoV-2 serology

Serum samples from study participants were then tested for IgG and IgA reactivity to two SARS-CoV-2 proteins using our in-house ELISA: the full-length extracellular domain (amino acids 14-1213) of Spike glycoprotein, including a

replacement of the furin cleavage site R684-R689 by a single alanine residue and replacement of K986-V987 by PP, produced in mammalian cells; and full-length untagged Nucleocapsid protein (NCP) produced in *E. coli* (Uniprot ID P0DTC9 (NCAP_SARS2))²⁷⁻²⁹. High binding microtitre plates (Immulon 4HBX; Thermo Scientific, 6405) were coated overnight with proteins diluted in phosphate buffered saline, washed with 0.05% PBS-Tween, and blocked for one hour with 200 µL/well casein buffer. Following optimisation, sample dilutions used were 1:200 for the IgG assay or 1:100 for the IgA assay²⁴. Plates were emptied and 100 µL/well of sample or control loaded. After two hours incubation, plates were washed and loaded with goat anti-human IgG-HRP conjugate (Invitrogen, 62-8420) at 1:500, or goat anti-human IgA-HRP conjugate (Invitrogen, 11594230) at 1:1000, for one hour. Plates were washed and developed for 10 minutes with 100 µL/well TMB substrate (KPL, 5120-0074). Development was stopped with 100 µL/well HCl Stop solution (KPL, 5150-0021), and absorbance read at 450nm. All steps were performed at room temperature.

A calibration curve of sera pooled from convalescent SARS-CoV-2 NAAT-confirmed patients with high antibody titres for both spike and NCP was included on plates to allow quantification of antibody concentrations. The calibration curve was generated by serially diluting in 1.75× steps from a starting concentration of 1:200 for the IgG assay or 1:100 for the serum IgA assay. When the WHO International Standard for anti-SARS-CoV-2 immunoglobulin (NIBSC, 20/136) later became available, the calibration curve was run in parallel for the IgG assay²⁴. Data for the IgG assay are therefore given in WHO antibody units, whereas IgA assay data are given in arbitrary antibody units.

Sample size

To meet the primary objective of measuring the SARS-CoV-2 IgG seroprevalence, we calculated a sample size of 1,000 HCWs would provide +/-1.4% precision based on a seroprevalence estimate that ~4% of the UK population may have been infected by April 2020, with a two-sided 95% confidence interval (with n=753, Binomial exact 95%CI has been estimated to be 2.7-5.6%)³⁰.

Statistical modelling

We considered three statistical models, i) a seroprevalence model, ii) an antibody kinetics model, and iii) a heterogeneous sensitivity model. For the seroprevalence model, we used the serostatus of all participants at first blood draw in a sensitivity- and specificity-adjusted Bayesian multilevel logistic regression model. Using seropositivity as the binary response variable, we considered three different Bayesian Hierarchical Regression model subtypes all with explanatory demographic variables age, race and gender, and each model with a different primary exposure; job location, contact with COVID-19 patients, and job type²⁴. In addition, we fitted a symptomatic prevalence model, where the data used were seropositive persons only, and the binary response variable was asymptomatic or symptomatic infection.

For the antibody kinetics model, we included samples from individuals who were seropositive at both bleeds, in a Bayesian multilevel linear regression model in two parts: i) using log2 antibody units (logAU) at the first blood draw as the response variable and ii) using the change in antibody titre at the follow up bleed (median 28 days) as the response variable. Age, ethnicity, gender and symptom severity (asymptomatic or symptomatic) were used as covariates and each model was run separately for four different antibody-antigen combinations; Spike-IgG, NCP-IgG, Spike-IgA, NCP-IgA. The time until seroreversion was calculated for each covariate group and antibody-antigen interactions by i) sampling a starting titre value and a rate of decline from the two models, and then ii) calculating the time until the minimum observed antibody value was reached for that antibody-antigen interaction, assuming a continuous rate of decrease.

In our heterogeneous sensitivity and specificity model, we explored how estimates for the sensitivity and specificity derived from our assay validation dataset generalise to covariate groups, e.g. participant age. To model the generalisability of these performance measures, we compared the seropositivity classification of our study dataset according to our in-house antibody assay, with the predicted seropositivity classification from hypothetical assays with an assumed sensitivity and specificity. Our model considers the different distribution of the A_{450} values in the assay validation and HERO study datasets to model how reliably the sensitivity and specificity given by the manufacturers of the assays generalise to specific subpopulations. Using the assay validation dataset, we

estimated an A_{450} cut-off value for every chosen sensitivity value, and then used this A_{450} cut-off to classify seropositivity in the study dataset. We then estimated the implied sensitivity on the HERO dataset by comparing seropositivity classification based on the estimated A_{450} cut-off value, with the seropositivity classification from our in-house assay (which for ease of comparison, we assume represents the maximum possible sensitivity and specificity (i.e. 100%) in this model), we estimate an “implied” sensitivity on the HERO dataset which would arise if the commercial assay alone had been used to detect seropositivity. This framework allowed us to estimate the hypothetical performance of serological assays reported in the literature on our HERO dataset, along with co-variate specific sensitivity.

All analysis was performed in R version 4.0.2 ³¹ and cmdstanr version 0.2.0 ³². An R package containing all the analysis in this study is available at <https://doi.org/10.5281/zenodo.6320552>.

Regulatory review

Following internal scientific review, local R&D (5 May 2020 ref: STH21394) and HRA and Health and Care Research Wales (HCRW) approval were given (29 April 2020 ref: 20/HRA/2180, IRAS ID: 283461). Anonymised serum samples from hospitalised COVID-19 patients and serum collected prior to 2017 during routine clinical care were used for assay validation with approval from STH R&D office.

Results

Serology assay development

We found our in-house ELISA had a sensitivity of 99.47% (95% confidence interval (CI) 97.10% - 99.99%) and specificity of 99.56% (95% CI 98.71% - 99.91%) for our IgG assay (*Extended data*: Figure S1a). Compared with IgG, we saw more rapid waning of the IgA response following SARS-CoV-2 infection, as well as higher levels of cross-reactivity in pre-pandemic samples. These factors complicated defining seropositivity based on an A_{450} threshold, as there was no clear separation between titres in these two groups. We therefore opted to use our spike and NCP IgA ELISA solely to compare IgA titres of individuals classified as

seropositive by our IgG assay (*Extended data*: Figure S2). Antibody units at each given dilution of the calibration curve are shown in Table S2 (*Extended data*).

Registration and study visits

1478 STH staff consented to take part between 13 May and 5 June 2020 (*Extended data*: Figure S3). Of these, 1277 attended for a first visit (V1) between 15 May 2020 and 12 June 2020. As two samples were contaminated in transit, we obtained a valid serostatus for 1275 samples. 1174 attended a second visit (V2) between 15 June and 10 July 2020, however eight samples were excluded from the V2 analysis as they were either unlabelled (n=2), lost (n=1) or the participant had participated in a COVID-19 vaccine trial (and seroconverted) (n=5) (*Extended data*: Figures S3 and S4).

Demographics, job role, work locations, and environment

The majority of participants were female (n=1008/1275, 79.1%) and most described their ethnicity as white (n=1130/1275, 88.6%, Table 1). Nurses (433/1275, 33.9%), doctors (232/1275, 18.2%), health care assistants (163/1275, 12.8%), and domestic services staff (136/1275, 10.7%) constituted the largest proportion of job roles. Almost half (593/1275, 46.5%) of HCWs worked in parts of the hospital managing acute COVID-19 admissions including ED, AMU, Critical Care, and inpatient medical wards (respiratory, geriatric care, infectious diseases). Participants reported working in areas with the highest level of COVID-19 patient contact (red zones), either most days (n=423/1275, 33.2%) or occasionally (n=305/1275, 23.9%).

Unadjusted seroprevalence

Analysis of V1 samples revealed that 24.4% (n=311/1275) of HCWs were seropositive by 12 June 2020 (Table 1). Of these, 39.2% (n=122/311) did not report a prior illness consistent with COVID-19. The second blood draw occurred a median of 28 days following the first visit (IQR 27-31) and 1166 had a valid V2 serology result (*Extended data*: Figure S3). Comparison of serology data from both visits demonstrated that 16 out of 964 participants had seroconverted and 9 out of 311 participants seroreverted (i.e. loss of reactivity against either spike ($A_{450} < 0.175$) or NCP ($A_{450} < 0.1905$)).

Adjusted seroprevalence estimates

The overall adjusted seroprevalence in the cohort was 23.1% (95% CrI 14.1–33.3), but varied across job type, job location and COVID-19 zone (Figure 1; *Extended data*: Tables S3 to S7). A relatively high seroprevalence was seen in occupational and physiotherapists (39.2%, 95% CrI 24.4–56.5), and low seroprevalence in allied medical staff (9.2%, 95% CrI 1.4–21.3). Between wards, there was higher seroprevalence in the AMU (41.1%, 95% CrI 30.0–52.9) compared to other wards. Across COVID-19 zones, working in the areas with the highest degree of COVID-19 patient contact (zone 6) was associated with a slightly higher seroprevalence of 28.6% (95% CrI 24.0–33.5) compared to the other five groups (Figure 1). The adjusted proportion of asymptomatic cases was 38.9% (95% CrI 23.6–57.3) (*Extended data*: Figure S5). The proportion of asymptomatic cases remained relatively consistent across all covariates except for job type, where it ranged from 21.4% for occupational and physiotherapists up to 61.5% for domestic services staff (*Extended data*: Figure S5).

Antibody kinetics model

Differences in antibody concentration between samples were calculated for four different antibody-antigen interactions (spike-IgG, NCP-IgG, spike-IgA, NCP-IgA). Though there was a positive correlation between Spike-IgG and NCP-IgG across all samples ($R^2 = 0.53$), the correlations between serum IgG and IgA were much weaker (R^2 between 0.17 and 0.3) (*Extended data*: Figure S6).

For both serum IgG and IgA, older age groups showed higher antibody titres (Figure 2); E.g., the median log2 titre of spike-IgG in those ≤ 30 years was 6.6 (95% CrI 6.2–7.0), and at 60+ years was 7.1 (95% CrI 6.6–7.8), while spike-IgA titre in those ≤ 30 years was 6.8 (95% CrI 6.3–7.3), and at 60+ years was 7.6 (95% CrI 7.0–8.3). Symptomatic cases showed similar titres compared to asymptomatic cases across IgG and IgA-serum measures. The reduction in antibody titre at the second blood draw was less in the spike-IgG (mean -0.15 (95% CrI -0.25– -0.06)) compared to NCP-IgG (mean -0.49 (95% CrI -0.58– -0.540)) and Spike-IgA/NCP-IgA. These estimated rates of decline remained consistent across all covariate groups studied. Consequently, the estimated time until seroreversion for seropositive

samples from symptomatic participants was around 100 weeks for the spike-IgG, and 52 weeks for NCP-IgG and IgA serum measures (Figure 2). When considering seropositive samples from symptomatic participants, there was little difference in the decrease in antibody levels as time post-symptom onset increased (*Extended data*: Figure S7).

Heterogeneous sensitivity model

The heterogeneous sensitivity model demonstrates that using varying A_{450} cut-offs (corresponding to varying sensitivity values) to categorise seropositivity in the HERO dataset will result in a lower sensitivity than that defined using our assay validation dataset (Figure 3a). The model also shows that there is no difference in implied sensitivity between using spike or NCP as the antigenic target in the ELISA assay.

The relationship between the A_{450} cut-off value and the sensitivity and specificity for the assay validation datasets for each antigen were plotted with the associated ROC curves (*Extended data*: Figures S8 and S9). We hypothesised that the higher A_{450} values seen in older adults suggest that some commercially available serological assays may have a higher sensitivity in detecting COVID-19 antibodies in older age groups compared with younger age groups. We therefore used our model to estimate age-specific implied sensitivity for assays of different sensitivity profiles in estimating seroprevalence in our HERO dataset. We found that the sensitivity of a serological assay decreases with age due to the higher antibody titres seen in older people, with a clearer trend in an NCP-based assay compared to a spike-based assay (Figure 3b). Assuming a theoretical assay validation set sample sensitivity of 80% for the NCP protein, the resulting median implied sensitivity for age groups <30, 30–39, 40–49, 50–59, and 60+ years was 61%, 77%, 70%, 85%, and 89% respectively.

Discussion

We found a high SARS-CoV-2 seroprevalence in HCWs at a large UK hospital trust compared to national seroprevalence estimates, following the first pandemic wave in the UK ³³. In addition, we identified important risk factors associated with

occupational exposure to COVID-19, and described a significant association between age and the likelihood of a positive serological result which has important implications for the validation of SARS-CoV-2 antibody assays and the hitherto interpretation of population-level COVID-19 serology data.

Over 20% of HCWs at STH had evidence of SARS-CoV-2 infection within just over 100 days of the first confirmed COVID-19 patient being admitted to our NHS trust. This high proportion over a short space of time is likely representative of the much higher exposure to SARS-CoV-2 infection among certain subpopulations of the workforce that we tested. Although data from other settings and countries suggested infection risk in HCWs is similar to community exposure, this seroprevalence is much higher than estimated seropositivity in the UK population at a similar time (6.0%, 95 CrI 5.8–6.1 in July 2020) ^{33, 34}.

Despite universal PPE and IPC guidelines across STH, our data show that HCWs working in AMUs are at significantly higher risk of infection with SARS-CoV-2, with seropositivity rates above that of other wards, consistent with other UK studies ^{5, 6, 35}. EDs face a similar patient turnover yet have lower HCW seroprevalence rates in both ours and previous reports ^{5, 6, 35, 36}. One factor contributing to the difference seen between ED and AMU, may be that our ED implemented a suspected / confirmed COVID-19 cohort area on 19th March 2020, where level 2 airborne PPE was mandated. In addition, on AMU there were likely longer stays of asymptomatic/ paucisymptomatic cases prior to universal PPE use, more fomites (e.g. bedside tables, chairs), and more frequent interspeciality interactions resulting in transmission between HCWs ³⁷. In the event of a further wave or outbreak, infection prevention and control (IPC) interventions to reduce risk in these areas could include targeted IPC training and auditing (particularly of PPE use and break areas), serial staff testing, pop-up isolation units in bay areas and optimising staff-to-patient ratios. At the other end of the spectrum, we and others have found that HCWs in critical care units have some of the lowest seropositivity rates, which likely reflects 'Level 3 Airborne' PPE use in Critical Care units from an early point in the pandemic and the increased availability of negative pressure rooms ^{5, 6, 8, 35, 36}.

Occupational and physiotherapists (OT/PT) had the highest rates of seroprevalence across all of the job roles included in our cohort (45.5%), which is consistent with some other UK studies ^{6,38}. OT/PT work involves prolonged close contact with patients in addition to PT performing chest physiotherapy and open suctioning of the respiratory tract. In addition, OT/PT work across multiple inpatient and outpatient areas in our Trust, which could increase risk of transmission from both patients and other HCWs.

Increasing age was associated with seropositivity, with over a third of our HCWs aged >60 testing seropositive, and with higher antibody titres. We demonstrate that the sensitivity of a serological assay increases with increasing age due to the higher antibody titres seen in older people, and with a clearer trend in NCP- compared to spike-based assays. Our data complements the existing literature, which shows antibody titres against SARS-CoV-2 and other coronaviruses are higher in older individuals, which could be due to a higher risk of exposure to the virus, greater antigenic load or boosting of antibodies from cumulative seasonal coronavirus infections throughout their lifetime ^{18–22}. Several of the commercial SARS-CoV-2 antibody assays available (e.g. Roche Elecsys, Abbott SARS-CoV-2 IgG and Wantai ELISA) were validated with patient sera collected from those with more severe disease early on in the pandemic (i.e. those who presented to health services) ^{13–15}. Patients with severe COVID-19 have been shown to have higher antibody titres than those with milder disease (Extended data : Figure S1b), and it would be reasonable to assume these cases were likely to also be older in age ^{13–17, 39, 40}. Our antibody kinetic modeling data suggest that using such samples from severe COVID-19 cases for the purposes of assay calibration may result in an assay with lower or insufficient sensitivity when applied to less severe or younger (often community) populations. We also found that NCP-IgG is likely to wane more quickly than Spike-IgG. Depending on the sampling time frame relative to pandemic wave, serological testing based on NCP-IgG alone may further underestimate seroprevalence. With increasing vaccine coverage, use of spike IgG to determine seroprevalence also becomes more problematic when distinguishing whether an individual is seropositive from vaccination or previous infection. Assays which combine antibody responses to membrane protein with NCP antibodies may overcome these challenges ^{41, 42}.

We note the limitations of our study, which include a potential for selection bias due to participants self-enrolling for convenience, rather than using systematic sampling. While we cannot measure the extent of this effect on the measured seroprevalence, we think any volunteer bias would have been equal across all categories compared, and so not altering the validity of these comparisons. In addition, we recognise that our cohort has relatively low numbers of HCWs from minority ethnic backgrounds (~10%), compared to the Sheffield general population (19%)⁴³.

With the ongoing global devastation caused by the COVID-19 pandemic and its lasting effect on healthcare services, understanding the risk factors leading to HCW exposure is paramount to ensuring the continuity of effective and safe patient care. Our real-world data suggest that NHS HCWs face high levels of exposure to SARS-CoV-2, plus highlights locations and job roles at greatest risk during the first wave of the pandemic. Population seroprevalence data can help guide decision makers on risk management. Using assays that have been validated using serum samples from a broad population, combined with antibody kinetic modelling and/or with age-adjusted cut-offs could overcome the potential limitations we have highlighted.

	Recruited with an initial valid serostatus	Seropositive at V1 (%)	Asymptomatic (% of seropositive at V1)	Completed both V1 and V2 (% of recruited)	Seroincident cases (% of seronegative at V1)
Total	1275	311 (24.4)	122 (39.2)	1166 (87.5)	16 (1.7%)
Job location					
Emergency Department (ED)	103	26 (25.2)	13 (50.0)	90 (87.4)	1 (1.2)
Acute Medical Unit (AMU)	83	38 (45.8)	17 (44.4)	66 (79.5)	0 (0.0)
Critical Care	100	18 (18.0)	7 (38.9)	95 (95.0)	0 (0.0)
Geriatric Care	23	3 (13.0)	1 (33.3)	22 (95.7)	1 (5.0)
Infectious Disease Ward	139	26 (18.7)	11 (42.3)	121 (87.1)	7 (6.2)
Other	664	157 (23.6)	56 (35.7)	621 (93.5)	2 (0.3)
Respiratory Geriatric Ward	92	27 (29.3)	10 (37.0)	85 (92.4)	2 (3.1)
Respiratory Ward	58	13 (22.4)	5 (38.5)	54 (93.1)	2 (4.4)
Job role					
Admin	127	26 (20.5)	12 (46.2)	118 (92.9)	1 (0.9)
Allied medical ¹	38	0 (0.0)	—	37 (97.4)	0 (0.0)
Domestic services	136	39 (28.7)	24 (61.5)	127 (93.4)	4 (4.1)
Healthcare assistants	163	39 (23.9)	21 (53.8)	140 (85.9)	3 (2.4)
Doctors	232	52 (22.4)	18 (34.6)	211 (90.9)	0 (0.0)
Nurses	433	116 (26.7)	34 (29.3)	391 (90.3)	7 (2.2)
Other	31	5 (16.1)	2 (40.0)	29 (93.5)	0 (0.0)
Pharmacists	35	8 (22.8)	5 (62.5)	33 (94.3)	1 (3.7)
Occupational and physiotherapists	33	15 (45.5)	3 (20.0)	33 (100.0)	0 (0.0)
Radiographers	42	9 (21.4)	2 (22.2)	42 (100.0)	0 (0.0)
COVID-19 zone²					
1 (lowest COVID-19 contact)	104	22 (21.2)	10 (54.5)	96 (92.3)	1 (1.2)
2	248	50 (20.2)	27 (46.0)	232 (93.5)	0 (0.0)
3	41	7 (17.1)	6 (14.3)	39 (95.1)	1 (2.9)
4	153	35 (22.9)	24 (31.4)	142 (92.8)	1 (0.8)

5	305	69 (22.6)	46 (33.3)	280 (91.8)	3 (1.3)
6 (highest COVID-19 contact)	423	128 (30.3)	76 (40.6)	376 (88.9)	10 (3.4)
Age (years)					
<30	267	67 (25.1)	32 (47.8)	236 (88.4)	6 (3.0)
30–39	306	69 (22.5)	22 (31.8)	279 (91.2)	5 (2.1)
40–49	314	72 (22.9)	29 (40.2)	293 (93.3)	2 (0.8)
50–59	314	76 (24.2)	28 (36.8)	288 (91.7)	1 (0.4)
60+	74	27 (36.5)	11 (40.7)	70 (94.6)	2 (4.3)
Ethnicity					
White	1130	281 (24.9)	108 (38.4)	1035 (91.6)	15 (1.8)
Black/Black British	33	6 (18.2)	3 (50.0)	30 (90.9)	0 (0.0)
Asian/Asian British	76	17 (22.4)	7 (41.2)	70 (92.1)	1 (1.7)
Other	33	7 (21.2)	4 (57.1)	30 (90.9)	0 (0.0)
Gender³					
Female	1008	253 (24.1)	105 (41.5)	922 (91.5)	14 (1.9)
Male	265	58 (21.9)	17 (29.3)	242 (91.3)	2 (0.9)

Table 1. Characteristics and serostatus of recruited participants who had a valid baseline result.

1. Allied Medical includes Speech and Language Therapists, Cardiac Physiologists, Dental Hygienists, Dietitians, ECG technicians, Orthotists, Podiatrists, Rehabilitation assistants
2. COVID-19 Zones are defined in extended data²⁴.
3. Participants were able to define their gender as non-binary, transgender or could choose not to disclose

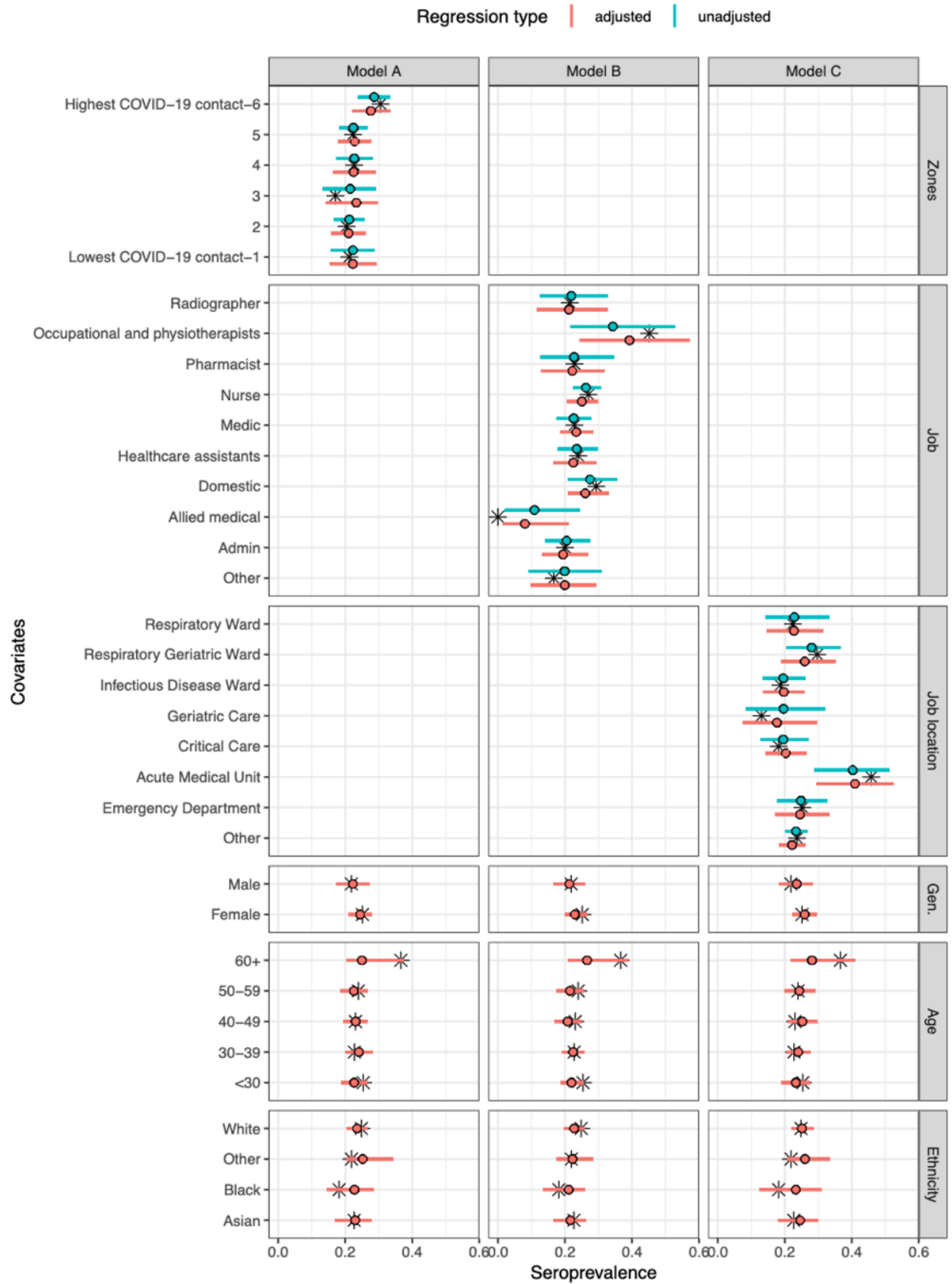


Figure 1. Model-predicted seroprevalence estimates for three different models (A–C), adjusted and unadjusted with covariates.

Black stars represent point values from the data. The point and whiskers represent the mean value and 95% CrI of the posterior distribution. The three models differed by their primary exposure, Model A used COVID-19 zones (1 refers to lowest COVID-19 contact and 6 refers to highest COVID-19 contact²⁴), Model B the job role, and Model C the job location. Each model was evaluated either unadjusted (primary exposure only) or adjusted (primary exposure with age, gender, and ethnicity).

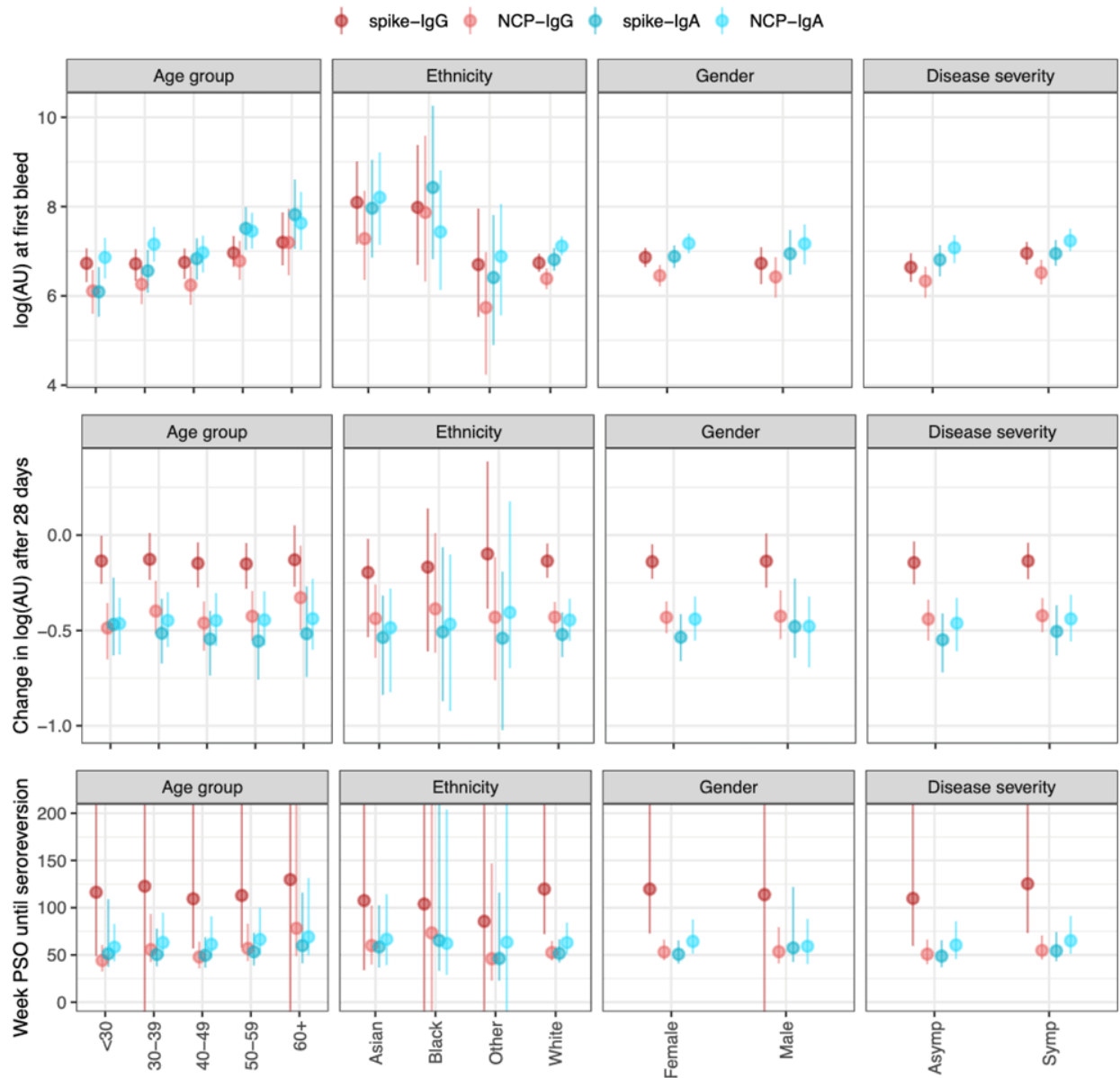


Figure 2. Outputs from the antibody kinetics model for four antibody-antigen interactions (spike-IgG, NCP-IgG, spike-IgA, and NCP-IgA).

The IgG measures are in the WHO standard universal log₂ antibody units, whereas the IgA measures are in log₂(AU) units scaled relative to the values in the study. The dots show the median and the line segments show the 95% credible interval of the posterior distributions. Top panel shows the log₂(AU) at the first bleed across four different covariates (Age group, ethnicity, gender, and disease severity). Middle panels show the change in log₂(AU) after 30 days. The bottom panels show the time until seroreversion in weeks. Asymp (asymptomatic participants), Symp (symptomatic participants) PSO (post symptom onset).

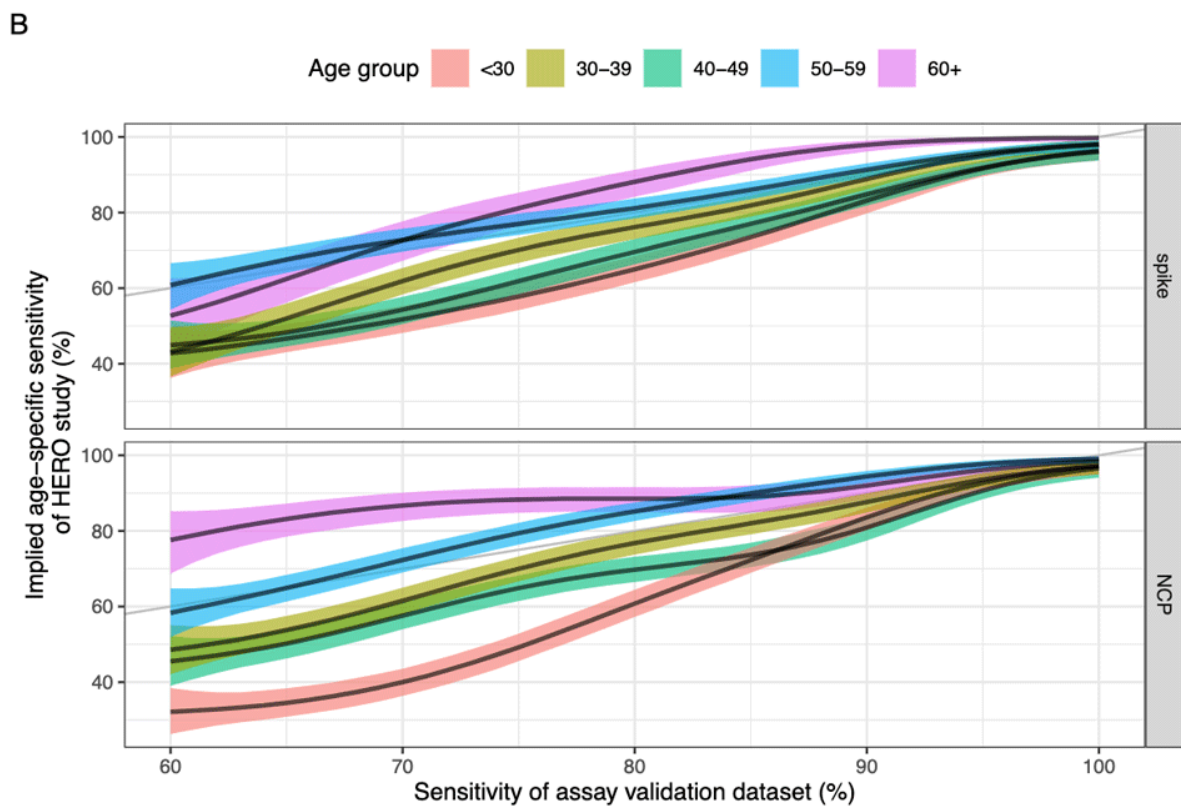
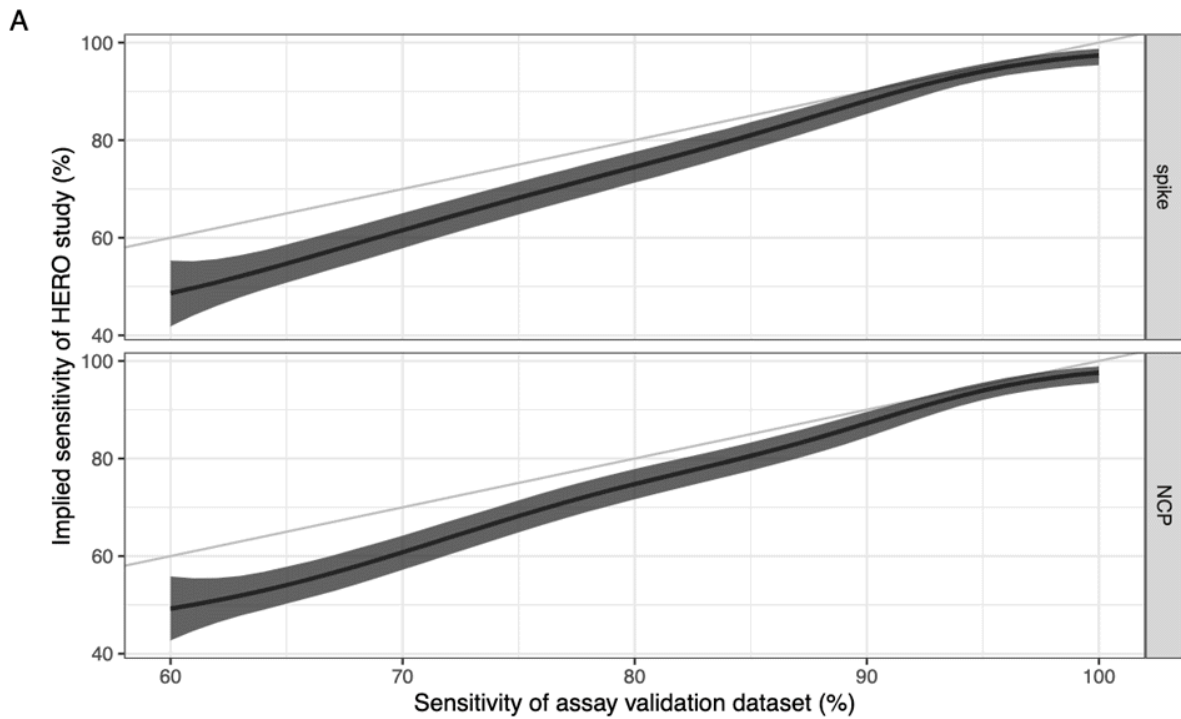


Figure 3.

(a) Sensitivity of the assay validation dataset against the implied sensitivity of the HERO dataset for spike and nucleoprotein. (b) Sensitivity of the assay validation dataset against the implied age-specific sensitivity in the HERO dataset for spike and nucleoprotein. Black line and ribbon shows median and 95% CrI for the posterior distributions respectively.

Underlying data

Zenodo: dchodge/hero-study: Version submitted with WOR reviewer comments (v1.1.0) <http://www.doi.org/10.5281/zenodo.6320552> ²⁵.

The project contains the following underlying data:

- datafit.RData
- fit_mcmc_asymp.RData
- fit_mcmc_change.RData
- fit_mcmc_prev.RData
- fit_mcmc_sens.RData
- fit_mcmc_spec.RData
- fit_mcmc_start.RData
- fit_mcmc_time.RData
- sensspec.RData
- sero_clean.RData

Extended data

Zenodo: dchodge/hero-study: Version submitted with WOR reviewer comments (v1.1.0) <http://www.doi.org/10.5281/zenodo.6320552> ²⁵.

The project contains the following extended data:

- Table S1. Details of the samples used to set thresholds during assay validation
- Table S2. Comparison of antibody units in assay calibration curve sera assigned to the assay with WHO international standard antibody units
- Table S3. Summary of the response variables and the covariates used in the regression model.
- Table S4. Summary of the model parameters used in the regression model.
- Table S5. Summary of the response variables and the covariates used in the regression model.
- Table S6. Summary of the model parameters used in the regression model.
- Table S7. Summary of the model parameters used in the Heterogenous sensitivity model.

- Figure S1a. ROC curves of the spike and NCP assays
- Figure S1b. Spike- and NCP-specific IgG response in inpatients vs outpatients
- Figure S2. Comparison of IgA assay A450 based on IgG Serostatus
- Figure S3. Study flow diagram.
- Figure S4. Histogram (overlaid) showing the symptom onset, date of first bleed (all cases and symptomatic cases only), and time at second bleed (all cases and symptomatic cases only).
- Figure S5. Model-predicted proportion of asymptomatic estimates for three different models (A-C), adjusted and unadjusted with covariates gender, age group and ethnicity.
- Figure S6. Correlation between the four different antibody measures for 264 serological samples.
- Figure S7. Rate of decline for the antibody concentrations post-symptom onset for the four antibody measures. The fitted line is from a linear regression, with the 95% CI shown in red.
- Figure S8. Relationship between sensitivity/specificity and the cutoff value for the control dataset.
- Figure S9. ROC curves with the A_{450} cut-off value indicated in red for the control dataset. x-axis shows the False Positive Rate, y-axis is the sensitivity.
- Figure S10. ROC curves for different age groups and antigen proteins, with the A_{450} cut-off value indicated in various colours for the control dataset.
- Figure S11. (a) Specificity of the control data set against the implied specificity of the HERO dataset for spike and nucleoprotein. (b) Specificity of the control data set against the implied age-specific specificity of the HERO dataset for spike and nucleoprotein.
- HERO Completed STROBE Checklist[60].doc

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0). A previous version of the Extended data is available: Zenodo: dchodge/hero-study: Version submitted for WOR (v1.0.0) <http://www.doi.org/10.5281/zenodo.5215671>. The underlying data remains the same under both DOIs.

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Paper 3

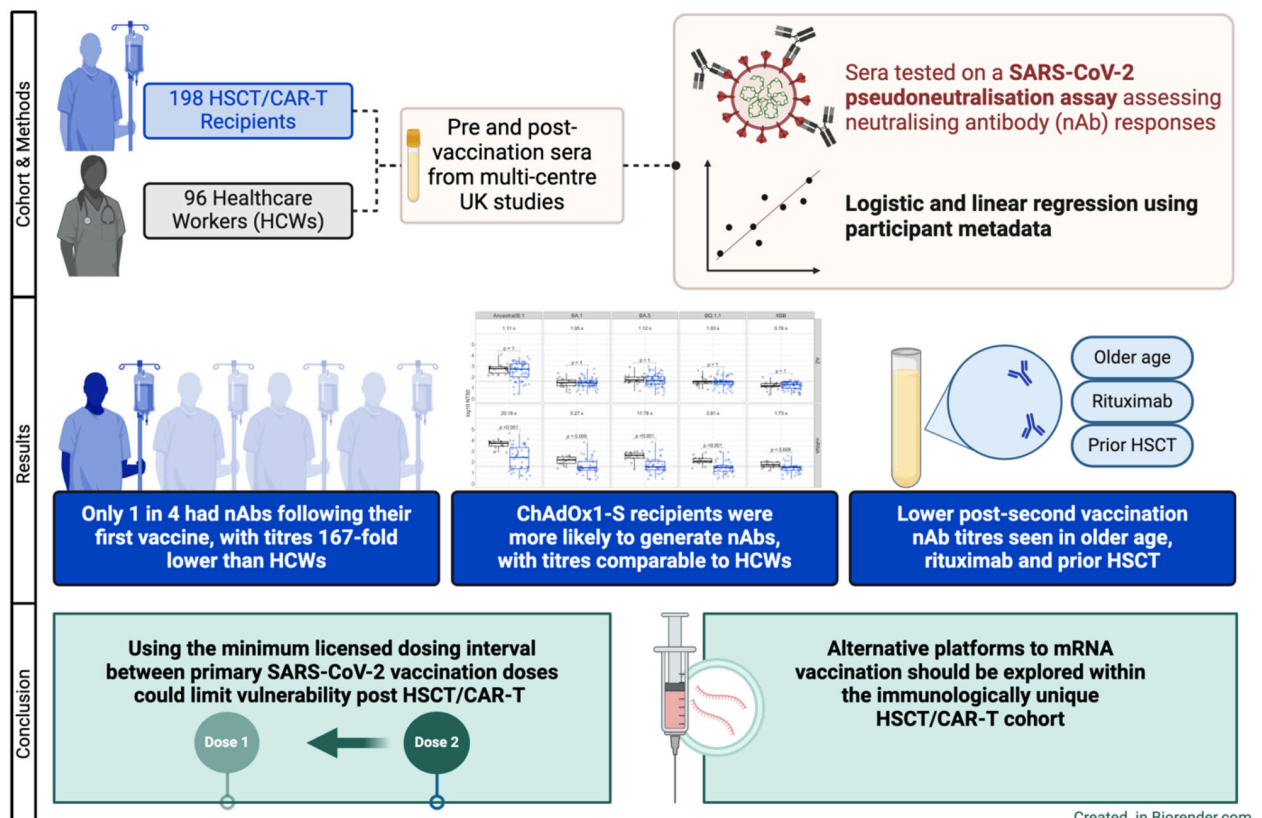
Colton H, Barratt N, Temperton N, Hornsby H, Angyal A, Grouneva I, Lindsey BB, Kearns P, Barnes E, Goodyear CS, Richter A, Thomas D, Cook G, McInnes IB, Willicombe M, Siebert S, Orchard K, Selby R, Bowden S, Collini PJ, Pope A, Kirkham A, Kronsteiner B, Dunachie SJ, Miller P, Clay J, Hurst E, Malladi R, Kesavan M, Kinsella F, Sanderson R, Yong KL, Rea D, Parry H; PITCH Consortium, OCTAVE Collaborative Group, OCTAVE-DUO Investigators, PROSECO Investigators; Lim SH, Snowden JA, de Silva TI. **Greater preservation of SARS-CoV-2 neutralising antibody responses following the ChAdOx1-S (AZD1222) vaccine compared with mRNA vaccines in haematopoietic cell transplant recipients.** Br J Haematol. 2024 Dec;205(6):2206-2218. doi: 10.1111/bjh.19874. Epub 2024 Nov 17. PMID: 39551718; PMCID: PMC11637739.

Summary

Whilst SARS-CoV-2 mRNA vaccines generate high neutralising antibodies (nAb) in most individuals, haematopoietic stem cell transplant (HSCT) and chimeric antigen receptor T-cell (CAR-T) recipients respond poorly. HSCT/CAR-T treatment ablates existing immune memory, with recipients requiring revaccination analogous to being vaccine naive. An optimal revaccination strategy for this cohort has not been defined. Factors predicting immunogenicity following three ancestral SARS-CoV-2 vaccines were assessed in 198 HSCT/CAR-T recipients and 96 healthcare workers (HCWs) recruited to multicentre studies. Only 25% of HSCT/CAR-T recipients generated nAbs following one dose, with titres 167-fold and 7-fold lower than that in HCWs after the first and second doses, respectively. Lower post-second dose nAb titres were associated with older age, rituximab use, and previous HSCT. ChAdOx1-S recipients were more likely to generate nAbs compared with mRNA vaccines, with titres comparable to HCWs. In contrast, nAbs were significantly lower in HSCT/CAR-T recipients than HCWs after mRNA vaccination. The poor first-dose immunogenicity in HSCT/CAR-T recipients suggests a minimum licensed dosing interval could limit the period of vulnerability following HSCT/CAR-T. The relative preservation of nAbs with ChAdOx1-S vaccination highlights the importance of evaluating alternative platforms to mRNA vaccination within this highly vulnerable clinical cohort.

Graphical Abstract

Knowledge of factors impacting immune responses after COVID-19 vaccination is essential for guiding revaccination strategies in haematopoietic stem cell transplant (HSCT) and chimeric antigen receptor T cells (CAR-T) recipients. Neutralising antibodies (nAbs) were assessed after three vaccine doses, and compared with healthcare workers (HCWs). Immunogenicity was particularly poor following one dose, with only 25% generating nAbs. Following two doses, recipients of ChAdOx1-S were eight times more likely to generate nAbs than recipients of mRNA vaccines, with titres similar to HCWs. Lower nAb titres were associated with older age, rituximab, and prior HSCT. Using the minimum licensed dosing intervals and exploring other vaccine platforms could benefit this vulnerable group.



Keywords: SARS-CoV-2; stem cell transplant; vaccination.

INTRODUCTION

Recipients of haematopoietic stem cell transplant (HSCT) and chimeric antigen receptor T cells (CAR-T) continue to be at the highest risk of severe COVID-19 in the SARS-CoV-2 Omicron era.¹⁻³ Despite a three-dose primary SARS-CoV-2 immunisation course, HSCT-recipients had a higher incidence of both COVID-19-related hospitalisation (11-fold) and death (5-fold) compared with the general population in England.¹ Unlike other immunosuppressed groups, new HSCT/CAR-T recipients are considered 'never vaccinated' as their immune memory is ablated during treatment, so require a repeat primary vaccine course regardless of prior vaccine status.⁴ Therefore, further optimisation of primary SARS-CoV-2 immunisation courses with new regimens and vaccine platforms is vital for these patients.

The three-dose primary SARS-CoV-2 vaccination schedule for unvaccinated clinically vulnerable adults in the United Kingdom (UK) was simplified to a two-dose course in 2023, with the rationale that many individuals had a degree of immune memory from prior infections.^{4,5} A longer interval between first and second doses is also being increasingly adopted, based on data from healthy individuals demonstrating greater antibody responses with extended intervals.⁶ Whether these strategies are appropriate in HSCT/CAR-T recipients is unclear. The multicentre studies OCTAVE, OCTAVE-DUO, and PROSECO demonstrated that many immunosuppressed groups were less likely to develop detectable SARS-CoV-2 binding IgG responses following two vaccine doses, although some non-responders seroconverted after a third dose.⁷⁻⁹

To what degree vaccine type drives SARS-CoV-2 vaccine immunogenicity in HSCT/CAR-T recipients remains relatively under-explored. In many countries, only mRNA vaccines are recommended for revaccination schedules,^{5,10-12} and have been shown to generate higher humoral responses than other vaccines in many populations, including the OCTAVE cohort.^{7,13-17} Knowledge of which factors impact serum neutralising activity after each vaccine dose is important in guiding revaccination strategies in HSCT/CAR-T recipients. We report on the induction of neutralising antibody (nAb) and cellular immunity following the adenoviral vector vaccine ChAdOx1-S (AZD1222; AZ) or mRNA vaccine (BNT162b2 or mRNA-1273) in

planned subcohort analyses of HSCT/CAR-T recipients recruited to several UK multicentre studies of immune-vulnerable patients.

MATERIALS AND METHODS

Study cohorts

OCTAVE (ISRCTN12821688), OCTAVE-DUO (ISRCTN15354495), and PROSECO (NCT04858568) were prospective multicentre trials in the UK evaluating humoral and cellular responses against SARS-CoV-2 in clinically vulnerable adults.^{7-9, 18} Immune responses following first (V1) and second (V2) vaccinations were studied in OCTAVE, which recruited clinically vulnerable adults between 19 February 2021 and 01 October 2021.⁷ Participants were vaccinated through the National Health Service (NHS) roll out with either mRNA or ChAdOx-S vaccinations encoding ancestral SARS-CoV-2 spike.⁷ Immunogenicity following a third dose (V3) was studied in OCTAVE-DUO and PROSECO. OCTAVE-DUO randomised clinically vulnerable adults who failed to mount an adequate binding antibody response after two doses of SARS-CoV-2 vaccines to a third dose (primarily BNT162b2 or mRNA-1273) between 04 August 2021 and 31 March 2022.⁹ PROSECO recruited people with lymphoid malignancies between 11 January 2021 and 07 May 2021, of which a subset were HSCT-recipients vaccinated via the NHS rollout.^{8,19} All reported humoral immunity data from HSCT/CAR-T recipients in these cohorts to date were for binding antibody responses.^{7-9,19}

Healthy participant samples were from PITCH (ISRCTN11041050), a multicentre prospective cohort study of UK healthcare workers (HCWs) evaluating humoral and cellular responses against SARS-CoV-2, that began recruiting from 9 December 2020.¹⁸ Additional prevaccination samples from SARS-CoV-2 infection-naïve HCWs from the HERO study were also included.²⁰

Serum selection

All available sera taken within defined post-vaccine visit windows, with matched metadata, were used from adult HSCT/CAR-T recipients participating in OCTAVE (21–42 days post-V1 and post-V2) and OCTAVE-DUO (21–35 days post-V3). As OCTAVE-DUO only recruited HSCT-recipients with no (<0.8 AU/mL) or low (0.8–399 AU/mL) receptor binding domain (RBD) antibody responses after two

doses,⁹ post-V3 plasma from HSCT-recipients participating in PROSECO with anti-Spike IgG responses >300 BAU/mL after two vaccine doses were included for a 'normal-responder group'.⁸ Available post-vaccination sera from PITCH participants recruited to Sheffield and Oxford sites were selected to correspond with sampling windows in OCTAVE and OCTAVE-DUO. Prevacination samples from PITCH and HERO participants were used to characterise nAb in seronegative donors.

Pseudotyped virus neutralisation assay

NAb responses to ancestral (B.1) and Omicron (BA.1, BA.5, BQ.1.1, XBB) SARS-CoV-2 variants were assessed using a pseudotyped virus neutralisation assay.

Pseudotyped viruses were produced by co-transfecting stocks of human embryonic kidney 293 T (HEK-293 T) cells with plasmids encoding Spike variants, a p8.91-lentiviral vector, and a firefly luciferase reporter plasmid (pCSFLW) as previously described.²¹ Chinese hamster ovarian cells expressing TMPRSS2 and ACE2 (CHO-ACE2-TMPRSS) were used as target cells in nAb assays and were maintained in Ham's F-12 Nutrient Mix (Gibco 21765029) supplemented with 10% fetal bovine serum (Cytiva SV30160.03) and 1% penicillin–streptomycin (Sigma-Aldrich P4333).

Sera were diluted 1:20 and mixed 1:1 with pseudotyped viruses (pre-titrated concentration to achieve 5×10^6 relative light units; RLU) in white Nunc™ MicroWell™ 96-Well plates (Thermo Fisher Scientific Inc.) in duplicate, resulting in an initial nAb screening serum dilution of 1:40. Following a 1-h incubation at 37°C and 5% CO₂, 20 000 CHO-ACE2-TMPRSS cells were added to wells prior to a further 48 h incubation. Supernatant was removed and 30 µL of Bright-Glo™ Luciferase Assay System (Promega E2650) diluted 1:1 with phosphate buffered saline was added to each well. Luminescence in RLU was read on a GloMax® Explorer Microplate Reader (Promega) after 5 min. All samples resulting in a mean RLU ≤50% of the pseudotype virus (no serum) control condition were repeated at serial serum dilutions of 1:40–1:78 732 in duplicate to estimate a half-maximal neutralising titre (NT50). Each plate included negative (anti-Spike IgG negative) and positive (pooled SARS-CoV-2 convalescent) serum controls.

Binding antibody and cellular responses

RBD and nucleocapsid binding antibody responses were generated previously in OCTAVE and OCTAVE-DUO (Elecsys AntiSARS-CoV-2, Roche) and PITCH (V-PLEX COVID-19 Coronavirus Panel 3, Meso Scale Discovery),^{7,18} whilst anti-Spike IgG was measured during PROSECO (Meso Scale Discovery).¹⁹ T-cell responses in OCTAVE were assessed using the T-SPOT DISCOVERY SARS-CoV-2 assay (Oxford Immunotec) as previously described in a subset of participants.⁷

Statistical analysis

Sera with an NT50 < 40 were considered non-neutralising based on non-specific inhibition seen in anti-Spike IgG negative sera from SARS-CoV-2-naïve HCWs and assigned a random NT50 < 40. Statistical analysis was performed using R version 4.3.1. Comparisons of NT50 across groups were performed using the Mann–Whitney U test, with Bonferroni correction for multiple testing. Differences between groups in the presence of neutralising activity was compared using Fisher's exact test. Correlations were performed using Spearman correlation. Logistic regression was performed to explore covariates predicting the presence of binding or nAb responses after two doses. Linear regression was performed to explore covariates predicting binding antibody or nAb (NT50) titre following two doses. In both cases, variables that either had a *p*-value of <0.1 on univariate analysis or an a priori hypothesis of impacting vaccine responses were taken forward to multivariable regression. NT50 were log10 transformed for visualisation and statistical analysis. Fold changes in nAb between groups were calculated using absolute NT50 values to ease interpretation.

Ethical approval

OCTAVE was approved by the UK Medicines and Healthcare Products Regulatory Agency (MHRA) on 5 February 2021 and by the London and Chelsea Research Ethics Committee (REC, 21/HRA/0489) on 12 February 2021. OCTAVE-DUO was approved by the MHRA on 19 July 2021. PITCH participants in Oxford were recruited under the GI Biobank Study 16/YH/0247, approved by the Yorkshire & The Humber Sheffield REC on 29 July 2016, which has been amended for this purpose on 8 June 2020. In Sheffield, PITCH participants were recruited under the Observational Biobanking study STHObs (18/YH/0441), which was amended for this study on 10 September 2020. PROSECO and HERO were approved by the UK

Health Research Authority (IRAS IDs 283461 and 294739/233768). Written informed consent was obtained for all participants enrolled in the above studies. All studies were conducted according to the principles of the Declaration of Helsinki (2008) and the International Conference on Harmonisation Good Clinical Practice guidelines.

RESULTS

Participant information

Data from 198 HSCT/CAR-T recipients recruited to OCTAVE, OCTAVE-DUO, and PROSECO are included in this paper (Table 1). Fourteen individuals were in both OCTAVE and OCTAVE-DUO studies. HSCT/CAR-T recipients were predominantly male (116, 58.6%), of white ethnicity (174, 87.9%) and aged under 65 years (150, 75.8%; median 55 years). Most had undergone allogeneic HSCT (145, 73.2%), with fewer receiving an autologous HSCT (43, 21.7%) or CAR-T therapy (8, 4.0%). From OCTAVE, sera were available for nAb assays from 72/82 post-V1 visits and 138/179 post-V2 visits.⁷ Previously generated post-V2 RBD IgG data (133) and T-SPOT data (44) from HSCT/CAR-T recipients were also analysed.⁷ From OCTAVE-DUO, 33 HSCT-recipients had post-V3 sera available for use in nAb assays.⁹ Post-V3 plasma from an additional five HSCT-recipients were available from PROSECO.⁸

Samples from 96 HCWs from PITCH were included, comprising 51 post-V1, 89 post-V2, and 71 post-V3 sera (Table 1). Healthy controls were predominantly female (75, 78.1%), with a median age of 43 years. Preimmunisation control sera (59) from PITCH and HERO HCWs were also used (Figure 1A; Table S1).

A two-dose ChAdOx1-S vaccine course was received by the majority of HSCT/CAR-T (105, 53.0%) and HCW (54, 56.3%) groups. Almost all participants received an mRNA vaccine for their third dose (38 HSCT-recipients, 100.0%; 70 HCWs, 98.6%).

Binding antibody responses

Although the majority of HSCT/CAR-T recipients had detectable anti-RBD IgG following vaccination (post-V1: 45, 55.5%; post-V2: 114, 85.7%), significantly fewer had a titre of ≥ 400 AU/mL (post-V1: 4, 4.9%; post-V2: 75, 56.4%). Only 19 of the 33 individuals with a titre of < 400 AU/mL after two vaccine doses, had antibody titres of > 400 AU/mL after the third dose (57.6%), and four remained seronegative (12.1%).

After adjustment for multiple covariates, post-V2 levels of ≥ 400 AU/mL in HSCT/CAR-T recipients were less likely with older age (compared with < 45 year olds, odds ratio [OR] 0.05 and 95% confidence interval [95% CI] 0.00–0.44, $p = 0.025$ for 45–64 year olds; OR 0.03 and 95% CI 0.00–0.35, $p = 0.015$ for ≥ 65 years olds) and underlying lymphoid malignancy (OR 0.07, 95% CI 0.00–0.56, $p = 0.036$; Table S2). Only weak correlations were seen between V1–V2 dosing interval and post-V2 anti-RBD IgG level for mRNA vaccinees ($R = 0.27$, $p = 0.06$) and ChAdOx1-S vaccinees ($R = 0.24$, $p = 0.034$; Figure S1).

Neutralising antibody responses against ancestral SARS-CoV-2

Prevaccination neutralising activity (NT50 ≥ 40) in sera against ancestral SARS-CoV-2 was seen in SARS-CoV-2 convalescent HCWs only, with limited or absent activity against Omicron lineage pseudoviruses (Figure 1A). HSCT/CAR-T recipients were less likely to have neutralising activity in sera against the vaccine antigen ancestral SARS-CoV-2 compared with HCWs following their first two vaccinations (25% vs. 94.1% post-V1, $p < 0.001$; 71.0% vs. 100.0% post-V2, $p < 0.001$; Table S3). Quantitative nAb titres were also lower in HSCT/CAR-T recipients than HCWs, with a greater difference after one dose (167-fold, $p < 0.001$) than two doses (7-fold, $p < 0.001$; Table S4; Figure 1B). Following a third dose, normal-responder HSCT/CAR-T recipients had similar neutralising activity to HCWs (Figure 2; Tables S3 and S4). In contrast, post-V3 sera from low/non-responders were less likely to neutralise ancestral SARS-CoV-2 than HCWs ($p < 0.001$; Table S3) and median nAb titres were 11-fold lower than in HCWs (Table S4; $p < 0.001$).

After adjustment for other factors, receiving ChAdOx1-S (OR 8.63, 95% CI 1.67–57.69, $p = 0.015$) and prior COVID-19 infection (OR 18.15, 95% CI 1.68–424.46, $p = 0.034$) increased the likelihood of HSCT/CAR-T recipients having neutralising activity after two doses (Figure 3A; Table S5). Prior COVID-19 infection was also associated with

higher nAb titres (+1.04 log₁₀ NT50, $p = 0.003$; Table S6; Figure 3B). HSCT/CAR-T recipients who had undergone a previous HSCT were both less likely to have neutralising activity (OR 0.18, 95% CI 0.03–0.93, $p = 0.049$) and have lower nAb titres (–0.52 log₁₀ NT50, $p = 0.031$), whilst older age (–0.81 log₁₀ NT50, $p = 0.009$ for 45–64 years; –0.84 log₁₀ NT50, $p = 0.016$ for ≥ 65 years; compared with aged <45 years) and receipt of rituximab (–0.85 log₁₀ NT50, $p = 0.016$) were both associated with lower nAb titres (Table S6; Figure 3B). In allogeneic HSCT recipients, lymphocyte depletion with anti-thymocyte globulin (ATG) reduced the likelihood of having neutralising activity (OR 0.00, 95% CI 0.00–0.09, $p = 0.044$; Table S5).

In HCWs, after adjusting for other covariates, post-V2 nAb titres were significantly lower in those vaccinated with ChAdOx1-S compared with mRNA vaccines (–0.51 log₁₀ NT50, $p < 0.001$, Table S6). This difference was not observed in HSCT/CAR-T recipients ($p = 0.455$; Figure 3A,B; Table S6). In infection naïve participants, nAb titres following two doses of ChAdOx1-S were similar in HSCT/CAR-T recipients and HCWs (1.11-fold, $p = 1.00$), but were significantly higher in HCW compared with HSCT/CAR-T recipients in mRNA vaccinees (20-fold, $p < 0.001$; Figure 4). Compared with HCWs, post-V3 nAb titres in low/non-responder HSCT-recipients were 50-fold lower for those who received mRNA for V1-2 ($p < 0.001$), but only 4.6-fold lower in those who received ChAdOx1-S ($p = 0.090$; Figure 2).

NAb responses against omicron SARS-CoV-2 lineages

HSCT/CAR-T recipients were significantly less likely to neutralise the selected Omicron pseudotypes following their first two vaccinations than HCWs (Table S3), with NAb titres up to 10-fold lower post-V2 (BA.1 4.5-fold, $p < 0.001$; BA.5 10.0-fold, $p < 0.001$; BQ.1.1 4.4-fold, $p < 0.001$; XBB 1.8-fold, $p < 0.001$; Table S4). Larger fold change differences between HSCT/CAR-T recipients and HCWs were also observed in mRNA vaccinees than ChAdOx1-S vaccinees (Figure 3C; Figure S2). Low/non-responder HSCT/CAR-T recipients after two doses were less likely to have anti-Omicron neutralising activity post-V3 than HCWs ($p < 0.001$ for all lineages, Table S3). Although numbers were small, normal-responder HSCT/CAR-T recipients had more similar post-V3 anti-Omicron nAb titres to HCWs (Tables S3 and S4).

NAb responses and COVID-19

25 HSCT/CAR-T recipients were reported to have had COVID-19 following their second vaccination, with varying severity (asymptomatic $n = 4$, symptomatic but not hospitalised $n = 5$, hospitalised with no oxygen $n = 3$, intensive care $n = 1$, unknown $n = 12$). We did not detect a significant difference in nAbs between those who had a post-V2 COVID-19 infection and those who did not (Figures S4–S6).

T-cell responses post-V2

No factors predicted the likelihood of HSCT/CAR-T recipients developing a T-cell response following two doses of vaccination, although numbers were limited ($n = 44$; Table S7). No differences in median SFC/ 10^6 PBMCs were observed between vaccine groups ($p = 0.58$; Figure S3).

DISCUSSION

Using participants recruited to several multicentre studies in the UK, we demonstrate that HSCT/CAR-T recipients have lower SARS-CoV-2 nAb responses compared with healthy controls following a primary vaccination course. This difference is more marked after one dose compared with two, highlighting the need for at least two vaccine doses post-HSCT/CAR-T to generate any appreciable nAb response. A particularly interesting and novel finding was that HSCT/CAR-T recipients receiving two ChAdOx1-S vaccines were more likely to have anti-SARS-CoV-2 nAb than those who received mRNA vaccines. In infection naïve participants, nAb titres between HSCT/CAR-T recipients and HCWs receiving ChAdOx1-S were remarkably similar, in contrast to the much higher titres seen in healthy adults following mRNA vaccines. Given that new HSCT/CAR-T recipients have their immune memory ablated, this finding is particularly relevant when considering how to improve vaccine regimens in these groups.

Multiple studies demonstrate that mRNA vaccination results in enhanced humoral responses compared with other vaccine platforms in healthy individuals and other immunosuppressed groups.^{5,7,13-17,22,23} There is however a paucity of published data in HSCT/CAR-T recipients for any vaccines other than homologous

mRNA regimens.^{24,25} In two recent meta-analyses, only 111/5906 (1.9%) and 79/2899 (2.7%) HSCT/CAR-T recipients received a homologous viral vector vaccine course, with a further 22/5906 (0.4%) and 12/2899 (0.4%) receiving a mixed ChAdOx1-S/mRNA regimen.^{17,24-36} One previously reported study of HSCT/CAR-T recipients receiving ChAdOx1-S ($n=34/55$, 61.8%) found that allogeneic HSCT-recipients receiving a single dose of ChAdOx1-S were more likely to have detectable binding antibodies than those receiving a single dose of BNT162b2.²⁶ Analysis of the entire cohort of clinically vulnerable adults in OCTAVE also demonstrated that ChAdOx1-S was more likely to induce T-cell responses.⁷ Our data represent the largest comparison of ChAdOx1-S and mRNA primary vaccine courses in a single study of HSCT/CAR-T recipients to date.

It is currently unclear why the SARS-CoV-2 nAb responses appear preserved in HSCT/CAR-T recipients receiving ChAdOx1-S compared with mRNA vaccines. In healthy individuals, differences in the immune response following ChAdOx1-S and mRNA vaccinations have been observed.³⁷ For instance, following a single dose, ChAdOx1-S induces a memory-like circulating T follicular helper cell and plasmablast response, whereas BNT162b2 does not.³⁷ Further mechanistic studies are required to carefully dissect whether the different pathways of immune induction after viral vector and mRNA vaccines are affected differently by HSCT/CAR-T-therapy.³⁸ In addition, as ChAdOx1-S and other viral vector vaccines are no longer available in most countries, our findings should encourage the evaluation of other viral vector platform vaccines in development in these patient groups. We also observed that priming with ChAdOx1-S vaccines in HSCT/CAR-T low/non-responders to two doses resulted in higher nAb titres after an mRNA 3rd dose, compared with those primed with an mRNA two-dose course. A heterologous prime-boost regimen is therefore worth evaluating in future clinical trials in HSCT/CAR-T recipients.

UK guidelines for vaccinating severely immunosuppressed adults considered vaccine naïve diverged in 2023 from other (inter-) national guidelines, from three doses of mRNA vaccination (0, 3, and 7–16 weeks), to two doses (0 and 8–12 weeks) with a third dose during the next seasonal campaign.^{5,10,39,40} Although the guidance allows the interval between the first two doses to be reduced to 3 weeks on specialist advice, a preference for a longer dosing interval is stated due to

findings in healthy adults showing greater post-dose two antibody.^{5,6} We find that a longer dosing interval did not result in a statistically significant increase in neutralising activity in HSCT/CAR-T recipients when adjusted for other factors. Previously published data in allogeneic HSCT-recipients also suggest that a similar proportion have detectable binding antibodies with a short (21 days, 86%) and a long dosing interval (median 76 days, 86%).^{7,41} Given that we and others have found that humoral responses are very poor in HSCT/CAR-T recipients following a single SARS-CoV-2 vaccination, adhering to a minimum licensed interval between first and second doses would offer the best course of action for individual patients.^{26,28,41-43}

Our study has several limitations, including small numbers in some groups (e.g. CAR-T recipients, post-V2 COVID-19 severity data, T-SPOT data) limiting the power to identify true differences. Our data were generated using SARS-CoV-2 ancestral vaccines, whilst Omicron lineage monovalent vaccines are currently in use. Nevertheless, our main findings are focused on vaccine-matched nAb to ancestral SARS-CoV-2, therefore, we would expect HSCT/CAR-T recipients receiving Omicron lineage vaccines to generate similar vaccine-matched Omicron-specific nAb data. Our data are observational and may not have accounted for unknown confounders affecting differences in groups.

Whilst COVID-19 is now a mild infection for many individuals with considerable immune memory from prior infections and vaccines, new HSCT/CAR-T recipients remain extremely immune-vulnerable. Randomised clinical trials of new SARS-CoV-2 vaccines should consider dosing intervals and vaccine platforms to prioritise these and other immunosuppressed groups to reduce the gap in protective anti-SARS-CoV-2 immunity.

	HSCT/CAR-T recipients ^a		Healthy controls
	Post-V1 and V2	Post-V3	
Participants who had post-vaccination sera available, <i>n</i>	174 (OCTAVE)	33 (OCTAVE-DUO) 5 (PROSECO)	96 (PITCH)
Age, years, median (range)	55 (19–75)	61 (33–72)	43 (21–70)
Sex, <i>n</i> (%)			
- Male	100 (57.5)	23 (60.5)	21 (21.9)
- Female	74 (42.5)	15 (39.5)	75 (78.1)
Ethnicity, <i>n</i> (%)			
- White	156 (89.7)	32 (84.2)	60 (62.5)
- Asian	6 (3.4)	1 (2.6)	3 (3.1)
- Black	2 (1.1)	0 (0.0)	0 (0.0)
- Mixed ethnicities	0 (0.0)	0 (0.0)	2 (2.1)
- Other	0 (0.0)	0 (0.0)	1 (1.0)
- Unknown	10 (5.7)	5 (13.2)	30 (31.3)
Vaccination type (V1 and V2), ^b <i>n</i> (%)			
- mRNA/mRNA	76 (43.7)	14 (36.8)	42 (43.8)
- ChAdOx1-S/ChAdOx1-S	93 (53.4)	24 (63.2)	54 (56.3)
- ChAdOx1-S/mRNA	3 (1.7)	0 (0.0)	0 (0.0)
- Unknown	2 (1.1)	0 (0.0)	0 (0.0)
Dosing interval between V1 and V2, days, median (range)	76 (19–421)	77 (29–188)	63 (34–84)
Vaccination type (V3), ^b <i>n</i> (%)			
- mRNA	NA	38 (100.0)	70 ^c (98.6)
- ChAdOx1-S	NA	0 (0.0)	1 ^d (1.4)
Evidence of COVID-19 prior to post-V2 visit, ^e <i>n</i> (%)			
- Yes	21 (12.1)	1 (2.6)	42 (43.8)
- No	117 (67.2)	37 (97.4)	49 (51.0)
- Unknown	36 (20.7)	0 (0.0)	5 (5.2)
HSCT-type, <i>n</i> (%)			
- Allogeneic	125 (71.8)	30 (78.9)	NA
- Autologous	40 (23.0)	7 (18.4)	
- CAR-T	8 (4.6)	0 (0.0)	
- Unknown	1 (0.1)	1 (2.6)	
Time between D0 and V1, days, median (range)	241 (20–8278)	379 (106–8287)	NA
Indication, <i>n</i> (%)			
- Myeloid	116 (66.7)	20 (52.6)	NA
- Lymphoid	46 (26.4)	16 (42.1)	
- Neurological ^f	6 (3.4)	0 (0.0)	

- Other ^g	6 (3.4)	2 (5.3)	
Disease status, <i>n</i> (%)			
- Complete remission	121 (69.5)	29 (76.3)	NA
- Stable disease	10 (5.7)	1 (2.6)	
- Partial remission	13 (7.5)	2 (5.3)	
- Progressive/relapsed disease	7 (4.0)	2 (5.3)	
- Not applicable (neurological)	6 (3.4)	0 (0.0)	
- Unknown	17 (9.8)	4 (10.5)	
Conditioning, ^h <i>n</i> (%)			
- Reduced intensity	73 (42.0)	17 (44.7)	NA
- Myeloablative	25 (14.7)	6 (15.8)	
- Non-myeloablative	12 (6.9)	1 (2.6)	
- Unknown/not applicable	64 (36.8)	14 (36.8)	
Lymphocyte depletion, ^h <i>n</i> (%)			
- Campath	56 (32.2)	18 (47.4)	NA
- Anti-thymocyte globulin	29 (16.7)	7 (18.4)	
- Post-transplant cyclophosphamide	13 (7.5)	2 (5.3)	
- None	10 (5.7)	3 (7.9)	
- Unknown/not applicable	66 (37.9)	8 (21.1)	
Other treatments received, <i>n</i> (%)			
- Total body irradiation	29 (16.7)	6 (15.8)	NA
- Previous HSCT	36 (20.7)	3 (7.9)	
- Rituximab	19 (10.9)	4 (10.5)	
- Tocilizumab	6 (3.4)	0 (0.0)	
GVHD			
- Acute at V2	7 (4.0)	Data not available	NA
- Chronic at V2	15 (8.6)		
Post-V1 results available			
- RBD IgG	81 (46.6)	NA	0 (0.0)
- NAb	72 (41.3)		51 (53.1)
- T-SPOT	0 (0.0)		0 (0.0)
Post-V2 results available			
- RBD IgG	133 (76.4)	NA	0 (0.0)
- NAb	138 (79.3)		89 (92.7)
- T-SPOT	44 (25.3)		0 (0.0)
Post-V3 results available			
- RBD IgG	NA	0 (0.0)	0 (0.0)
- NAb		38 (100.0)	71 (74.0)
- T-SPOT		0 (0.0)	0 (0.0)

Table 1. Details of participants included from the HSCT/CAR-T recipients in OCTAVE, OCTAVE-DUO, and PROSECO and healthy controls from PITCH. The number of participants with available data from post-V1, post-V2, and post-V3 visits are detailed separately.

Abbreviations: CAR-T, chimeric antigen receptor T cells; D0, day zero of HSCT/CAR-T; GVHD, Graft-versus-host disease; HSCT, haematopoietic stem cell transplant; NA, not applicable; nAb, neutralising antibodies; RBD, receptor binding domain; V1, first vaccination; V2, second vaccination; V3, third vaccination.

- a. Fourteen participants in OCTAVE were recruited into OCTAVE-DUO.
- b. mRNA includes BNT162b2 (BioNTech Pfizer) and mRNA-1273 (Moderna).
- c. Regimens for V1-V2: mRNA $n = 41$, ChAdOx1-S $n = 29$.
- d. Regimens for V1-V2: ChAdOx1-S $n = 1$.
- e. Defined as previous PCR positivity or detection of SARS-CoV-2 anti-nucleocapsid IgG from Roche Elecsys (OCTAVE and OCTAVE-DUO) or Meso Scale Discovery (PROSECO and PITCH).
- f. Neurological conditions include neuromyelitis optica, multiple sclerosis, chronic inflammatory demyelinating polyneuropathy, myasthenia gravis, and stiff person syndrome.
- g. Other conditions: aplastic anaemia $n = 5$, amyloidosis $n = 1$.
- h. Data for conditioning and lymphocyte depletion is only available for allogeneic HSCT recipients in OCTAVE and OCTAVE-DUO.

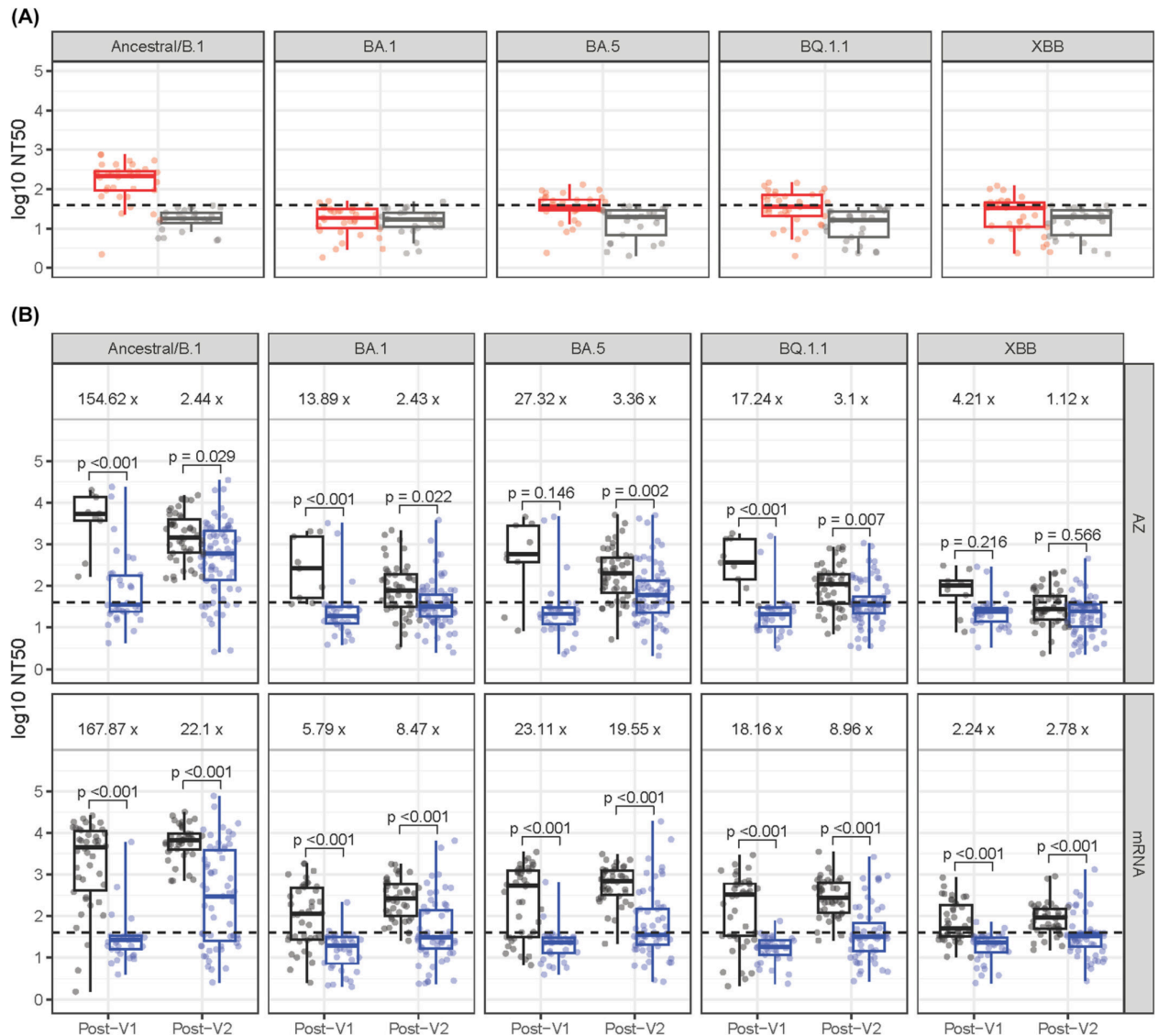


Figure 1

Neutralising antibody titres against SARS-CoV-2 ancestral and Omicron variants in HSCT/CAR-T recipients and healthy controls. (A) Data in healthy controls prevaccination stratified by SARS-CoV-2 naive and convalescent serostatus. (B) Data comparing HSCT/CAR-T recipients with healthy control participants following first and second doses, stratified by receipt of ChAdOx1-S (AZD1222; AZ) or mRNA vaccines (BNT162b2 or mRNA-1273). Y-axes denote log₁₀ reciprocal dilution at which 50% neutralisation was observed (NT50). Boxplots demonstrate median and interquartile ranges (IQR25 and IQR75), with whiskers showing the range (Mann–Whitney *U* test and Bonferroni correction for multiple comparisons). Dashed line shows an NT50 of 40, considered the threshold at which true neutralising activity in serum is detected. CAR-T, chimeric antigen receptor T cell;

HSCT, haematopoietic stem cell transplant; V1, first vaccination; V2, second vaccination.

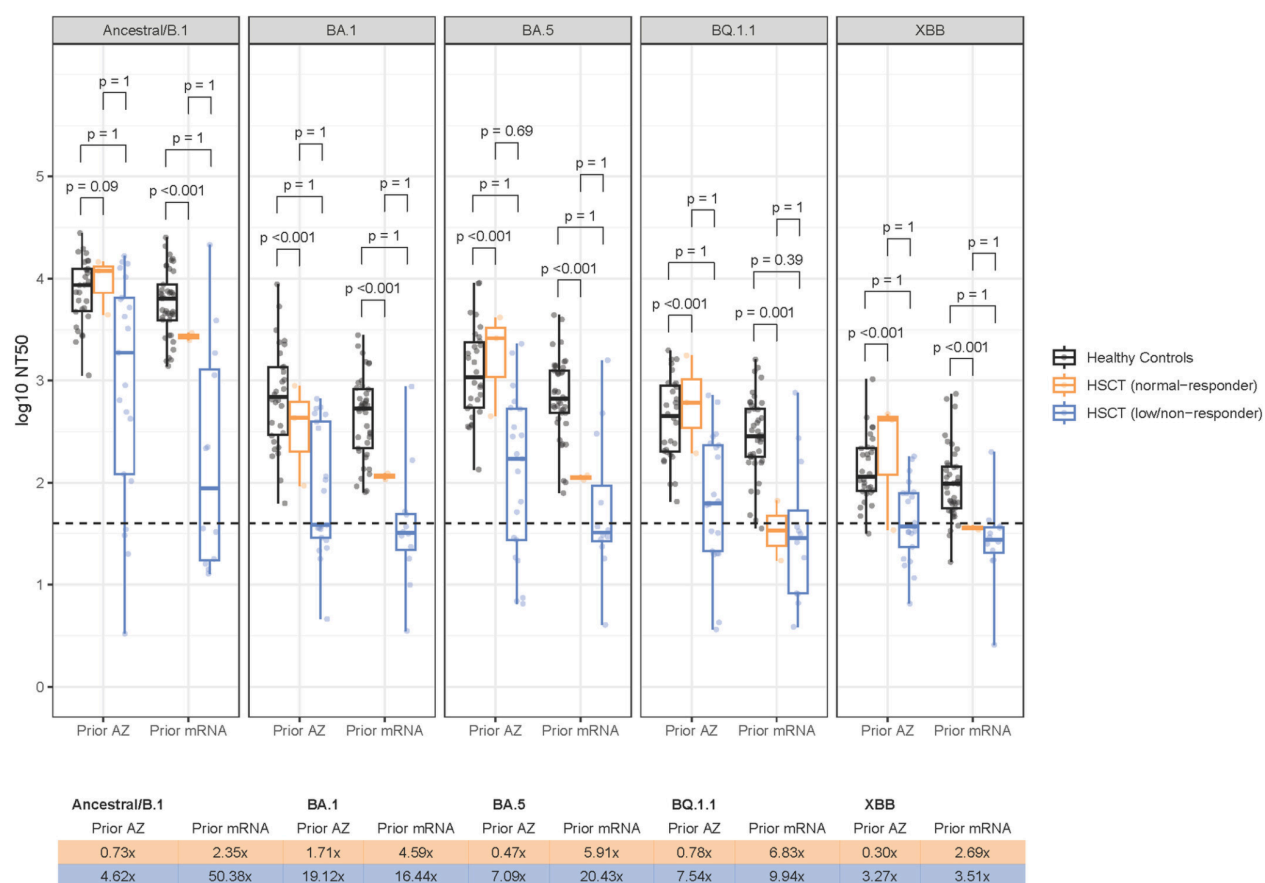


Figure 2

Data comparing HSCT-recipients with healthy controls following a third vaccine dose (BNT162b2 or mRNA-1273) split by whether they received ChAdOx1-S (AZD1222; AZ) or mRNA for their first two doses. HSCT-recipients are stratified by those with a low/no response (<400 AU/mL on anti-RBD IgG, Roche) or normal response (>300 BAU mL⁻¹ Anti-Spike IgG, Meso Scale Discovery) following two doses of either mRNA or ChAdOx1-S vaccination. One healthy control received ChAdOx1-S for their third dose therefore is not shown in this graph. Y-axes denote log₁₀ reciprocal dilution at which 50% neutralisation was observed (NT₅₀). Boxplots demonstrate median and interquartile ranges (IQR₂₅ and IQR₇₅), with whiskers showing the range. Fold changes of the median for normal and low/non-responder HSCT-recipients compared with healthy controls are displayed in the table, whilst statistical comparisons are displayed above the box plots (Mann–Whitney U test and Bonferroni correction for multiple comparisons). Dashed line shows an NT₅₀ of 40, considered the threshold at which true neutralising activity in serum is detected. HSCT, haematopoietic stem cell transplant.

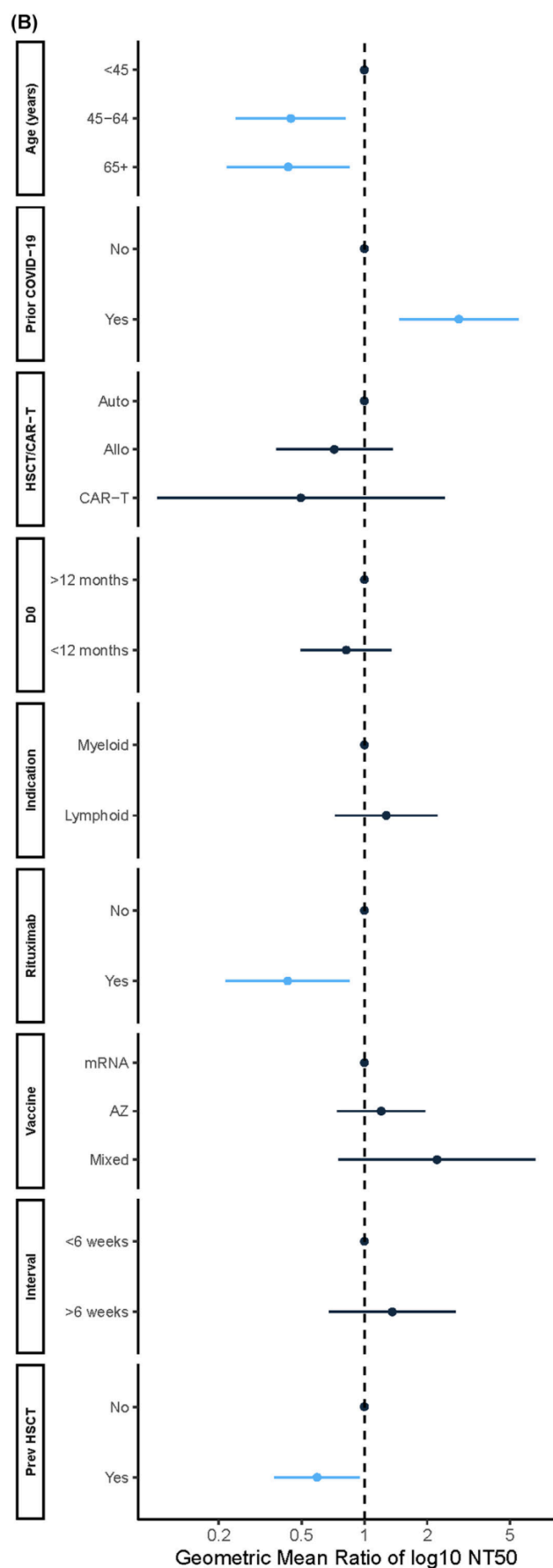
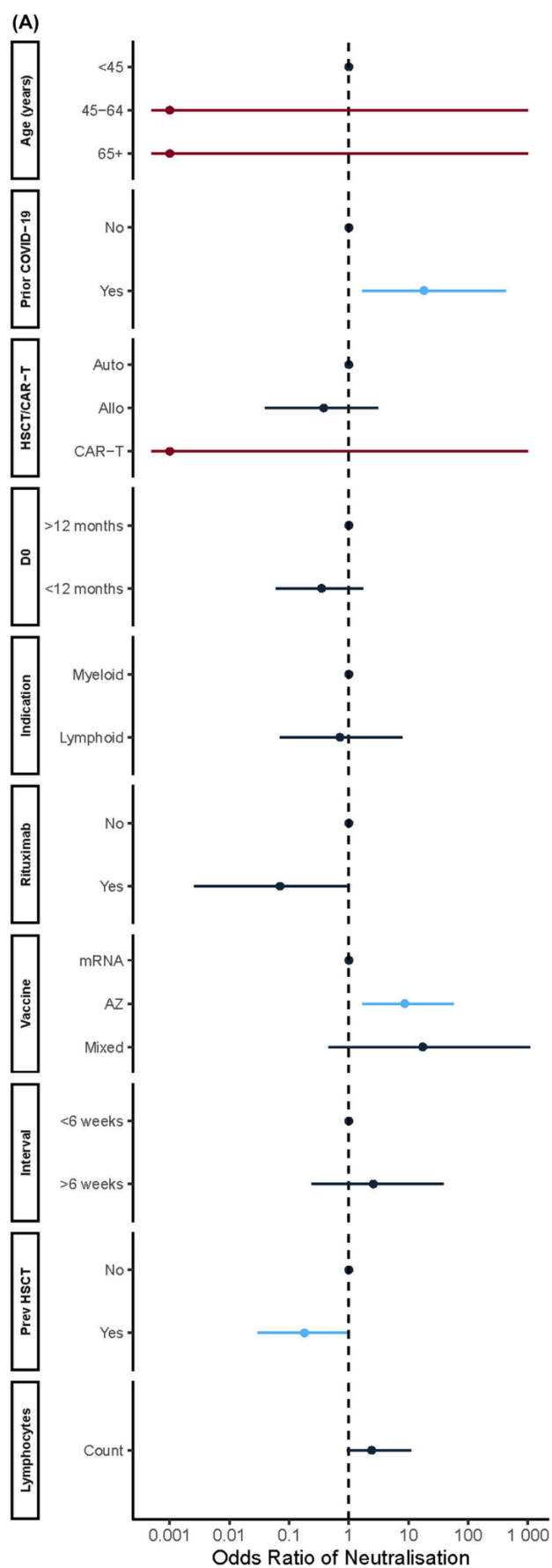


Figure 3

Forest plots from multivariable regression analyses demonstrating factors that impact neutralising antibody (nAb) responses after two SARS-CoV-2 vaccine doses in HSCT/CAR-T recipients to the vaccination target ancestral SARS-CoV-2 (B.1). (A) Adjusted odds ratio and 95% confidence intervals (CI) from multivariable logistic regression exploring covariates that predict the presence of neutralising activity in serum. Variables with p-values < 0.05 are shown in light blue, whilst burgundy represents factors with outlier odds ratios with incalculable confidence intervals as per Table S3 (B) Covariates impacting nAb titre following a second SARS-CoV-2 vaccine dose from a multivariable linear regression. Shown are geometric mean differences in log₁₀ reciprocal dilution at which 50% neutralisation was observed (NT₅₀) between the reference group (1.0) and other factors for each variable, along with 95% CI. Variables with p-values < 0.05 are shown in light blue. In both cases, factors with a p-value < 0.1 in univariate regression or those with an a priori hypothesis of impacting nAb were taken forward to multivariable analyses (Tables S6 and S7). Allo, allogeneic HSCT; Auto, autologous HSCT; AZ, ChAdOx1-S vaccine (AZD1222); CAR-T, chimeric antigen receptor T cell; HSCT D0, time between HSCT/CAR-T and first vaccine dose; HSCT, haematopoietic stem cell transplant; Indication, type of haematological malignancy; Interval, interval between first and second vaccine doses; Lymph, total lymphocyte count ($\times 10^9/L$) at the time of second vaccination (± 7 days); Prev HSCT, previous HSCT prior to most recent HSCT/CAR-T; Prior COVID-19, history or serological evidence of prior COVID-19 prior to vaccination; Rituximab, receipt of Rituximab in 12 months prior to recruitment.

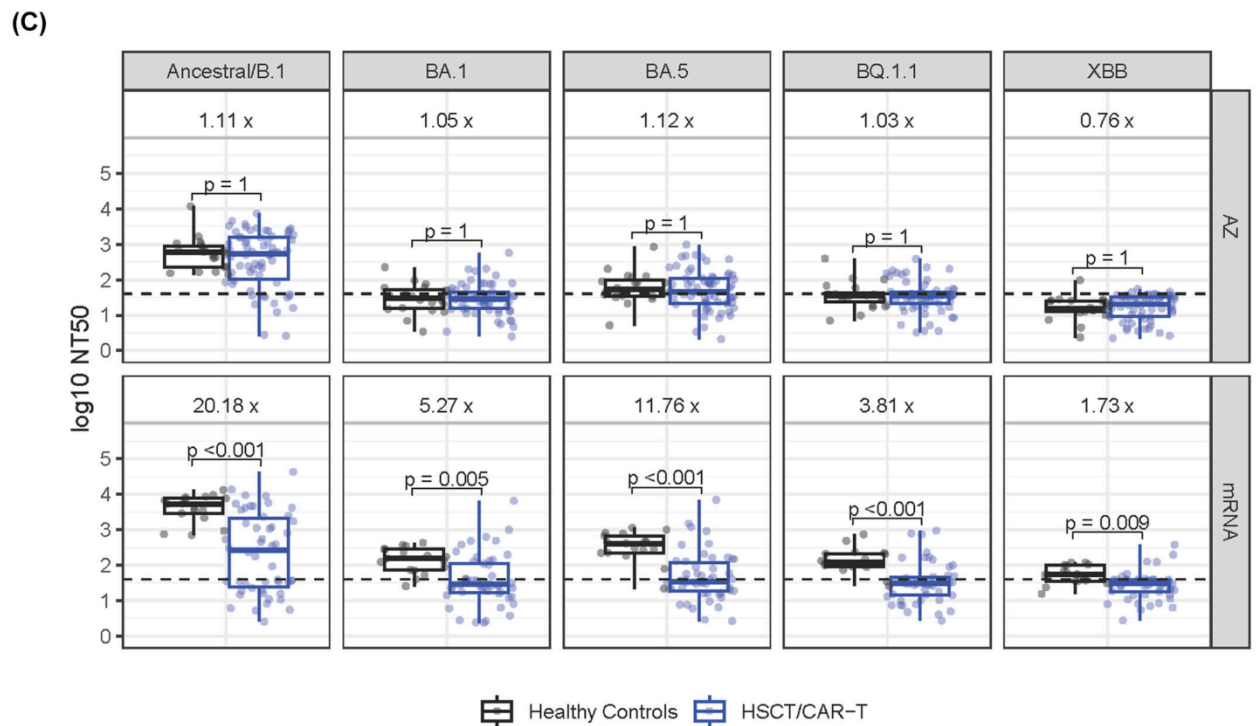
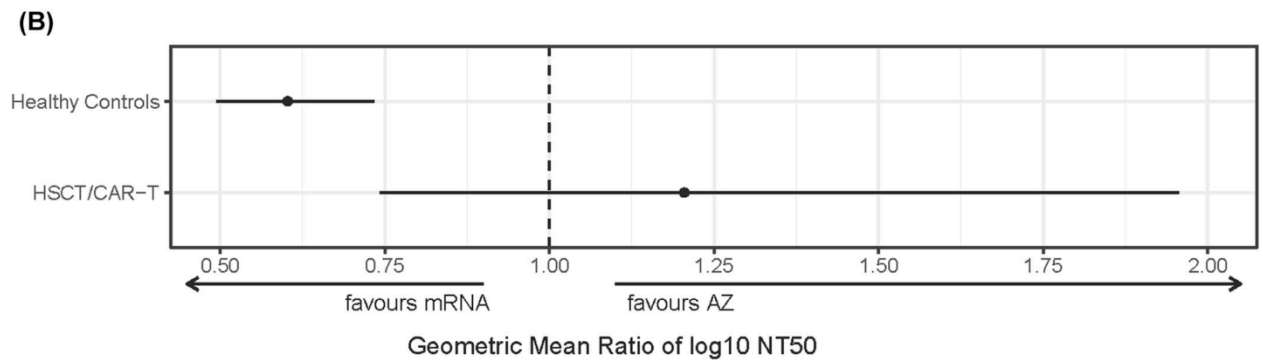
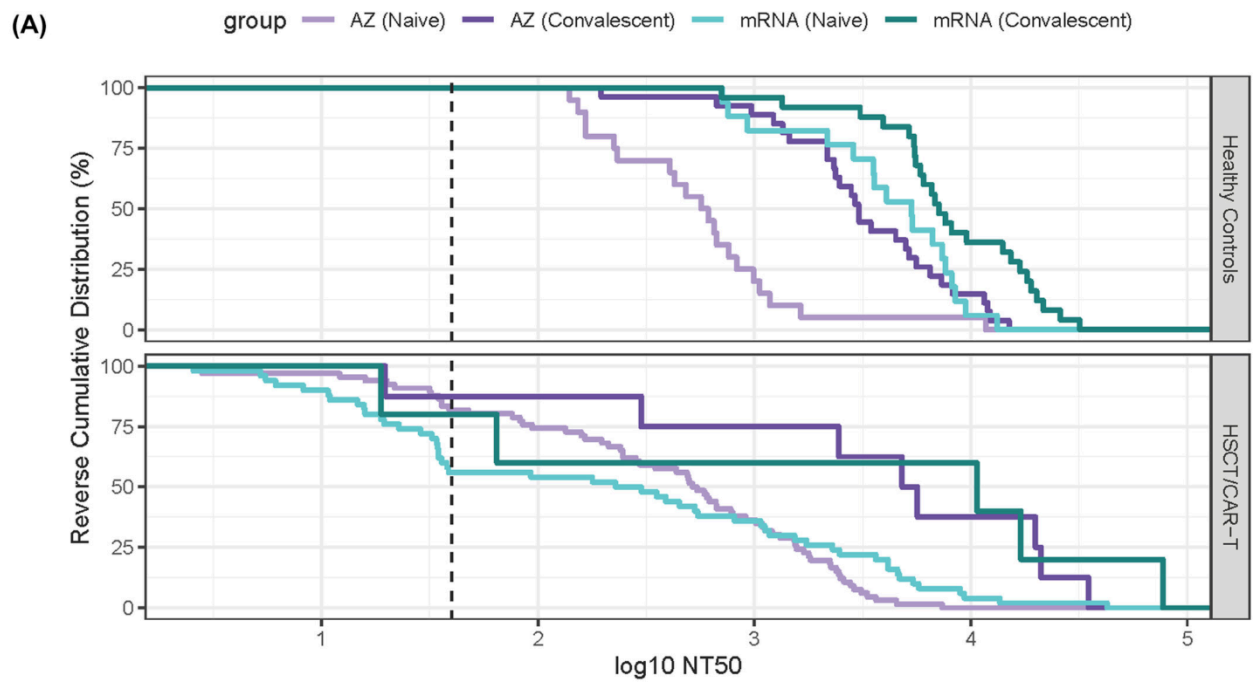


Figure 4

Comparison of neutralising antibody responses following a two-dose SARS-CoV-2 vaccine course with ChAdOx1-S (AZD1222; AZ) vaccine or mRNA vaccines (BNT162b2 or mRNA-1273) in HSCT/CAR-T recipients and healthy control participants. (A) Reverse cumulative plots of neutralising antibody (nAb) titres in healthy controls (top panel) and healthy controls (bottom panel), stratified by individuals who were naive or convalescent on SARS-CoV-2 anti-nucleocapsid IgG testing prior to the first SARS-CoV-2 vaccine dose. NAb titres are expressed as the log₁₀ reciprocal dilution at which 50% neutralisation was observed (NT50). Shown are the percentage of participants (y axis) with nAb activity above NT50 thresholds (x-axis) against SARS-CoV-2 ancestral/B.1 pseudotype virus. Dashed line shows an NT50 of 40, considered the threshold at which true neutralising activity in serum is detected. Reverse cumulative plot created using `stat_ecdf` within `ggplot2` package in R. (B) The geometric mean ratio (and 95% confidence intervals) of log₁₀ NT50 against ancestral/B.1 SARS-CoV-2 between individuals receiving ChAdOx1-S and mRNA vaccine courses, adjusted for other covariates in multivariable linear regression analyses. (C) Comparison of nAb titres in SARS-CoV-2 naive healthy controls and HSCT/CAR-T recipients, stratified by receipt of ChAdOx1-S (top panel) or mRNA (bottom panel) vaccine courses. Shown are NT50 values (Y-axes) against ancestral/B.1 and Omicron lineage viruses (BA.1, BA.5, BQ.1.1, XBB). Boxplots demonstrate median and interquartile ranges (IQR25 and IQR75), with whiskers showing the range. Fold change of the median absolute NT50 values and statistical comparisons between HSCT/CAR-T recipients and healthy controls are displayed (Mann–Whitney *U* test with Bonferroni correction). Dashed line shows an NT50 of 40, considered the threshold at which true neutralising activity in serum is detected. The HSCT groups in the plots include both HSCT and CAR-T recipients. CAR-T, chimeric antigen receptor T cell; HSCT, haematopoietic stem cell transplant.

AUTHOR CONTRIBUTIONS

HC selected and tested samples on the pseudotype neutralisation assay, formally and visually analysed the data, and wrote the original draft. NT developed the pseudotype neutralisation assay and supplied the SARS-CoV-2 pseudotype plasmids. TIdS supervised the project and adapted the pseudoneutralisation assay with NB. RS, AP, BK, NB, HH, AA, IG, and SHL collated and shipped participant samples. BBL wrote the bespoke R script to analyse the luminescence files and generate NT50 values. AK, BK, and SHL curated the metadata. IBM, EB, CSG, PK, AR, SHL, TIdS, JAS, KO, PM, SJD, and PJC conceptualised one or more of the main studies. IBM (OCTAVE, OCTAVE-DUO), PK (OCTAVE-DUO), SHL (PROSECO), SJD (PITCH), and PJC (HERO) were chief investigators in the main studies. EB, TIdS, JAS, CSG, AR, DT, GC, IBM, MW, SS, KO, JC, EH, RM, MK, FK, RS, VP, KLY, DR, HP, and SB contributed to patient recruitment and data collection. All authors reviewed the manuscript (except PM who died in October 2022).

CONFLICT OF INTEREST STATEMENT

SHL has received speaker's honoraria and is on an advisory board for AstraZeneca. JAS declares honoraria for educational events from Jazz, Gilead, Janssen, for advisory board membership from Medac, Vertex, Jazz, and BMS, and for trial IDMC membership from Kiadis Pharma.

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The collaborators of the PITCH Consortium, OCTAVE Collaborative Group, OCTAVE-DUO Investigators, and PROSECO Investigators are listed in Appendix S2.

DATA AVAILABILITY STATEMENT

Data are not publicly available due to datasets containing information that could compromise research participant privacy/consent. Reasonable requests for data may be considered as per the processes for each study as previously described.^{7-9,18} R scripts used for regression and graphical analyses are publicly available, alongside a dummy dataset in order to run the code (<https://github.com/xhayles/hsct-cart-covid-pseudoneutralisation/>).

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Paper 4

Colton H, Greenfield DM, Snowden JA, Miller PDE, Morley NJ, Wright J, Darton TC, Evans CM, de Silva TI. **Long-term survivors following autologous haematopoietic stem cell transplantation have significant defects in their humoral immunity against vaccine preventable diseases, years on from transplant.** *Vaccine*. 2021 Aug 9;39(34):4778-4783. doi: 10.1016/j.vaccine.2021.07.022. Epub 2021 Jul 20. PMID: 34294480.

Abstract

Current international guidelines recommend routinely vaccinating haematopoietic stem cell transplant (HSCT) recipients. Despite significant infection-related mortality following autologous HSCT, routine vaccination programmes (RVP) completion is poor. For recovered HSCT recipients, it is uncertain whether catch-up vaccination remains worthwhile years later.

To determine potential susceptibility to vaccine preventable infections, we measured antibody titres in 56 patients, a median of 7 years (range 0–29) following autologous HSCT, who had not completed RVP. We found that almost all participants had inadequate titres against diphtheria (98.2%) and pneumococcal infection (100%), and a significant proportion had inadequate titres against measles (34.5%). Of those subsequently vaccinated according to available guidelines, many mounted adequate serological responses.

These data suggest a pragmatic catch-up approach for autologous HSCT recipients who have not completed RVP is advisable, with universal vaccination against some pathogens (e.g. *Streptococcus pneumoniae* and diphtheria) and serologically-guided approaches for others (e.g. measles and varicella zoster virus).

Introduction

Despite advances in haematopoietic stem cell transplantation (HSCT), infection remains a substantial cause of late mortality in both allogeneic HSCT and autologous HSCT recipients [1,2]. As impaired humoral immunity can persist for years post-transplant, several best-practice international guidelines recommend routine vaccination programmes (RVP) in the months following HSCT to reduce the risk of vaccine preventable diseases (VPD) [1,3].

Although humoral antibody responses to VPD may reconstitute more readily following autologous HSCT compared to allogeneic HSCT, autologous recipients commonly lose their immunity, particularly if multiple chemotherapy regimens were given pre-transplantation [1]. Previous surveys of centres in the UK have shown that only ~50% recommended RVP following autologous HSCT, and RVP completion rates are often poor despite clinician awareness of their importance [4,5].

With increasing life expectancy post-HSCT, 'late-effects' clinics are increasingly important for managing transplant-related late sequelae. We were faced with a cohort of autologous HSCT recipients, some even decades post-transplant, who for various reasons missed RVP. Given there are no clear guidelines on how to approach these patients, we explored whether humoral immune responses against common VPD reconstitutes naturally in the absence of specific vaccination. In seronegative patients, we aimed to see whether a complete catch-up RVP or a more pragmatic targeted vaccination programme would be more appropriate.

Methods

Study participants and procedures: Following institutional approval, a 1-year observational study (15th May 2017 to 23rd May 2018) was conducted at Sheffield Teaching Hospitals NHS Foundation Trust of consecutive autologous HSCT patients attending a late-effects clinic as part of routine NHS care. These patients had not undergone a previous RVP post-transplant.

During clinic attendance, patients were asked about childhood vaccinations and blood was drawn to measure antibody titres against diphtheria, MMR (measles, mumps, rubella), varicella zoster virus (VZV), *Streptococcus pneumoniae* (12 Danish serotypes: 1, 3, 4, 5, 6B, 7F, 9 V, 14, 18C, 19A, 19F, 23), tetanus and *Haemophilus influenzae* type B (Hib). Other VPD were not included in our study as testing is either based on the presence of specific risk factors (e.g. birthplace for hepatitis B), or antibody testing is not routinely available (e.g. polio, pertussis).

Antibody titre measurement and thresholds (Supplementary Table 1): Serology was performed using standard practice for our NHS laboratory. Definitions of protective thresholds were based on accepted levels in the literature, or on assay specific values. Thresholds for anti-tetanus IgG, anti-Hib IgG and anti-rubella IgG (≥ 0.1 IU/ml, ≥ 0.15 μ g/ml and ≥ 10 IU/ml, respectively) were based on previously defined cut-offs [6]. Although a protective anti-diphtheria IgG is frequently cited as ≥ 0.1 IU/ml, we used the WHO's threshold of ≥ 1.0 IU/ml, which is associated with long term protection [6,7]. Anti-measles and anti-mumps IgG were assay-specific cut offs (15 AU/ml and 10 AU/ml, respectively), and reported qualitatively. Defining protective correlates to *S. pneumoniae* was more challenging, as protective levels vary by serotype, patient age and comorbidities [6,8]. The WHO recommends a threshold of ≥ 0.35 μ g/mL, however this is based on studies on infants [8]. The threshold for adults is unknown, as they have a higher level of circulating IgG yet have a greater incidence of pneumococcal disease, and even less is known about immunocompromised individuals [8]. A putative threshold of ≥ 1.0 μ g/ml has been suggested by multiple experts and was used in this study [9]. The protective VZV IgG threshold varies by age, ethnicity and whether immunity was acquired following infection or vaccination, however we opted to use our assays' threshold of 150 mIU/ml which was based on previous studies [10,11].

Vaccinations (Supplementary Table 1): Recommendation for vaccination and post-vaccination serological testing was sent to the patient's general practitioner (GP) in accordance with published guidelines [1,3,8,12]. Where vaccination was not available in primary care settings, patients were referred and vaccinated through the outpatient clinics in the infectious diseases department.

Follow up: One year following clinic attendance (15th May 2018 to 23rd May 2019), post-vaccination serology was reviewed and compared to pre-vaccination titres.

Analysis: Non-normally distributed continuous data were log transformed prior to analysis with parametric tests. Normally distributed continuous data were analysed using either a *t*-test (two group comparison), analysis of variance (three group comparison) or Pearson correlation. Categorical variables were analysed using chi-squared test. Univariate linear and logistic regression models were constructed to assess if any characteristics were associated with antibody titres (or seropositivity where results were qualitatively reported) for each VPD. Associations with p values ≤ 0.2 were assessed with multivariate analysis for each VPD. Statistical analysis was performed using R Studio version 1.1.463.

Results

Demographic and clinical details (Table 1): 60 sequential attendees were identified as suitable. As one patient had completed RVP elsewhere and three patients' blood samples were not received by the laboratory, a total of 56 patients were included in the final analysis. Since live vaccines are contraindicated within two years of HSCT, one patient was excluded from the analysis for MMR and VZV [3]. Another patient was excluded from the analysis for tetanus and Hib as incomplete testing occurred at clinic attendance.

The median patient age was 48 years (range 22 – 72), and most had received HSCT due to haematological malignancy ($n = 42$, 75%). The median time from HSCT was 7 years, but some were decades post-transplant (range 0 – 29 years). Most patients had received pre-HSCT chemotherapy ($n = 52$, 92.9%), and the majority reported completing their childhood vaccination schedule ($n = 34$, 60.7%).

Proportion with adequate antibody titres (Table 2): The proportion of patients with antibody titres below defined protective thresholds varied across the VPDs tested.

Almost all patients did not achieve the 1.0 IU/ml threshold for diphtheria (n = 55, 98.2%, 95% CI 90.4–100.0%) and every patient had titres below 1.0 µg/mL for all 12 Danish pneumococcal serotypes (n = 56, 100%, 95% CI 93.6–100%). Only two patients (3.57%) even achieved the lower threshold of ≥ 0.35 µg/mL for all 12 pneumococcal serotypes (Fig. 1, Supplementary Table 2a).

In contrast, titres below the thresholds for Hib, VZV and tetanus were seen in fewer individuals (5.5%, 10.9% and 20% respectively). The MMR results were varied, with 34.5% of patients considered seronegative to measles, 50.9% to mumps and 29.1% to rubella.

Factors associated with antibody status (Supplementary Tables 3a and b): Significant associations on univariate analysis were found between measles seropositivity and increasing age (current age OR 1.09, p = 0.005, 95% CI 1.03–1.15; age at transplant OR 1.07, p = 0.008, 95% CI 1.02–1.13), and those who received BEAM conditioning were less likely to be mumps seropositive (OR 0.21, p = 0.028, 95% CI 0.05–0.80). On multivariate analysis, these associations remained significant, and in addition those who received BEAM or TBI were more likely to be measles seronegative.

Follow up serology (Supplementary Tables 4a and b): In most cases, GPs were willing to facilitate administration of recommended vaccinations and follow up serology. Serology for at least one VPD was performed in 42 patients (75%), either by their GP or at subsequent clinic attendances. Increases were seen in post-vaccination titres against diphtheria and *S.pneumoniae* (Figs. 1 and 2, Supplementary Fig. 1). Univariate analysis revealed male patients were more likely to have an antibody response to diphtheria vaccination (OR 9.8, p = 0.017, 95% CI 1.72–83.05), although this did not remain significant on multivariate analysis.

Discussion

Our cohort of autologous HSCT patients with a range of underlying diagnoses, who for various reasons missed post-transplant RVP, had significant defects in their humoral immunity against a number of common VPD (median 7, range 0 – 29 years post-transplant).

A prominent finding was an almost universal absence of adequate antibody titres against vaccine-serotype strains of *S. pneumoniae*, with only 3.57% achieving pre-vaccination titres greater than the WHO's paediatric threshold to all 12 Danish serotypes. This is concerning as invasive pneumococcal disease incidence is substantially higher in autologous HSCT recipients compared to the general population, and is a significant cause of morbidity and mortality [8,12]. Although serotype-specific antibody levels increased post-vaccination, only 20% achieved the WHO's paediatric threshold for all 12 serotypes. It is worth noting that while we advised the UK standard recommendation of 2 doses of pneumococcal vaccine, other guidance suggests more may be required to elicit a protective immune response [3,12].

Low levels of anti-diphtheria IgG was another common finding, and it was reassuring that our cohort responded well serologically post-vaccination. In other studies men were found to have higher circulating levels of anti-diphtheria IgG which fits with our post-vaccination findings [13].

Our measles results were varied, however a link was seen between increasing age and measles seropositivity. This supports other studies which found that individuals with previous measles exposure, who are usually older in age, were more likely to be measles seropositive post-HSCT when compared to their vaccinated counterparts [14]. MMR vaccination is not currently recommended for individuals who are seronegative to mumps or rubella if they are measles seropositive, so a significant proportion of our cohort could remain potentially susceptible to mumps and/or rubella (Supplementary Fig. 2) [1,3]. The rationale may be that the correlates of protection for mumps and rubella are not as reliably defined as measles, plus the epidemiology and severity of each infection differs

[6]. While there have been multiple outbreaks of measles and mumps in the UK, mumps case fatality is low in the HSCT population, whereas measles has a significantly higher morbidity and mortality [6,8,14]. The signal between conditioning regimens, and measles and mumps seronegativity could be explained by more intensive treatment and/or underlying transplant indications; however numbers within our study were small and this requires further investigation in a larger cohort.

On the whole, antibody titres within our post-HSCT cohort were lower than the published data available for the UK population and other immunosuppressed individuals who did not receive HSCT, however direct comparison is challenging due to the different thresholds used (Table 2) [13,15–19]. Interestingly, rates of diphtheria seronegativity were similar in our cohort to a study in Austria of patients with haematological malignancy who did not undergo HSCT, who used the same threshold and testing kit to our cohort [19].

The suboptimal antibody titres, particularly against diphtheria and *S. pneumoniae*, in our cohort who missed RVP favours efforts to optimise immunity in the months post-transplant through RVP. For HSCT recipients who missed RVP, other transplant centres may find ‘late-effects’ clinics as a good platform to screen and recommend pragmatic vaccination advice.

We suggest different approaches for different VPD for those who have not undergone RVP post-transplant. Universal pneumococcal and diphtheria vaccination without checking antibody titres may be a practical approach given the scarcity of adequate titres in our cohort. This recommendation would have the benefit of covering for other VPD within the diphtheria combination vaccine, which has particular relevance to our aging population of patients who likely have waning immunity to tetanus and pertussis, plus pertussis has had a recent upsurge in some developed countries [13,20]. Given most of our cohort had adequate antibody titres to measles and VZV, performing antibody testing prior to vaccination seems sensible given the incidence, morbidity and mortality of both infections [15,17]. As Hib titres were almost always adequate, our data suggests there may be little benefit to check titres or vaccinate, although equally there is

probably no harm, and indeed Hib may be recommended in disease specific guidelines.

More evidence is needed to vaccinate based on immunity to mumps and rubella, and to determine the optimal pneumococcal vaccination dosing and timing. There is scope for future studies to compare antibody titres in those who did receive RVP versus those who did not, the immunogenicity of a pre- vs post-HSCT RVP, and to review post-vaccination antibody responses in the autologous HSCT population on a larger scale, particularly following SARS-CoV-2 vaccination.

Conclusion

Recovered autologous HSCT recipients who had not received RVP post-transplant had significant defects in their humoral immunity against a number of common VPD despite being years post-transplant. Catch-up vaccination resulted in an increase in antibody titres. Whilst further evidence is required to guide vaccination in this setting, our study contributes to existing evidence that earlier strategies to optimise immunity in autologous HSCT patients are warranted.

For those who missed RVP, we suggest different approaches for each VPD, using late-effects clinics as a platform to screen and recommend vaccination. A pragmatic strategy could be to universally vaccinate against diphtheria and pneumococcal infection, and to target measles and VZV vaccination based on serostatus.

Age	Median in years (range)
- At clinic attendance	48 (22–72)
- At HSCT	37.5 (14–69)
Time from diagnosis to HSCT	1 (0–6)
Time since HSCT	7 (0–29)
Sex	n (%)
- Male	26 (46.4)
- Female	30 (53.6)
Childhood vaccination status	n (%)
- Fully vaccinated	34 (60.7)
- Not fully vaccinated	2 (3.6)
- Patient unsure	20 (35.7)
Diagnoses	n (%)
- Haemato-oncological Malignancies [‡]	42 (75.0)
- Lymphoma	36
- Myeloma	3
- Acute Leukaemia	3
- Solid Tumours [†]	10 (17.9)
- Autoimmune Diseases ^Ψ	4 (7.1)
Treatments received pre-HSCT	n (%)
- Chemotherapy [#]	52 (92.9)
- Radiotherapy [∅]	21 (37.5)
- Rituximab	11 (19.6)
Conditioning regimens^{&}	n (%)
- BEAM	35 (62.5)
- Other chemotherapy-only conditioning	13 (23.2)
- TBI-containing conditioning	4 (7.1)
- ATG-containing conditioning	4 (7.1)

Table 1

Clinical demographics characteristics of HSCT patients.

The Abbreviations: HSCT- Haematopoietic Stem Cell Transplant, TBI - total body irradiation, ATG -anti-thymocyte globulin

‡ Lymphomas: Hodgkin n = 18, Diffuse Large B-Cell n = 8, Follicular n = 6, NonHodgkin n = 1, T-Cell Central Nervous System n = 1, Enteropathy-Associated T-Cell n = 1, Anaplastic Large Cell n = 1. Acute Leukaemias: Acute Myeloid Leukaemia n = 2, Acute T-Cell Lymphoblastic Leukaemia n = 1.

† Solid tumours: Gestational Trophoblastic Disease n = 5, Testicular Teratoma / Germ Cell n = 3, Neuroendocrine n = 2.

Ψ Autoimmune diseases: Multiple Sclerosis n = 4.

Regimens included ESHAP (etoposide, methylprednisalone, cytarabine, cisplatin; n = 17), cyclophosphamide (n = 9), CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone; n = 8), ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine; n = 7), methotrexate (n = 7), RCHOP (rituximab plus CHOP; n = 6), Ch1VPPI (chlorambucil, procarbazine, prednisolone, vinblastine; n = 6), EP/EMA (etoposide/cisplatin, etoposide/methotrexate/dactinomycin; n = 5),

PAB10EI (prednisalone, doxorubicin, bleomycin, vincristine, etoposide; n = 5), rituximab (n = 5), TP/TE (paclitaxel/cisplatin, paclitaxel/etoposide; n = 4), VAPEC-B (vincristine, doxorubicin, prednisolone, etoposide, cyclophosphamide, bleomycin; n = 3), R-ESHAP (rituximab plus ESHAP; n = 2), IVE (ifosfamide, epirubicin, etoposide; n=2), DHAP (dexamethasone, cytarabine, cisplatin; n = 2), BEP (bleomycin, etoposide, cisplatin; n = 2) and R-CVP (rituximab, cyclophosphamide, vincristine, prednisolone; n = 2).

Ø Radiotherapy - Mediastinal only n = 5, Mantle n = 4, Pelvis n = 3, Neck only n = 3, Mediastinum/Neck n = 2, Whole Brain n = 2, Para-aortic Nodes n = 1, Stereotactic Radiosurgery of Brain n = 1

& BEAM conditioning regimen includes BiCNU (Carmustine), Etoposide, Ara-C (Cytarabine) and Melphalan. Other conditioning chemotherapy agents were used either alone or in combination - melphalan, cyclophosphamide, carboplatin, etoposide, paclitaxel, ifosfamide and busulfan.

	Study group		UK General population [13,15–18]		Immunosuppressed individuals (NO HSCT)[19]		
	%	Threshold	%	Threshold	Solid malignancies %	Haematological malignancies %	Threshold
Diphtheria							
All ages	98.2	1.0 IU/ml	N/A	-	N/A	N/A	-
All ages	76.8	0.1 IU/ml	59	0.1 IU/ml	40.51	76.09	0.1 IU/ml
<35 years	37.5	0.1 IU/ml	30	0.1 IU/ml	N/A	N/A	-
>35 years	82.6	0.1 IU/ml	69–76	0.1 IU/ml	N/A	N/A	-
Measles	34.5	15 AU/ml	1–10	N/A	3.70 *	13.04*	275 IU/ml*
Mumps	50.9	10 AU/ml	N/A	-	12.73*	39.13*	22 RU/ml*
Rubella	29.1	10 IU/ml	N/A	-	17.17*	30.43*	26 IU/ml*
VZV	10.9	150 mIU/ml	1.6–10	N/A	11.83*	32.61*	400 IU/ml*
Pneumococcal	96.4	0.35 µg/ml	N/A	-	N/A	N/A	-
	100	1.0 µg/ml	N/A	-	N/A	N/A	-
Tetanus	20.0	0.1 IU/ml	17	0.1 IU/ml	11.11	13.04	0.1 IU/ml
Hib	5.5	0.15 µg/ml	5	N/A	N/A	N/A	-

Table 2

Seronegativity in our study cohort compared to other cohorts in the literature.

* Different testing methods and thresholds were used to define serostatus so are difficult to directly compare

N/A – Not available.

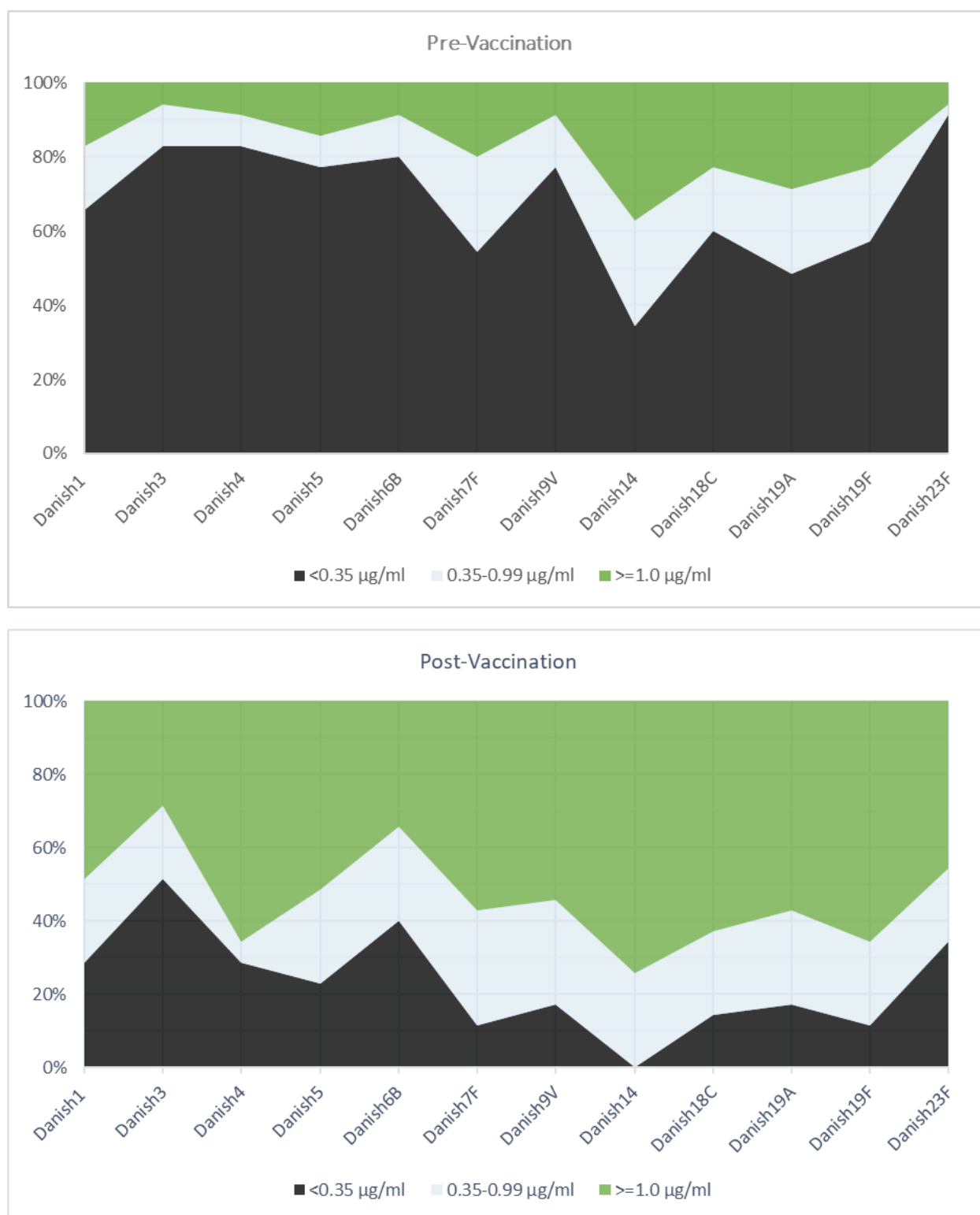


Figure 1. Percentage of patients with antibody titres against each Pneumococcal serotype, Pre- and Post- Vaccination.

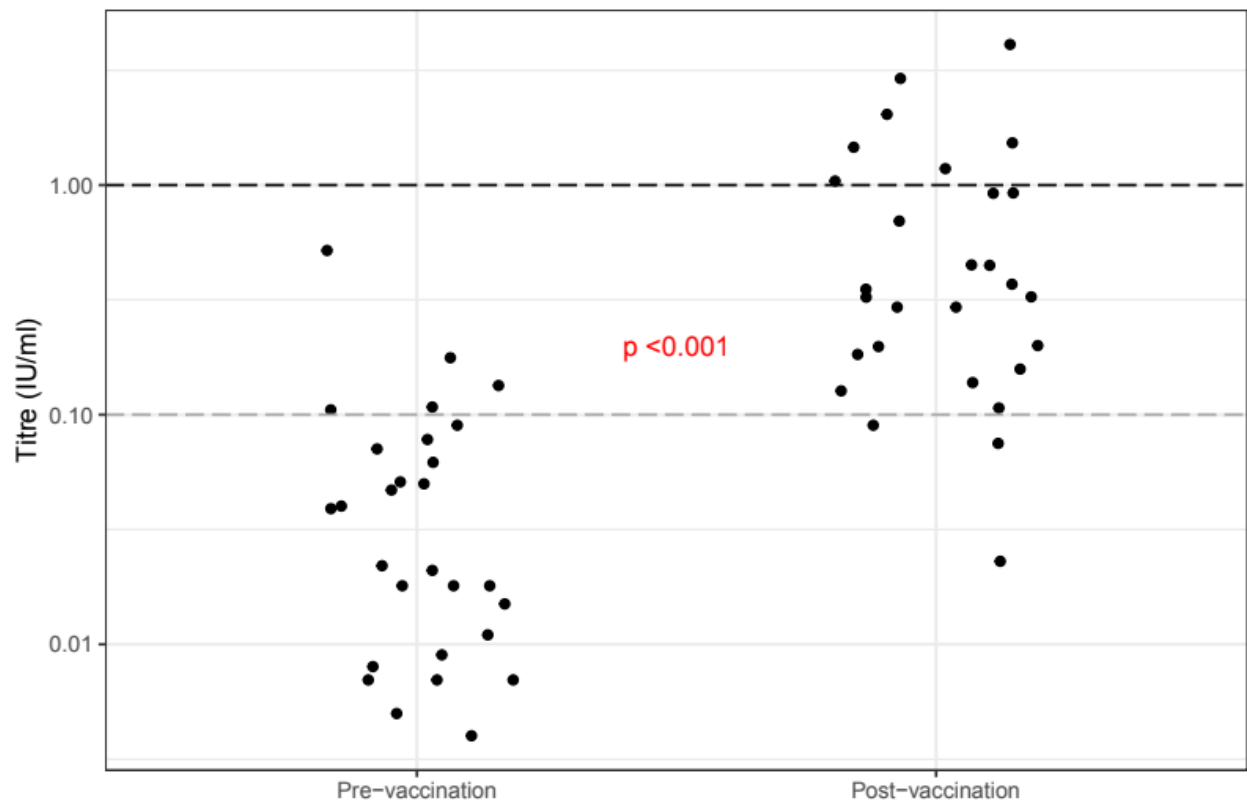


Figure 2. Comparison of Diphtheria titres pre and post vaccination

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Diana Greenfield is a National Institute for Health Research (NIHR) Senior Nurse Research Leader. The views expressed in this article are those of the author and not necessarily those of the NIHR, or the Department of Health and Social Care.].

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