JCI insight

Fatal COVID-19 outcomes are associated with an antibody response targeting epitopes shared with endemic coronaviruses

Anna L. McNaughton, … , Sunetra Gupta, Craig P. Thompson

JCI Insight. 2022. https://doi.org/10.1172/jci.insight.156372.

Research In-Press Preview **Immunology** **Infectious disease**

The role of immune responses to previously seen endemic coronavirus epitopes in severe acute respiratory coronavirus 2 (SARS-CoV-2) infection and disease progression has not yet been determined. Here, we show that a key characteristic of fatal coronavirus disease (COVID-19) outcomes is that the immune response to the SARS-CoV-2 spike protein is enriched for antibodies directed against epitopes shared with endemic beta-coronaviruses, and has a lower proportion of antibodies targeting the more protective variable regions of the spike. The magnitude of antibody responses to the SARS-CoV-2 full-length spike protein, its domains and subunits, and the SARS-CoV-2 nucleocapsid also correlated strongly with responses to the endemic beta-coronavirus spike proteins in individuals admitted to intensive care units (ICU) with fatal COVID-19 outcomes, but not in individuals with non-fatal outcomes. This correlation was found to be due to the antibody response directed at the S2 subunit of the SARS-CoV-2 spike protein, which has the highest degree of conservation between the beta-coronavirus spike proteins. Intriguingly, antibody responses to the less cross-reactive SARS-CoV-2 nucleocapsid were not significantly different in individuals who were admitted to ICU with fatal and non-fatal outcomes, suggesting an antibody profile in individuals with fatal outcomes consistent with an original antigenic sin type-response.

Title:

Fatal COVID-19 outcomes are associated with an antibody response targeting epitopes shared with endemic coronaviruses

Authors:

Anna L McNaughton[1,2]*, Robert S Paton[1,3]*, Matthew Edmans[1,2,3], Jonathan Youngs[4], Judith Wellens[1,5,6], Prabhjeet Phalora[1,2], Alex Fyfe[1,3], Sandra Belij-Rammerstorfer[7], Jai S Bolton[1,3], Jonathan Ball[8], George Carnell[9], Wanwisa Dejnirattisai[10], Christina Dold[11], David W Eyre[,12], Philip Hopkins[13], Alison Howarth[14], Kreepa Kooblall[15], Hannah Klim[1,3,16], Susannah Leaver[8], Lian Lee[1,2], César López-Camacho[10], Sheila F Lumley[1,2,14], Derek Macallan[4], Alexander J Mentzer[10], Nicholas M. Provine[17], Jeremy Ratcliff[1,2], Jose Slon-Compos[10], Donal Skelly[1,18,19], Lucas Stolle[20], Piyada Supasa[10], Nigel Temperton[21], Chris Walker[22], Beibei Wang[10], Duncan Wyncoll[23], OPTIC consortium, SNBTS consortium, Peter Simmonds[1,2], Teresa Lambe[7], Kenneth Baillie[24], Malcolm G Semple[25], Peter JM Openshaw[26], ISARIC4C Investigators, Uri Obolski[27,28], Marc Turner[29], Miles Carroll[10,30], Juthathip Mongkolsapaya[10,31,32], Gavin Screaton[10,32], Stephen H Kennedy[33], Lisa Jarvis[29], Eleanor Barnes[1,2,5], Susanna Dunachie[1,14,34], José Lourenço[1,3], Philippa C Matthews[1,2,14], Tihana Bicanic[4], Paul Klenerman[1,2,6], Sunetra Gupta[1,3], Craig P Thompson[1,3,35]

* These authors contributed equally.
Corresponding author: Craig Thompson (craig.thompson@warwick.ac.uk),
Warwick Medical School, University of Warwick, Coventry, CB7 7AL, 024 7657 4880

Associations:

1. Peter Medawar Building for Pathogen Research, South Parks Road, Oxford, UK
2. Nuffield Department of Medicine, University of Oxford, Oxford, UK
3. Department of Zoology, University of Oxford, Oxford, United Kingdom
4. Institute of Infection & Immunity, St. George's University of London, London, UK
5. Translational Gastro-intestinal Unit, Nuffield Department of Medicine, John Radcliffe Hospital, Oxford
6. Translational Research for Gastrointestinal Diseases, University hospitals Leuven, Herestraat, Leuven, Belgium
7. The Jenner Institute Laboratories, University of Oxford, Oxford, UK
8. Intensive Care Medicine, St. George's University Hospital NHS Trust, London UK
9. Department of Veterinary Medicine, University of Cambridge, Cambridge, UK
10. Wellcome Centre for Human Genetics, Nuffield Department of Medicine, University of Oxford, Oxford, UK
11. Oxford Vaccine Group, Oxford Vaccine Group, Department of Paediatrics, University of Oxford, UK
12. Nuffield Department of Population Health, University of Oxford, Oxford, UK
13. Centre for Human & Applied Physiological Sciences, School of Basic & Medical Biosciences, Faculty of Life Sciences, & Medicine, King's College, London, UK
14. Department of Microbiology/Infectious Diseases, Oxford University Hospitals NHS Foundation Trust, John Radcliffe Hospital, Oxford, UK
15. Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, University of Oxford, Oxford, UK
16. Future of Humanity Institute, Department of Philosophy, University of Oxford, UK
17. Translational Gastroenterology Unit, Nuffield Department of Medicine, John Radcliffe Hospital, Oxford
18. Nuffield Department of Clinical Neurosciences, University of Oxford

19. Oxford University Hospitals NHS Foundation Trust
20. Department of Biochemistry, University of Oxford, Oxford, UK
21. Viral Pseudotype Unit, Medway School of Pharmacy, University of Kent, Chatham, UK
22. Meso-Scale Diagnostics, Maryland, USA.
23. Intensive Care Medicine, Guys and St Thomas' Hospital NHS Foundation Trust, London, UK
24. Roslin Institute, University of Edinburgh, Edinburgh, UK
25. NIHR Health Protection Research Unit in Emerging and Zoonotic Infections, Institute of Infection, Veterinary and Ecological Sciences, Faculty of Health and Life Sciences, University of Liverpool, Liverpool, UK
26. National Heart and Lung Institute, Imperial College, London, UK
27. School of Public Health, Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel
28. Porter School of Environmental and Earth Sciences, Faculty of Exact Sciences, Tel-Aviv University, Tel-Aviv, Israel
29. National Microbiology Reference Unit, Scottish National Blood Transfusion Service, Edinburgh, UK
30. National Infection Service, Public Health England (PHE), Porton Down, Salisbury, UK
31. Siriraj Center of Research Excellence in Dengue & Emerging Pathogens, Dean Office for Research, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand
32. Chinese Academy of Medical Science (CAMS) Oxford Institute (COI), University of Oxford, Oxford, UK
33. Nuffield Department of Women's & Reproductive Health, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom
34. Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, UK
35. Warwick Medical School, University of Warwick, Coventry, Coventry CV4 7HL, UK

Abstract

The role of immune responses to previously seen endemic coronavirus epitopes in severe acute respiratory coronavirus 2 (SARS-CoV-2) infection and disease progression has not yet been determined. Here, we show that a key characteristic of fatal coronavirus disease (COVID-19) outcomes is that the immune response to the SARS-CoV-2 spike protein is enriched for antibodies directed against epitopes shared with endemic beta-coronaviruses, and has a lower proportion of antibodies targeting the more protective variable regions of the spike. The magnitude of antibody responses to the SARS-CoV-2 full-length spike protein, its domains and subunits, and the SARS-CoV-2 nucleocapsid also correlated strongly with responses to the endemic beta-coronavirus spike proteins in individuals admitted to intensive care units (ICU) with fatal COVID-19 outcomes, but not in individuals with non-fatal outcomes. This correlation was found to be due to the antibody response directed at the S2 subunit of the SARS-CoV-2 spike protein, which has the highest degree of conservation between the beta-coronavirus spike proteins. Intriguingly, antibody responses to the less cross-reactive SARS-CoV-2 nucleocapsid were not significantly different in individuals who were admitted to ICU

with fatal and non-fatal outcomes, suggesting an antibody profile in individuals with fatal outcomes consistent with an original antigenic sin type-response.

Introduction

Four human coronaviruses (HCoV) are currently considered endemic. These include two beta-coronaviruses, HCoV-OC43 and HCoV-HKU1, as well as two alpha-coronaviruses, HCoV-229E and HCoV-NL63. Infection by these viruses causes a mild respiratory illness in the majority of people (1). Over the past two decades, two further beta-coronaviruses have also emerged, SARS-CoV-1 and MERS-CoV. Whilst both viruses have been more pathogenic than endemic coronaviruses, their transmission and subsequent spread has remained limited (2). In 2019 a fifth beta-coronavirus, SARS-CoV-2, emerged which has led to a pandemic with over 100 million cases and upwards of 3 million deaths confirmed to date (3). Several studies have shown that prior infection with other HCoVs induces both cross-reactive antibody and T-cell responses to SARS-CoV-2 (4–7). However, the response to shared epitopes and their relationship to disease progression has not been defined (8).

The spike protein of SARS-CoV-2, which is the primary vaccine target, consists of the S1 and S2 subunits (9, 10). The S1 subunit contains a more variable receptor-binding domain (RBD), which mediates viral entry during the infection process via interaction with the angiotensin-converting enzyme 2 (ACE2) receptor. Antibodies targeting the RBD can be neutralising and have been shown to correlate with protection (11). Endemic HCoV induced antibody responses do not appear to cross-react with the SARS-CoV-2 RBD (9, 12), or at least do so infrequently (10, 13, 14). In contrast, it has previously been reported by several studies that antibodies induced by prior HCoV infections cross-react with the SARS-CoV-2 S2 subunit (7,8,9,10), which is more conserved between beta-HCoV viruses. Several recent studies have shown that prior immunity induced by endemic beta-coronavirus infection to conserved epitopes of the S2 subunit of the spike protein inversely correlates with the production of an antibody response to novel parts of SARS-CoV-2 spike protein, such as the RBD (17, 18).

The targeting of previously seen parts of SARS-CoV-2 in preference to more novel parts of the virus occurs via a mechanism known as ‘original antigenic sin’ (OAS), first described in 1960 by Thomas Francis. OAS can be defined as an immune response

where a response to previously seen epitopes dominates the response to cognate antigens, when encountered at a later exposure (19).

Exposure to antigens shared between SARS-Cov-2 and related HCoVs may affect immunity and infection outcomes as a consequence of 'original antigenic sin' (OAS) (19). For OAS to manifest, antigens need to be shared between primary and secondary exposures (e.g. shared epitopes between HCoVs and SARS-CoV-2). Due to the development of memory, re-exposure to any of the antigens present in the first exposure will result in a robust memory response that will overwhelm and potentially block the development of immune responses to new antigens associated with the secondary exposure (20). If immunity targeting the novel antigens present in the second exposure is needed for protection, OAS could affect disease progression and will differ across populations based on antigenic exposure histories.

Cross-reactive T-cell responses have also been reported to be present in many individuals (5, 7, 21, 22), which may have been induced by prior infections with endemic HCoVs. These studies found CD4+ and CD8+ T cells reactive to SARS-CoV-2 spike peptide pools in blood samples from individuals unexposed to SARS-CoV-2 (5, 7, 21). This indicates that prior exposure to endemic HCoVs could confer a protective cross-reactive T-cell response in a subset of the population (23).

In this study, we determine if antibody responses to shared endemic coronavirus and SARS-CoV-2 epitopes could be used to characterise groups or cohorts with defined clinical outcomes. Consequently, we retrospectively tested samples against a panel of coronavirus antigens from individuals who previously had qRT-PCR-confirmed asymptomatic infection, as well as individuals admitted to intensive care units (ICU) with severe COVID-19, half of whom died (24).

We also analysed two large cohorts with SARS-CoV-2 neutralising antibodies obtained from UK seroprevalence studies: one containing sera from blood donors and the other sera collected from pregnant women sampled at <14 weeks gestation (25, 26). These two cohorts did not have a precise clinical definition of SARS-CoV-2 infection severity. As a third control cohort, we included SARS-CoV-2 seronegative individuals from the same blood donor seroprevalence study (26). Further details of the cohorts can be found in Table 1 and Figures S6 and S7.

We show that in comparison to individuals admitted to ICU with non-fatal COVID-19 outcomes, those with fatal outcomes have a lower antibody response to the SARS-CoV-2 RBD and N-terminal domain of the spike protein. However, there was no significant difference in antibody responses to the S2 domain of the spike protein, which is more conserved among endemic beta-coronaviruses. Individuals with fatal COVID-19 outcomes also showed no difference in antibody responses to the less cross-reactive nucleocapsid (27). To our knowledge this is the first-time that the response to shared endemic beta-coronavirus and SARS-CoV-2 epitopes has been shown to be a marker of fatal COVID-19 outcomes.

Results

Individuals with fatal COVID-19 outcomes make lower antibody responses to the SARS-CoV-2 spike protein but not the SARS-CoV-2 nucleocapsid

We used a multi-spot assay system (MSD V-PLEX) to quantify total antibody responses to the SARS-CoV-2 nucleocapsid (N), the SARS-CoV-2 RBD, N-terminal domain (NTD) of the spike, the full-length spike as well as the spike proteins of the four HCoVs and SARS-CoV-1 (Figure 1; (28)). In-house indirect enzyme-linked immunosorbent assays (ELISAs) were also developed for the SARS-CoV-2 RBD and full-length spike, in addition to the full-length alpha- and beta-HCoV spike proteins to confirm the results produced by the MSD V-PLEX assay via a second independent method. Both the MSD assay and in-house ELISAs correlated well (Figure S1). A schematic of the proteins, their subunits and domains used in this analysis can be found in Figure S2, whilst the variability of the associated proteins is shown in Figures S3 & S4.

We found that the antibody titres to the SARS-CoV-2 spike and nucleocapsid were low in convalescent sera from individuals with asymptomatic infections and substantially higher in individuals admitted to ICU with severe COVID-19 outcomes (sampled during acute infection) (Figure 1). However, among those admitted to ICU with severe COVID-19, individuals with fatal outcomes consistently exhibited lower titres to SARS-CoV-2 spike antigens than those with non-fatal outcomes; responses to the SARS-CoV-2 RBD (Figure 1A, t test: $p = 0.01$), and NTD (Figure 1C, t test: $p = 0.02$), as well as the full-length spike (t test: $p = 0.02$) were all higher in the non-fatal cases. In contrast, no

significant difference in antibody responses to the second SARS-CoV-2 antigen, the nucleocapsid protein (N), were identified (Figure 1D, t test: p = 0.99).

Beta-coronavirus responses are enriched in individuals with fatal COVID-19 outcomes

We next compared responses to endemic coronavirus (HCoV) spike antigens in the cohorts to determine how they correlated with COVID-19 clinical outcome. All cohorts previously infected with SARS-CoV-2 showed increased responses to the endemic beta-HCoV spike proteins relative to the unexposed background cohort (Figure 1), suggesting that infection with SARS-CoV-2 induces increased cross-reactive beta-HCoV responses, as reported elsewhere (10, 12, 17).

We found that the increased reactivity to the beta-HCoV spike proteins was also broadly associated with COVID-19 severity. The response to the HCoV-OC43 spike antigen was significantly larger for individuals admitted to ICU with COVID-19 than either the infected (Figure 1F, t test: $p = 2.93\times10^{-6}$) or asymptomatic groups (Figure 1F, t test: $p = 3.73\times10^{-4}$). Similar increases were observed for the beta-HCoV HKU1 spike protein (Figure 1G), although these were smaller in magnitude in comparison to those associated with the HCoV-OC43 spike protein. Increases in responses to shared SARS-CoV-2/HCoV epitopes, termed "back boosts" by several papers, have been previously observed during natural infection and after vaccination (17, 29). Among individuals admitted to ICU with severe COVID-19, antibody responses to endemic beta-HCoV spike proteins were not statistically different between those with fatal and non-fatal outcomes, unlike responses to the SARS-CoV-2 spike protein (Figure 1B, t test: p = 0.83).

There was no comparative increase in antibody responses to alpha-HCoV (HCoV-NL63 and HCoV-229E, Figures 1H & I) spike proteins following SARS-CoV-2 infection in either the blood donor or asymptomatic cohorts. However, smaller increases in responses to alpha-HCoV spike protein were detected in the ICU fatal/non-fatal outcome groups as well as the antenatal control group. For all endemic HCoVs, the antenatal cohort had an elevated antibody spike protein response in comparison to the blood donor cohort, which could not be explained by biases in age or sex, but we postulate these trends could be due to environmental differences (Figure 1H & I).

The S2 subunit of the SARS-CoV-2 spike protein is conserved to a greater extent between beta-HCoVs than other more variable parts of the spike such as the RBD or N-terminal domain (17; Figure S2). As responses to the SARS-CoV-2 spike protein but not the beta-HCoV spike proteins were reduced in individuals with fatal COVID-19 outcomes relative to those with non-fatal outcomes, we next analysed responses to the S2 subunit of the SARS-CoV-2 spike (Figure 2A & B).

To test responses to the S2 subunit of the SARS-CoV-2 spike protein we developed an S2 subunit indirect ELISA. In contrast to the other SARS-CoV-2 spike antigens (the RBD, N-terminal of the spike as well as the full-length spike) measured by both the MSD V-PLEX assay and in-house ELISA, there was no difference in magnitude of the SARS-CoV-2 S2 ELISA responses between the fatal and non-fatal cohorts (Figure 2A: t test: $p = 0.99$).

Furthermore, we found that individuals admitted to ICU with fatal outcomes had antibody responses to the SARS-CoV-2 RBD, NTD, full-length spike and nucleocapsid that correlated strongly with the SARS-CoV-2 S2 antibody responses (Figure 2B). These correlations were absent in individuals in the ICU non-fatal COVID-19 outcomes group, and are denoted by a black cross.

The ratio of total antibody response to the beta-coronavirus spike was then determined as a proportion of the SARS-CoV-2 response (Figure 2C). This demonstrated that the antibody response to SARS-CoV-2 in individuals with fatal COVID-19 outcomes is enriched for antibodies that bind both SARS-CoV-2 and endemic beta-HCoV spike proteins.

ACE2-binding inhibition and pseudotyped SARS-CoV-2 microneutralisation assay responses correlate with disease severity

The neutralising antibody response has been shown to be a key correlate of protection against SARS-CoV-2 infection (11, 30, 31). Three assays were run to determine neutralising antibody responses: a pseudotyped SARS-CoV-2 microneutralisation assay as well as two R-PLEX competition assays measuring the binding capacity of ACE2 to the SARS-CoV-2 spike and RBD, respectively (Figure 3). The assays show entry inhibition, a widely used proxy for live virus SARS-CoV-2 neutralisation capacity (28, 32–34).

Neutralising antibody titres were comparable in both the ICU groups with fatal and non-fatal COVID-19 outcomes, as measured using a pseudotyped SARS-CoV-2 microneutralisation assay (Figure 3A, t test: p = 0.96). R-PLEX ACE2 competition assays using the full-length spike and RBD as antigens were also run. The R-PLEX assay showed no significant difference in binding inhibition between ACE2 and the spike protein (t test: p = 0.83). However, there was significantly lower inhibition of binding between the RBD and ACE2 in the MSD R-PLEX assay for individuals with fatal COVID-19 outcomes (Figure 3B; t test: p = 0.02).

Comparison of the ratio of neutralising antibody responses to total SARS-CoV-2 spike antibody responses showed that ACE2 binding inhibition responses were significantly lower in individuals with fatal COVID-19 outcomes in comparison to those with non-fatal COVID-19 outcomes when measured by the MSD R-PLEX full-length spike but not the RBD inhibition assays (Figure 3D, t test: RBD p = 0.25, Spike p = 0.018, 13). Again, there was no difference between neutralising responses in individuals admitted to ICU with fatal COVID-19 outcomes and those with non-fatal outcomes when neutralisation was measured by pseudotype neutralisation assay (Figure 3C, t test: p = 0.26).

The SARS-CoV-2 antibody response in individuals with fatal COVID-19 outcomes correlate with responses to endemic beta-coronavirus spike proteins

We then analysed how the SARS-CoV-2 antibody response correlated with the antibody response to beta-HCoV spike proteins. We calculated the Spearman's rank correlation coefficients for all pairs of antigens from the MSD V-PLEX assay, split by cohort (Figure 4A). Notably, in the ICU fatal COVID-19 outcome group, responses to the SARS-CoV-2 spike were strongly correlated with the HCoV-OC43 and HCoV-HKU1 spike antigens (Spearman rank correlation: $\rho = 0.89$, $p = 8\times10^{-8}$ and $\rho = 0.78$, $p = 4\times10^{-5}$, respectively). This correlation was present not only for the full-length spike antigen but also for the NTD (Spearman rank correlation: HCoV-OC43 $p = 5\times10^{-7}$; HCoV-HKU1 $p = 4\times10^{-4}$) and RBD (Spearman rank correlation: HCoV-OC43 $p = 2\times10^{-5}$; HCoV-HKU1 $p = 3\times10^{-4}$) of the spike, as well as the SARS-CoV-2 nucleocapsid (Spearman rank correlation: HCoV-OC43 $p = 5.\times10^{-7}$; HCoV-HKU1 $p = 4\times10^{-4}$; Figure 4). Notably, we could not identify statistically significant correlations in the similarly sized asymptomatic and ICU non-fatal COVID-19 outcome groups (Figure 4A).

A linear model fit on the log-scale was used to analyse the correlation of the magnitude of response to either the SARS-CoV-2 NTD or RBD of the spike, the full-length spike, and the SARS-CoV-2 nucleocapsid (Figure 4B) with the HCoV-HKU1 and HCoV-OC43 spike responses in the asymptomatic and ICU fatal/non-fatal COVID-19 outcome groups. Responses between SARS-CoV-2 antigens and the beta-HCoVs correlated strongly in the fatal COVID-19 outcome group with consistently high R^2 values, indicating that for those with fatal COVID-19 outcomes, the SARS-CoV-2 *de novo* antibody response is strongly linked with the responses to shared SARS-CoV-2/HCoV spike protein epitopes. Importantly, these trends were consistent when the linear models were age-adjusted (Table S1).

Our larger blood donor and antenatal control cohorts also showed a weaker correlation between SARS-CoV-2 antibody response and endemic beta-HCoV spike antibody responses, indicating that this phenomenon can also be found to a lesser extent in the general population if sample size is substantially increased, as reported elsewhere (17).

Preferentially targeted epitopes map to the HCoV-OC43 S2 subunit of the spike protein but not the HCoV-OC43 nucleocapsid

In cases of COVID-19 with fatal outcomes, responses to shared epitopes in the HCoV-OC43 spike protein increased to a greater extent than those to the HCoV-HKU1 spike protein (Figure 1F & G). Consequently, to identify the location of the beta-HCoV epitopes causing the correlation, we chose to subdivide the HCoV-OC43 spike protein into the NTD (amino acid [aa] 1-419) as well as the S1 (aa 1-794) and S2 (aa 766-1304) subunits (Figure 5A). The various domains and subunits of the HCoV-OC43 spike protein and the HCoV-OC43 nucleocapsid analysed can be found in Figure S8.

Responses to infection in both ICU fatal and non-fatal SARS-CoV-2 groups demonstrated an increase in response to all regions of the HCoV-OC43 spike protein analysed, although the response to the S2 subunit was considerably greater, indicating that the majority of shared SARS-CoV-2 and beta-HCoV epitopes reside in the S2 subunit.

There were no significant differences in the fold-change of responses to NTD, S1 or S2 subunits of the HCoV-OC43 spike protein between the ICU with non-fatal or fatal outcome groups (Figure 5A). There were median fold-increases of 6.93 (t test: $p = 1\times10^{-3}$), 2.48 (t test: $p = 4\times10^{-3}$), 31.4 (t test: $p = 2\times10^{-18}$) to the NTD, S1 and S2 HCoV-OC43

spike domains, respectively, across the ICU fatal and non-fatal outcome groups in comparison to the blood donor negative control group.

We then fitted a linear regression between the log-concentration of response (as measured by antibody titre) between the HCoV-OC43 responses (the NTD, S1, S2 domains of the spike as well as the nucleocapsid) and either the SARS-CoV-2 full-length spike protein or nucleocapsid (Figure 5B). In individuals with fatal COVID-19 outcomes, there was a strong correlation between antibody responses to the HCoV-OC43 S2 subunit and the SARS-CoV-2 spike (Spearman rank correlation: $p = 6.48 \times 10^{-07}$), which extended to the SARS-CoV-2 RBD (Spearman rank correlation: $p = 3.62 \times 10^{-05}$) and NTD of the spike protein (Spearman rank correlation: p-value = 3.38×10^{-06}), as well as the SARS-CoV-2 nucleocapsid (Spearman rank correlation: Figure 5B & C, p = 0.0018). The SARS-CoV-2 spike S2 subunit has previously been shown to be the major target of antibodies induced by prior endemic coronavirus infection (9,10). In contrast to the S2 spike subunit, antibody responses to both the HCoV-OC43 S1 subunit and NTD correlated poorly with SARS-CoV-2 antibody response in individuals with fatal COVID-19 outcomes (Figure 5C). These trends were also consistent when the linear models were age-adjusted (Table S2).

We next looked in more detail at the HCoV-OC43 nucleocapsid response. Wratil *et al.* have recently shown that responses to the endemic coronavirus nucleocapsids (HKU1, OC43, 229E and NL63) do not increase during COVID-19, and these responses can therefore be used as markers of immunity to endemic coronaviruses prior to SARS-CoV-2 infection (27).

To that end, we analysed the HCoV-OC43 nucleocapsid response in our cohorts. In agreement with Wratil *et al* and Aguilar-Bretones *et al*, we found that HCoV-OC43 nucleocapsid levels in both the ICU fatal and non-fatal COVID-19 outcome groups did not increase above background population levels. Background levels were determined by analysis of the control uninfected blood donor cohort (Figure 5A, indicated by the grey division). Furthermore, there was also no correlation between the HCoV-OC43 nucleocapsid levels and either the spike or the SARS-CoV-2 nucleocapsid (Figure 5B, Spearman's rank correlation: p = 0.99 and p = 0.9, respectively).

When comparing between the two ICU cohorts, we found that there was a significantly higher HCoV-OC43 nucleocapsid response in individuals with fatal COVID-19 outcomes

in contrast to individuals with non-fatal outcomes (Figure 5A; t test: $p = 4\times10^{-4}$), indicating that there was likely to be higher, or more recent exposure to HCoV-OC43 coronavirus prior to SARS-CoV-2 infection in individuals with fatal COVID-19 outcomes.

Discussion

Our study shows that in fatal COVID-19 outcomes, the antibody response to the SARS-CoV-2 spike is enriched for antibodies that bind to conserved epitopes shared with endemic beta-coronaviruses. The majority of these epitopes are found within the S2 subunit of the SARS-CoV-2 spike (Figure 2C & Figure 5).

Individuals with fatal COVID-19 outcomes have a lower *de novo* antibody response to the SARS-CoV-2 RBD and N-terminal domain of the SARS-CoV-2 spike protein (Figure 1A-C). These regions have been shown to be more divergent between the beta-CoVs than the S2 subunit (9, 17). In contrast, the response to the more conserved SARS-CoV-2 S2 subunit of the spike is not significantly different between individuals admitted to ICU, regardless of outcome (Figure 2A-B).

Importantly, antibody responses to a second SARS-CoV-2 antigen, the nucleocapsid protein, are not significantly different between individuals admitted to ICU with fatal and non-fatal COVID-19 outcomes (Figure 1D). As has been reported in Wratil *et al* and Aguilar-Bretones *et al*, we also found that unlike the HCoV-OC43 spike protein, antibody responses to the HCoV-OC43 nucleocapsid did not increase upon SARS-CoV-2 infection (Figure 5A). However, OC43 nucleocapsid antibody responses were higher in individuals admitted to ICU with fatal COVID-19 outcomes, compared to individuals with non-fatal outcomes. This could potentially indicate that individuals admitted to ICU with fatal outcomes had higher levels of immunity to HCoV-OC43 prior to SARS-CoV-2 infection than individuals admitted to ICU with non-fatal outcomes (Figure 5A; 26).

These observations are compatible with the 'original antigenic sin' (OAS) concept, whereby prior immune responses compromise *de novo* responses to a related antigen (27). In this case, prior immunity to the endemic beta-coronavirus HCoV-OC43 or HCoV-HKU1 epitopes shared with SARS-CoV-2 could impair the immune response to novel SARS-CoV-2 epitopes (35).

In this context, upon infection with SARS-CoV-2, memory B cells produced during an individual's previous exposure to either HCoV-OC43 or HCoV-HKU1 recognise conserved epitopes in SARS-CoV-2 and would outcompete naïve B cells, targeting novel parts of the spike protein, in the germinal centre reaction. This could, in theory, lead to diminishment of an effective antibody response, if less protective regions, such as the S2 region of the SARS-CoV-2 spike, are targeted by memory B cells (20).

Several studies have shown that antibodies targeting the RBD of SARS-CoV-2 are associated with protection (11, 31). In addition to these studies, Dejnirattisai *et al* found that antibodies that bound the S2 subunit were less potent neutralisers than antibodies that bound the RBD in focus reduction neutralization tests (FRNT; 33,37).

Therefore, the inhibition or reduction of RBD antibody responses could provide a mechanism by which OAS may lead to a worse clinical outcome. Similar phenomena have been observed for influenza viruses and for SARS-CoV-2 but have not yet been associated with clinical outcome (17, 37, 38).

It is unlikely that immunosenescence is responsible for the observations in this study as responses in the fatal and non-fatal groups to the second SARS-CoV-2 antigen, the nucleocapsid, are not significantly different (Figure 1D, t test; p=0.99). If *de novo* responses were generally impaired in the fatal COVID-19 outcome group, then responses to both SARS-CoV-2 antigens should be equally impaired.

However, it is important to note that whilst we describe an association, our data does not show direct correlation nor provide irrefutable evidence for an immunological mechanism. These findings suggest a number of potential hypotheses, which need validating in further cohorts.

Alternatively, the results outlined in this study could also be due to a yet undefined malfunctioning immune response whereby the immune response to certain novel antigens is inhibited. We cannot also exclude the possibility that some non-RBD binding antibodies could be disease enhancing.

Finally, individuals with severe COVID-19 admitted to ICU had much higher immune responses to both the spike and nucleocapsid than individuals with asymptomatic infection. This is partially due to the timing of the sampling of the asymptomatic

individuals in question (during the convalescent phase) but also due to the greater disease burden and length of infection imposed by severe infection, leading to a greater antibody response, which may make the underlining mechanisms easier to differentiate (31). We would expect that the levels of antibodies in individuals with severe COVID-19 would eventually drop over time to levels similar to those in our convalescent asymptomatic samples (39)

Our data is also in agreement with other studies such as Atyeo *et al.* which outlines that there is a greater spike response in individuals surviving severe COVID-19 in contrast to individuals who die as a consequence of their infections (Figure 1A-C; 30). They also noted that the antibody response in individuals with fatal COVID-19 outcomes are skewed towards nucleocapsid targetting. Consequently, our data agree with Atyeo *et al.* that the spike:nucleocapsid (S:N) ratio can be used as a measure of disease severity. Within our cohorts, the ratio of spike:nucleocapsid respone is skewed towards the nucleocapsid in individuals with fatal COVID-19 outcomes, in constrast to those with non-fatal outcomes (Figures 1B & D).

Our study also builds on Atyeo *et al.* by demonstrating that the response targeted against the spike can be divided into a cross-reactive S2 response which does not differ between ICU fatal and non-fatal COVID-19 outcome groups (Figure 2), and a *de novo* response targetting the RBD and N-terminal domain of the spike, which causes the difference in spike response between the two outcomes (Figure 1A-C). Furthermore our data also agree with Aguilar-Bretones *et al.* which showed IgG B-cell clones activated by prior coronavirus infection, were boosted to the greatest extent in individuals with severe COVID-19 (35).

However, there exist several limitations with our study. The timeframe of sampling has been suggested to be critical in the ability to identify differences in the responses amongst severely ill COVID-19 patients (31). The single timepoint sampled in this study limits the window in which the appearance of *de novo* responses can be examined in fatal COVID-19 cases (Figure S9). Consequently, an earlier timepoint might indicate that neutralising antibodies are generally lower, as opposed to only one out of three neutralisation or ACE2 binding assays showing this feature (Figure 2). This would match with the consistently lower IgG RBD, NTD and spike antibody responses measured by ELISA (Figure 3). The quality of the neutralising antibody response may be affected by OAS-induced blocking of antibody responses to new antigens expressed by SARS-CoV-2 absent in beta-coronaviruses (10, 20).

We also note that our S2 ELISA was run was in the pre-fusion form and that further works needs to be undertaken regarding the inhibition of fusions and other mechanisms, such as antibody-dependent cell cytotoxicity (ADCC), with regard to the role of S2 SARS-CoV-2 spike binding antibodies.

The study is further limited by the absence of longitudinal samples. Longitudinal samples would be ideal to determine the level of prior immunity to endemic HCoVs. In addition to this, whether the key contributing factors are age, sex, time since the last beta-coronavirus infection, infection with specific strains or just serendipity is beyond the scope of this study. Individuals in this study were likely to have been infected with early SARS-CoV-2 variants, and it is also intriguing to consider how these trends would manifest in those infected with more recent emergent variants. This may have implications impacting vaccine efficacy and to this end, the same phenomena should be studied in those vaccinated with spike proteins from early variants of SARS-CoV-2, then exposed to new variants or updated vaccines.

Methods

Enzyme-linked immunosorbent assay (ELISA)

SARS-CoV-2 spike, RBD as well as HCoV-229E, HCoV-NL63, HCoV-HKU1 and HCoV-OC43 spike IgG antibody responses were measured using in-house indirect ELISAs. Further work to characterise the location of the conserved epitopes between HCoV-OC43 and SARS-CoV-2 used the HCoV-OC43, NTD, S1 and S2 subunits. Spike and RBD proteins were produced as per Amanat *et al.* 2020 (41). Further information regarding the antigens used in the analysis can be found below (Supplementary Methods Table 1).

Nunc-Immuno 96-well plates (Thermo Fischer Scientific, USA) were coated with 1.0 μg ml^{-1} of antigen in phosphate buffered saline (PBS) and left overnight at 4 °C. Plates were washed 3x with 0.1% PBS–Tween (PBS/T), then blocked with casein in PBS for 1 hour at room temperature (RT). Serum or plasma was diluted in casein–PBS solution at dilutions ranging from 1:50 to 1:20,000 before being added to Nunc-Immuno 96-well plates in triplicate. Plates were incubated for 2 hours before being washed 6x with PBS/T. Secondary antibody rabbit anti-human whole IgG conjugated to alkaline phosphatase (A3187-1ML, Sigma, USA) was added at a dilution of 1:1000 in casein–PBS

solution and incubated for 1 hour at RT. After a final wash, plates were developed by adding 4-nitrophenyl phosphate substrate in diethanolamine buffer (Pierce, Loughborough, UK), and optical density (OD) was read at 405 nm using an ELx800 microplate reader (Cole Parmer, London, UK).

The positive reference standard was used on each plate to produce a standard curve. A monoclonal antibody standard was used for the RBD/spike ELISAs (34). Pooled HCoV highly reactive sera were used as a standard for the HCoV spike ELISAs.

MSD V-PLEX assay

IgG antibody responses to SARS-CoV-2 spike, RBD, NTD and nucleocapsid and the spike proteins of SARS-CoV-1, HCoV-229E, HCoV-NL63, HCoV-HKU1 and HCoV-OC43 were assessed using the Meso Scale Diagnostics (MSD) Multi-Spot Assay System (MSD, USA). Pre-coated plates ('Coronavirus panel 2') were incubated at RT with Blocker A solution for at least 30 minutes whilst being shaken at 500-700 rpm. Serum or plasma was diluted 1:500 to 1:50,000 and samples were added to the plates in duplicate. Plates were incubated for 2 hours at RT, whilst being shaken at 500-700 rpm throughout. A 1x working concentration of the SULFO-TAG anti-human IgG Detection Antibody was prepared (R32AJ-5, MSD, USA). After incubation with the samples, the plates were washed 3x with 1x MSD wash buffer. Prepared detection antibody solution was added to the plates, which were incubated at RT for 1 hour, whilst being shaken. Plates were then washed 3x with 1X MSD Wash buffer. To read the assay results, MSD GOLD Read Buffer B (provided ready to use) was added to the plate. The plates were read on a MESO QuickPlex SQ 120 (MSD, USA) immediately after adding the buffer.

A 7-point calibration curve of the standards was prepared, along with a negative control. An additional three positive controls provided with the kit were also run on every plate. All standards and controls were run in duplicate. Data from the assay was analysed using MSD Discovery Workbench software, which averaged all the duplicates, generated and fitted all the data to standard curves (28).

Some of the 10 MSD assay plates (enough for 350 samples) were gifted by MesoScale Diagnostics (Rockville Maryland, USA), in addition to the loan for the MESO QuickPlex SQ 120 assay system. No agreements were made with regarding to publication or promotion of the system.

MSD ACE2 competition assay

The ability of antibodies present in serum/plasma to inhibit the binding of angiotensin-converting enzyme 2 (ACE2) to the SARS-CoV full-length spike proteins and RBD domains was assessed using the COVID-19 ACE2 competition assay (MSD, USA). The assay can be used to estimate the neutralising activity of the antibodies present in the samples.

Pre-coated plates were incubated at RT with MSD blocker A solution for at least 30 minutes whilst being shaken at 500-700 rpm. Serum or plasma was diluted at 1:10 to 1:100 and samples were added to the plates in duplicate. Plates were incubated for 1 hour at RT, whilst being shaken at 500-700 rpm throughout. A 1x working concentration of the SULFO-TAG ACE2 detect was prepared. After incubation with the samples, the plates were washed 3x with 1x MSD Wash buffer. Prepared SULFO-TAG ACE2 solution was added to the plates, which were incubated at RT for a further 1 hour, whilst being shaken. Plates were then washed 3x with 1X MSD Wash buffer. To read the assay results, MSD GOLD Read Buffer B (provided ready to use) was added to the plate. Plates were read immediately after adding the buffer on a MESO QuickPlex SQ 120 (MSD, USA)

A 7-point calibration curve of the standards was prepared along with a negative control. All standards were run in duplicate. Data from the assay was analysed using MSD Discovery Workbench software, which averaged all the duplicates, generated and fitted all the data to standard curves.

Pseudotyped virus microneutralisation assay

A lentivirus-based pseudotyped virus system was used to display the SARS-CoV-2 spike protein on its surface using a synthetic codon optimised SARS-CoV-2 expression construct (NCBI reference sequence: YP_009724390.1). Pseudotyped viruses were generated by transfecting HEK293 T/17 cells (ATCC, USA) with 1.0 µg of codon optimised spike protein (plasmid pcDNA3.1), 1.0 µg of gag/pol (plasmid p8.91) and 1.5 µg of a luciferase reporter construct (plasmid pCSFLW) as part of a plasmid-OptiMEM/PEI solution. Transfections were performed in 10 ml of media DMEM 10% FCS, 1% penicillin–streptomycin, 20% L-glutamate and left for 24hrs at 37°C 5% CO_2. Fresh media was added to the cells before leaving them at 37°C 5% CO_2 for 48hrs. Supernatant was then harvested and stored at -80°C.

Target cells (HEK293T) were transfected using Fugene (Promega) 24 hours prior to assay setup with 2.75 ug of ACE2 expression plasmid and 250 ng of TMPRSS2 expression plasmid (24–26).

Phylogenetic analysis

Consensus sequences were generated by aligning sequences using Muscle and curated in AliView before generating consensus sequences using Bioconductor R package "ConsensusSequence". Amino acid sequences were aligned using MAFFT (https://mafft.cbrc.jp/alignment/software/). Maximum likelihood trees were generated using MEGA X (42) with 1000 bootstrap replicates. Trees were midpoint rooted and bootstrap support of greater >70% is indicated. Trees were visualised using Figtree (http://tree.bio.ed.ac.uk/software/figtree/).

Variability analysis

5,000 sequences were randomly selected from the GISAID SARS-CoV-2 protein database. These were curated so only whole sequences were included. All HCoV-HKU1 and HCoV-OC43 sequences were downloaded from NCBI. The proteins sequences were aligned using MUSCLE before being curated. The sequences were aligned to either the SARS-CoV-2 spike or nucleocapsid sequences. Only sequences containing full length sequences with run of uncalled bases <3 were used. The variability determined by counting the possible mismatches at each amino acid position was then determined via counting the number of different amino acids.

Statistics

Over dispersed variables were transformed onto the logarithmic scale (base 10) for between group comparisons for V-PLEX platform concentrations, ELISA optical density and neutralising titres. Unless otherwise specified, a t-test assuming unequal variances was used to test for differences in the mean responses; values were analysed on the logarithmic (base 10) scale unless otherwise stated. A Holm correction was applied to p-values for multiple comparisons. In cases where a fold change or ratio is calculated, the log-scale group means can be compared to zero using a t-test to determine if the group differs from equal concentrations of antigens. Data pertaining to difference in means were tested for normality using the Shapiro-Wilks test. We did not find violations of normality and hence used a Welch t-test for such differences. Reported correlations are Spearman's rank, as the measure is non-parametric and robust to transformation.

Study Approval

Ethical approval was obtained for the Scottish National Blood Transfusion Service (SNBTS) anonymous archive - IRAS project number 18005. SNBTS blood donors gave fully informed consent to virological testing, donation was made under the SNBTS Blood Establishment Authorisation and the study was approved by the SNBTS Research and Sample Governance Committee IRAS project number 18005.

The International Severe Acute Respiratory and emerging Infection Consortium (ISARIC) WHO Clinical Characterisation Protocol (CCP)-UK protocol was developed by international consensus in 2012-14 and activated in response to the SARS-CoV-2 pandemic on 17th January 2020. This is an actively recruiting prospective cohort study recruiting across the United Kingdom (43). Study materials including protocol, revision history, case report forms, study information and consent forms, are available online [https://isaric4c.net/protocols/]. Ethical approval was given by the South Central - Oxford C Research Ethics Committee in England (Ref: 13/SC/0149) and by the Scotland A Research Ethics Committee (Ref: 20/SS/0028).

The antenatal samples were collected during routine antenatal care appointments in the Oxfordshire area. Samples were taken during the first trimester of pregnancy (generally between 8–12 weeks' gestation) between 14 April and 15 June 2020. Ethical approval was obtained from the South-Central Research Ethics Committee (Ref: 08/H0606/139).

Patients and healthcare workers comprising the asymptomatic cohort were recruited from the John Radcliffe Hospital in Oxford, United Kingdom, between March and May 2020. Patients identified during the SARS-CoV-2 pandemic were screened and recruited into the Sepsis Immunomics (IRAS260007) and ISARIC WHO CCP-UK (IRAS126600). Patients were sampled at least 28 days from their positive PCR test. The ICU patients were enrolled as part of an ongoing prospective observational study AspiFlu (ISRCTN51287266) at St George's Hospital, London, UK. Researchers working with the samples in the laboratory were blinded to the clinical outcomes of the ICU patients during testing. None of the study subjects received convalescent plasma therapy.

Author Contributions

Conceptualisation, PK, CPT; Methodology, ALM, RSP, ME, PP, KK, NT, CW, SR, TL, KK; Software Programming, RSP, JB, UO, JL; Formal Analysis, RSP, CPT, KK; Investigation, ALM, ME, AF, JW, HK, JB, JR, LL, LS; Resources, SB-R, GC, WD, CD, DWE, AH, SL, CL-C, SFL, DM, AJM, NP, DS, PS, NT, CW, BW, DW, KK, MGS, JKB, PJMO ISARIC4C Investigators, OPTIC consortium, SBTS consortium, PS, MGS, TL, KB, MT, MC, GS, SHK, LJ EB, SD, PCM, TB; Data Curation Management, ALM, RSP, ME, JY, JW, SB-R, GC, WD, CD, DWE, AH, SL, CL-C, SFL, DM, AJM, NP, DS, PS, NT, CW, BW, DW, ISARIC4C Investigators, OPTIC consortium, SNBTS consortium; Writing – Original Draft Preparation, ALM, RSP, CPT; Writing – Review & Editing Preparation, all authors reviewed and approved the final manuscript; Visualization Preparation, RSP, UO, JL, CPT; Supervision, TL, SD, PK, CPT; Project Administration, ALM, CPT; Funding Acquisition, PK, SG, CPT, MGS, JKB, PJMO.

Acknowledgments

Please see Supplemental Acknowledgement for consortium details.

We would like to express our gratitude and acknowledge the contribution of the staff at Oxford University Hospitals NHS Foundation Trust, Scottish National Blood Transfusion Service and St George's University Hospitals NHS Foundation Trust (London) who were involved in the provision and preparation of samples analysed in this project. We acknowledge the wider support of the ISARIC4C, OPTIC and SBTS research consortia.

This work was supported by the Medical Research Council [grant MC_PC_19059], ME was supported by The Leona M. and Harry B. Helmsley Charitable Trust on May 31, 2021 for the project titled, "ICARUS –IBD: International study of COVID-19., Antibody Response Under Sustained immune suppression in Inflammatory Bowel Disease. RP was supported by funds provided under Professor RW Snow's Wellcome Trust Principal Fellowship (# 212176). Meso Scale Diagnostics (USA) provided loan of equipment, reagents and technical support. JSB was supported by funding from the Biotechnology and Biological Sciences Research Council (BBSRC) [grant number BB/M011224/1]. CPT was funded by an ERC research grant 'UNIFLUVAC' and two MRC CiC grants (Ref: BR00140). ALM is funded by a NIHR Research Capability Funding grant. ALM, RP and SG were supported by the Georg and Emily von Opel Foundation. ALM was supported a National Institute for Health Research (NIHR) [award CO-CIN-01]. HJK is supported by The Future of Humanity Institute at the University of Oxford DPhil Scholarship program.

DE is funded by the Robertson Foundation. PK was funded by a Wellcome Trust grant (ref 222426/Z/21/Z). EB is supported by the Oxford NIHR Biomedical Research Centre and is an NIHR Senior Investigator. The views expressed in this article are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health. The funders played no role in the design, execution or reporting of the study.

Competing interests:

DE declares lecture fees from Gilead. CPT and SG hold funding from Blue Water Vaccines.

References

1. Gaunt ER, et al. Epidemiology and clinical presentations of the four human coronaviruses 229E, HKU1, NL63, and OC43 detected over 3 years using a novel multiplex real-time PCR method. *J. Clin. Microbiol.* 2010;48(8):2940–2947.
2. Petrosillo N, et al. COVID-19, SARS and MERS: are they closely related?. *Clin. Microbiol. Infect.* 2020;26(6):729–734.
3. Li Q, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N. Engl. J. Med.* 2020;382(13):1199–1207.
4. Bacher P, et al. Low avidity CD4+ T cell responses to SARS-CoV-2 in unexposed individuals and humans with severe COVID-19. *Immunity* [published online ahead of print: 2020]; doi:10.1016/j.immuni.2020.11.016
5. Mateus J, et al. Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans. *Science.* 2020;370(6512). doi:10.1126/science.abd3871
6. Braun J, et al. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. *Nature* 2020;587(7833):270–274.
7. Le Bert N, et al. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. *Nature* 2020;584(7821):457–462.
8. Lipsitch M, et al. Cross-reactive memory T cells and herd immunity to SARS-CoV-2. *Nat. Rev. Immunol.* 2020;20(11):709–713.
9. Ng KW, et al. Preexisting and de novo humoral immunity to SARS-CoV-2 in humans. *Science.* 2020;eabe1107.
10. Anderson EM, et al. Seasonal human coronavirus antibodies are boosted upon SARS-CoV-2 infection but not associated with protection. *Cell* [published online ahead of print: 2021]; doi:10.1016/j.cell.2021.02.010
11. Addetia A, et al. Neutralizing antibodies correlate with protection from SARS-CoV-2 in humans during a fishery vessel outbreak with a high attack rate. *J. Clin. Microbiol.* 2020;58(11). doi:10.1128/JCM.02107-20
12. Westerhuis BM, et al. Homologous and heterologous antibodies to coronavirus 229E, NL63, OC43, HKU1, SARS, MERS and SARS-CoV-2 antigens in an age stratified cross-sectional serosurvey in a large tertiary hospital in The Netherlands. *medRxiv* 2020;2020.08.21.20177857.
13. Saunders KO, et al. Neutralizing antibody vaccine for pandemic and pre-emergent coronaviruses. *Nature* 2021;594(7864):553–559.
14. Liu Y, et al. An infectivity-enhancing site on the SARS-CoV-2 spike protein targeted by antibodies. *Cell* 2021;184(13):3452–3466.e18.
15. Ng K, et al. Pre-existing and de novo humoral immunity to SARS-CoV-2 in humans. *Science* 2020;370(6522):1339–1343.
16. Westerhuis BM, et al. Severe COVID-19 patients display a back boost of seasonal coronavirus-specific antibodies. *medRxiv* 2020;2020.10.10.20210070.

17. Aydillo T, et al. Antibody Immunological Imprinting on COVID-19 Patients. *medRxiv* 2020;2020.10.14.20212662.
18. Lapp SA, et al. Original antigenic sin responses to Betacoronavirus spike proteins are observed in a mouse model, but are not apparent in children following SARS-CoV-2 infection. *PLoS One* 2021;16(8 August). doi:10.1371/journal.pone.0256482
19. Francis TJ. On the Doctrine of Original Antigenic Sin. *Proc. Am. Philos. Soc.* 1960;104(6):572–578.
20. Brown EL, Essigmann HT. Original Antigenic Sin: the Downside of Immunological Memory and Implications for COVID-19. *mSphere* 2021;6(2). doi:10.1128/msphere.00056-21
21. Weiskopf D, et al. Phenotype and kinetics of SARS-CoV-2-specific T cells in COVID-19 patients with acute respiratory distress syndrome. *Sci. Immunol.* 2020;5(48). doi:10.1126/SCIIMMUNOL.ABD2071
22. Ogbe A, et al. T cell assays differentiate clinical and subclinical SARS-CoV-2 infections from cross-reactive antiviral responses. *Nat. Commun.* 2021;12(1). doi:10.1038/s41467-021-21856-3
23. Swadling L, et al. Pre-existing polymerase-specific T cells expand in abortive seronegative SARS-CoV-2. *Nature* 2022;601(7891):110–117.
24. Youngs J, et al. Identification of immune correlates of fatal outcomes in critically ill COVID-19 patients. *PLOS Pathog.* 2021;17(9):e1009804.
25. Lumley SF, et al. SARS-CoV-2 antibody prevalence, titres and neutralising activity in an antenatal cohort, United Kingdom, 14 April to 15 June 2020. *Euro Surveill.* 2020;25(42). doi:10.2807/1560-7917.ES.2020.25.41.2001721
26. Thompson CP, et al. Detection of neutralising antibodies to SARS-CoV-2 to determine population exposure in Scottish blood donors between March and May 2020. *Eurosurveillance* 2020;25(42). doi:10.2807/1560-7917.es.2020.25.42.2000685
27. Wratil PR, et al. Evidence for increased SARS-CoV-2 susceptibility and COVID-19 severity related to pre-existing immunity to seasonal coronaviruses. *Cell Rep.* 2021;37(13). doi:10.1016/j.celrep.2021.110169
28. Wellens J, et al. Combination therapy of infliximab and thiopurines, but not monotherapy with infliximab or vedolizumab, is associated with attenuated IgA and neutralisation responses to SARS-CoV-2 in inflammatory bowel disease. *Gut* 2021;gutjnl-2021-326312.
29. Amanat F, et al. SARS-CoV-2 mRNA vaccination induces functionally diverse antibodies to NTD, RBD, and S2. *Cell* 2021;184(15):3936–3948.e10.
30. Dispinseri S, et al. Neutralizing antibody responses to SARS-CoV-2 in symptomatic COVID-19 is persistent and critical for survival. *Nat. Commun.* 2021;12(1). doi:10.1038/s41467-021-22958-8
31. Lucas C, et al. Delayed production of neutralizing antibodies correlates with fatal COVID-19. *Nat. Med.* 2021;27(7):1178–1186.
32. Harvala H, et al. Convalescent plasma therapy for the treatment of patients with COVID-19: Assessment of methods available for antibody detection and their correlation with neutralising antibody levels. *Transfus. Med.* 2021;31(3):167–175.
33. Thompson CP, et al. Detection of neutralising antibodies to SARS coronavirus 2 to determine population exposure in Scottish blood donors between March and May 2020. *Eurosurveillance* [published online ahead of print: January 1, 2020]; doi:10.1101/2020.04.13.20060467
34. Dejnirattisai W, et al. The antigenic anatomy of SARS-CoV-2 receptor binding domain. *Cell* 2021;184(8):2183–2200.e22.
35. Aguilar-Bretones M, et al. Seasonal coronavirus–specific B cells with limited SARS-CoV-2 cross-reactivity dominate the IgG response in severe COVID-19. *J. Clin. Invest.* 2021;131(21). doi:10.1172/jci150613
36. Piccoli L, et al. Mapping Neutralizing and Immunodominant Sites on the SARS-

CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology.. *Cell* 2020;183(4):1024–1042.e21.
37. Mongkolsapaya J, et al. Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever. *Nat. Med.* 2003;9(7):921–927.
38. Linderman SL, et al. Potential antigenic explanation for atypical H1N1 infections among middle-aged adults during the 2013-2014 influenza season.. *Proc. Natl. Acad. Sci. U. S. A.* 2014;111(44):15798–15803.
39. Seow J, et al. Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans. *Nat. Microbiol.* 2020;5(12):1598–1607.
40. Atyeo C, et al. Distinct Early Serological Signatures Track with SARS-CoV-2 Survival. *Immunity* 2020;53(3):524–532.e4.
41. Amanat F, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. *Nat. Med.* [published online ahead of print: 2020]; doi:10.1038/s41591-020-0913-5
42. Kumar S, et al. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 2018;35(6):1547–1549.
43. Dunning JW, et al. Open source clinical science for emerging infections. *Lancet Infect. Dis.* 2014;14(1):8–9.

Figures

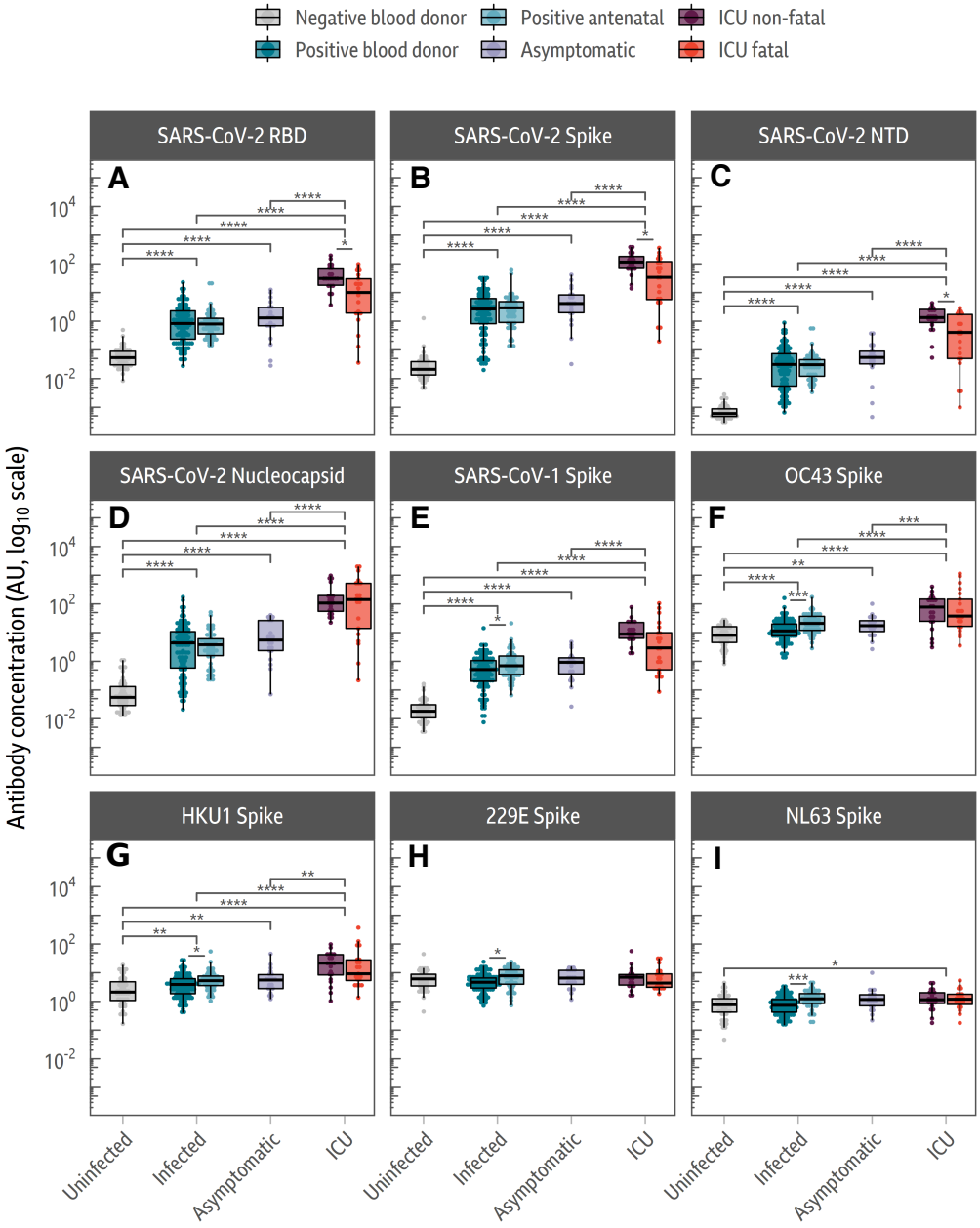


Figure 1. Individuals admitted to ICU with fatal COVID-19 outcomes make lower responses to the SARS-CoV-2 spike protein, but not the SARS-CoV-2 nucleocapsid. Boxplots comparing antibody concentrations for SARS-CoV-2, SARS-CoV-1 and HCoV antigens. Sample groups (background uninfected, infected, asymptomatic and ICU with fatal and non-fatal outcomes) are given on the x-axis. Sub-groups are denoted by colour. The average response to all SARS-CoV-2 antigens were elevated in individuals admitted to ICU with COVID-19, and no differences were observed between the infected and asymptomatic groups. Individuals admitted to ICU with fatal COVID-19 outcomes made a lower response to SARS-CoV-2 RBD (t test: p = 0.01), spike (t test: p = 0.02) and NTD (t test: p = 0.02) than individuals admitted to ICU with non-fatal COVID-19 outcomes. The data in this figure were generated using the MSD V-PLEX assay. t tests were used to assess significance, and the reported p-values were adjusted for multiple comparisons using the Holm-Bonferroni method.

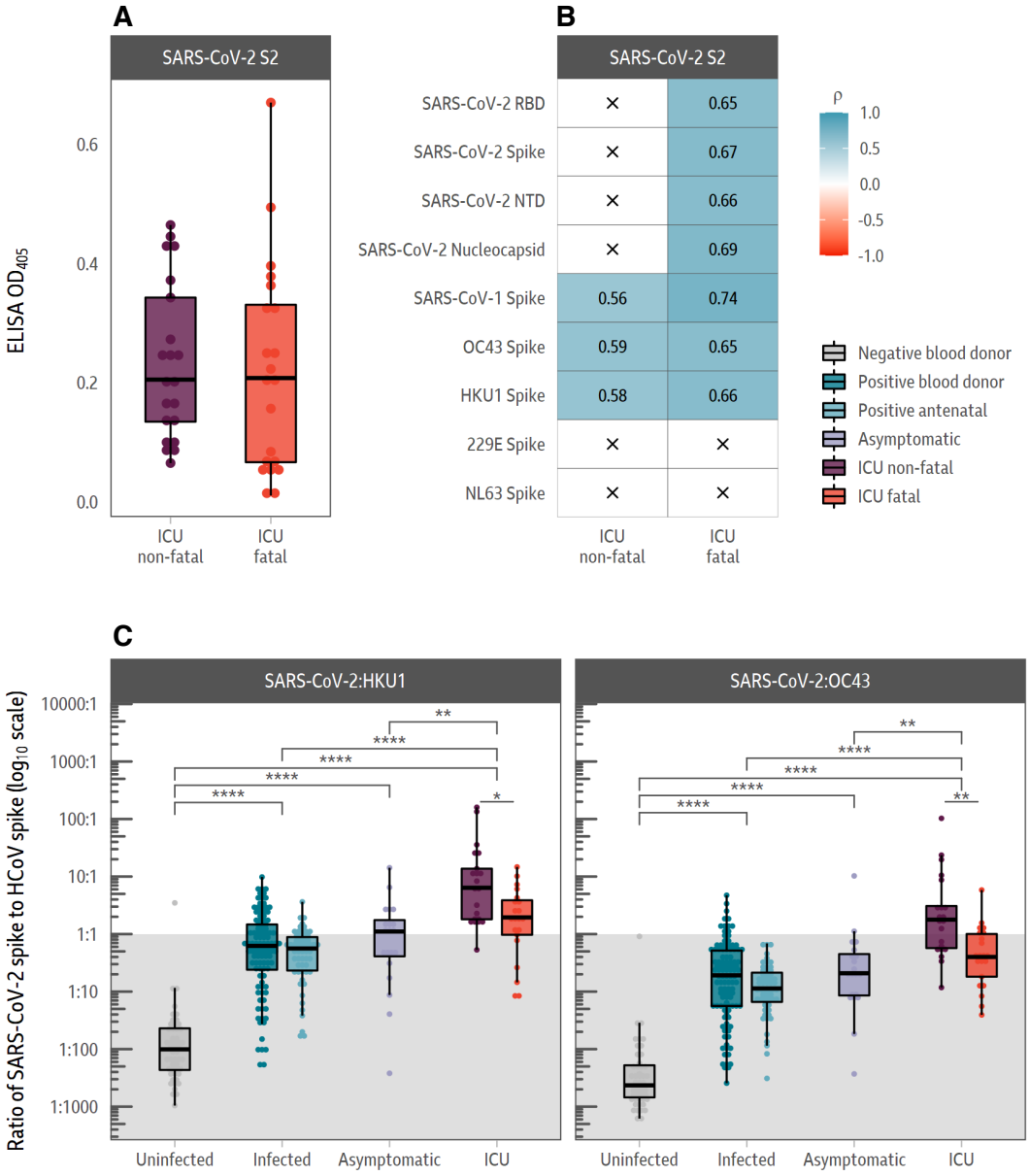
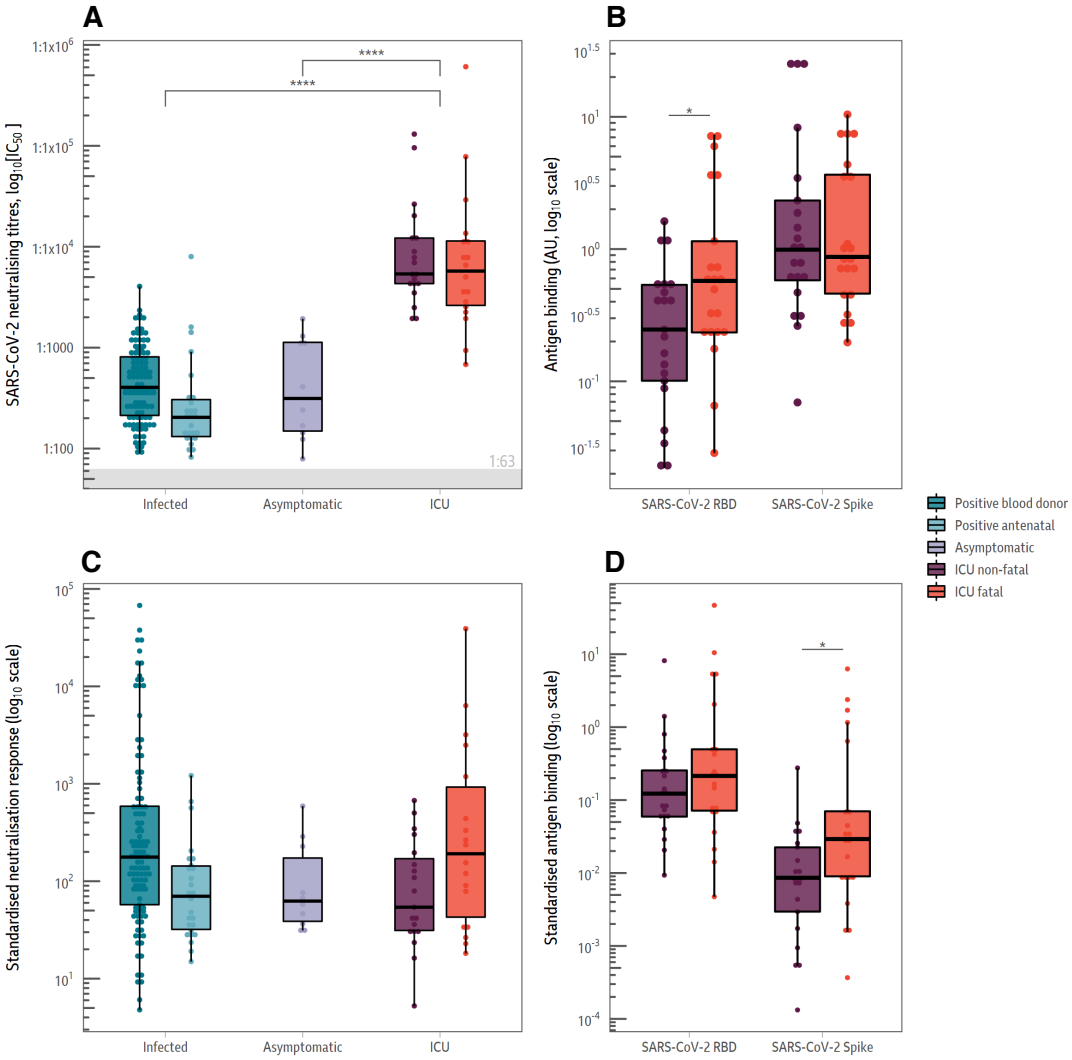
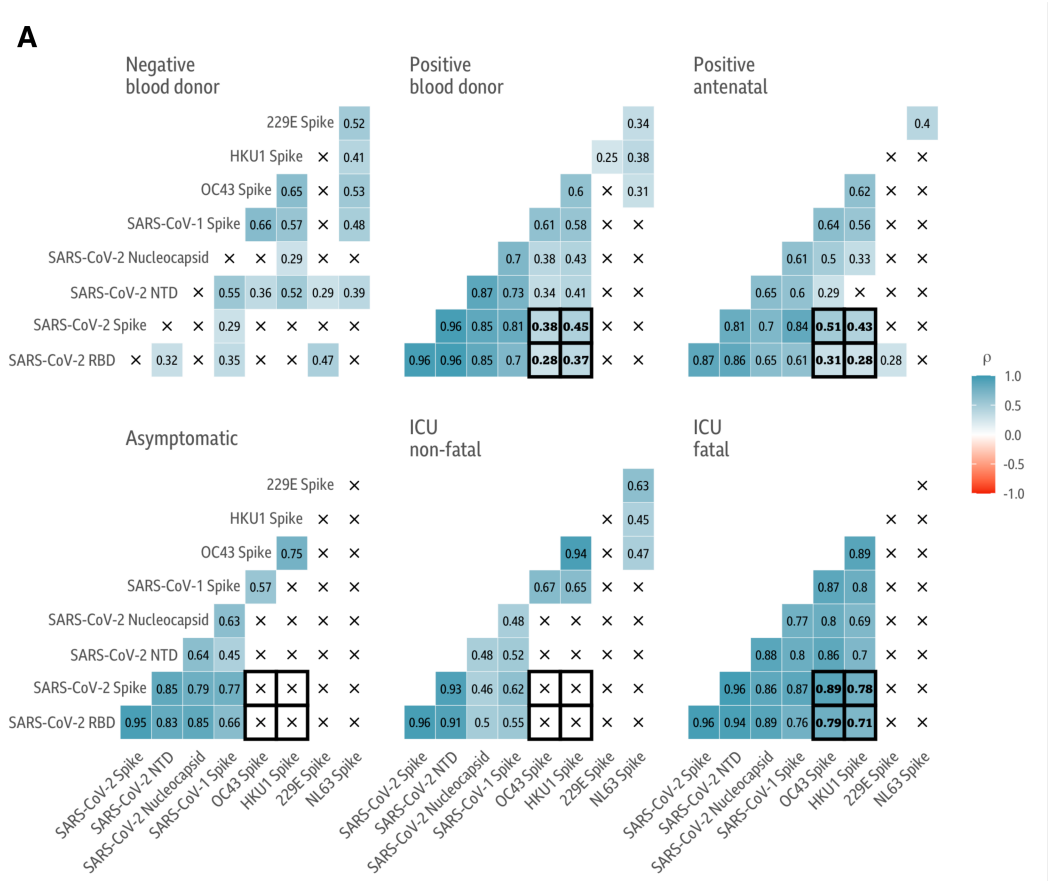


Figure 2. ***Individuals with fatal COVID-19 outcomes have immune responses enriched in antibodies targeting shared SARS-CoV-2 and endemic beta-coronavirus epitopes.*** (**A**) Antibody responses to the S2 subunit of the SARS-CoV-2 spike protein are not statistically different in individuals admitted to ICU with fatal or non-fatal COVID-19 outcomes. (**B**) S2 antibody responses correlate with the SARS-CoV-2 responses in individuals admitted to ICU with fatal COVID-19 outcomes, but not non-fatal outcomes. 'x' indicates the absence of a correlation. (**C**) Ratio of beta-HCoV (HCoV-HKU1 or HCoV-OC43) spike response to SARS-CoV-2 spike response. The grey division in the figure indicates the point at which the ratio of SARS-CoV-2 spike response to beta-HCoV response is lower than 1. t test was used to assess significance, and the reported p-values were adjusted for multiple comparisons using the Holm-Bonferroni method, in A, C. Spearman correlations are shown for each pair of antigens in B.



865 **Figure 3. Neutralising antibody levels correlate with disease severity. a. Samples were tested using a SARS-CoV-2 pseudotype microneutralisation assay**. (**A**) **Neutralising antibody levels. Neutralisation titres were higher in the individuals admitted to ICU with COVID-19.** There was no significant difference between individuals admitted to ICU with fatal or non-fatal COVID-19 outcomes (t test: p = 0.99).
870 (**B**) **ACE2 inhibition assay results.** Samples were also analysed with an MSD R-PLEX ACE2 inhibition assay. The level of ACE2 binding inhibition was not statistically significant for the full-length spike protein, but the individuals admitted to ICU with fatal COVID-19 outcomes show statistically lower ACE2-RBD binding inhibition in comparison to the non-fatal ICU cohort (t test: p = 0.02). (**C**) **Neutralising antibody**
875 **levels as a proportion of total spike antibody response.** There was no statistically significant difference between any of the groups. (**D**) **ACE2-binding inhibition as a proportion of total spike antibody response.** ACE2 binding inhibition responses were significantly lower in individuals with fatal COVID-19 outcomes in comparison to those with non-fatal COVID-19 outcomes when measured by the R-PLEX full-length spike but
880 not the RBD inhibition assays (t test: RBD; p = 0.25, Spike; p = 0.018). t tests were used to assess significance, and the reported p-values were adjusted for multiple comparisons using the Holm-Bonferroni method.


A
Negative blood donor
Positive blood donor
Positive antenatal
Asymptomatic
ICU non-fatal
ICU fatal
229E Spike
HKU1 Spike
OC43 Spike
SARS-CoV-1 Spike
SARS-CoV-2 Nucleocapsid
SARS-CoV-2 NTD
SARS-CoV-2 Spike
SARS-CoV-2 RBD
NL63 Spike
ρ
1.0
0.5
0.0
-0.5
-1.0

885

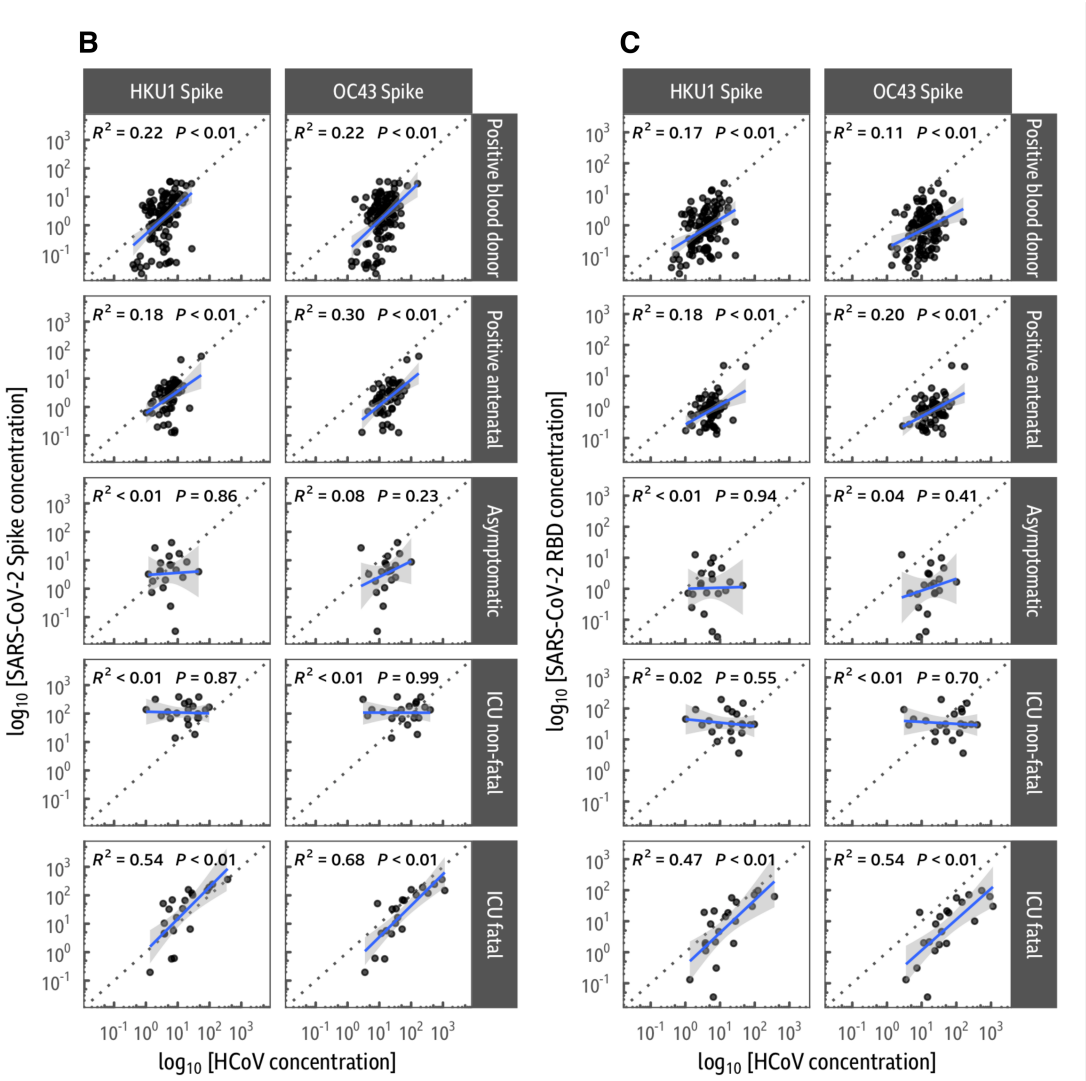


Figure 4. ***In fatal COVID-19 outcomes, antibody responses to SARS-CoV-2 are highly correlated with antibody responses to the endemic beta-coronavirus spike proteins.*** **(A) Correlation between SARS-CoV-2 and endemic coronavirus responses.** Spearman's rank correlations (ρ) are shown for each pair of antigens, split by sample group. There is a positive correlation between all SARS-CoV-2 antigens in all cohorts exposed to SARS-CoV-2. Significant correlations are found between SARS-CoV-2 epitopes and endemic beta-HCoVs (HCoV-OC43 and HCoV-HKU1) in the SARS-CoV-2 antibody positive blood donor, antenatal groups as well as the ICU fatal outcome group. These correlations are absent in the asymptomatic and non-fatal outcome from severe COVID-19 groups. The correlation between endemic beta-HCoVs and SARS-CoV-2 epitopes are considerably weaker in the larger positive blood donor and antenatal cohorts than in the ICU fatal outcome group. **b. and c. Responses to the SARS-CoV-2 spike (B) and receptor-binding domain (C) correlate with beta-coronavirus spike responses in individuals with fatal COVID-19 outcomes.** Correlations are shown with a linear model fit between the concentration of two SARS-CoV-2 antigens and the endemic viruses HCoV-OC43 and HCoV-HKU1. The best fit line is shown in blue with 95% confidence intervals in grey; the dotted grey division denotes a 1:1 response to both antigens. There is a strong positive association between SARS-CoV-2 Spike/RBD and the endemic HCoVs in the fatal outcomes from severe COVID-19 group, which is absent in the similarly-sized asymptomatic and non-fatal outcomes from severe COVID-19 groups.

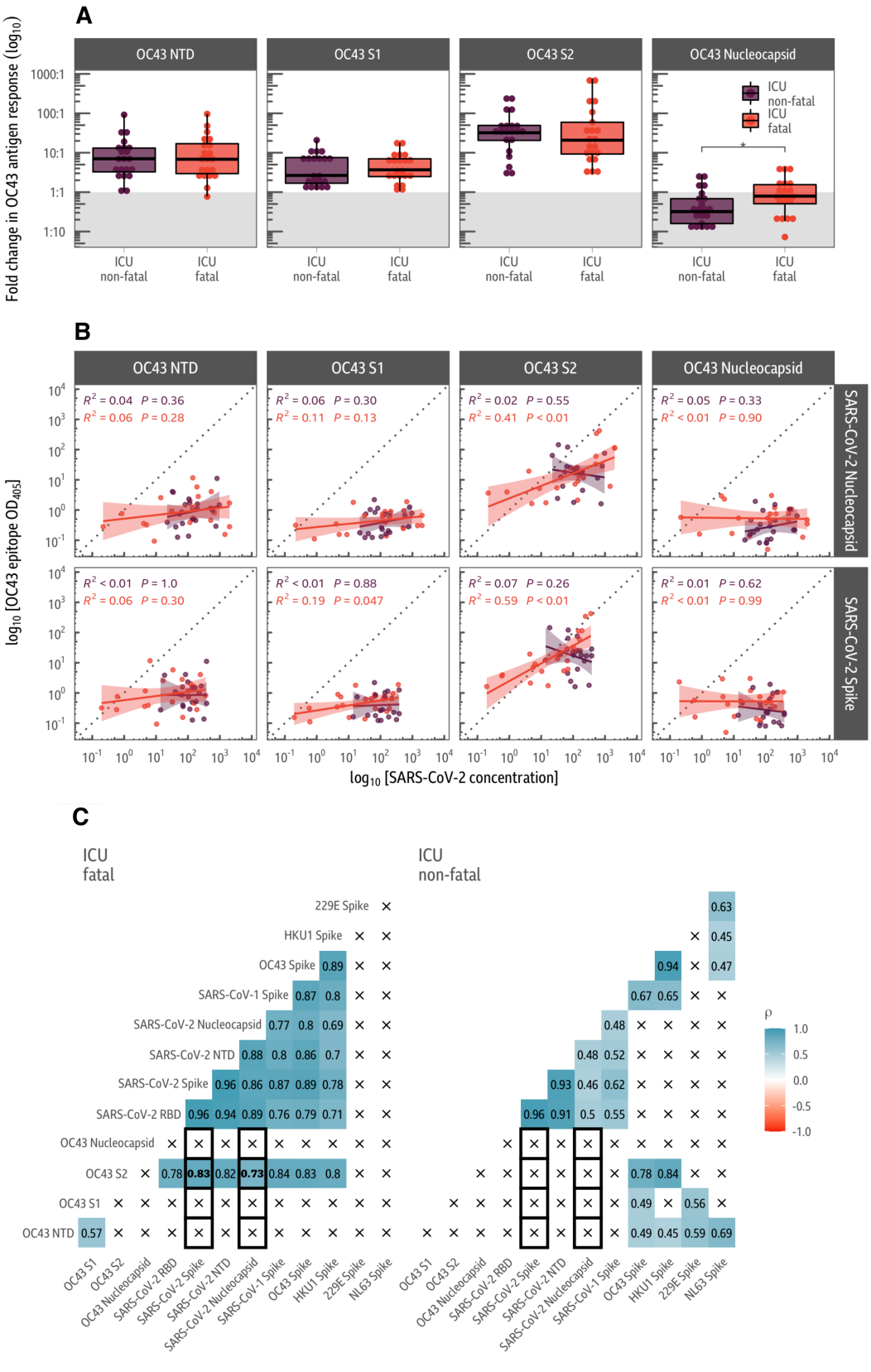

A
OC43 NTD
OC43 S1
OC43 S2
OC43 Nucleocapsid
Fold change in OC43 antigen response (log10)
1000:1
100:1
10:1
1:1
1:10
ICU non-fatal
ICU fatal
*
B
log10 [OC43 epitope OD405]
log10 [SARS-CoV-2 concentration]
SARS-CoV-2 Nucleocapsid
SARS-CoV-2 Spike
R2 = 0.04 P = 0.36
R2 = 0.06 P = 0.28
R2 = 0.06 P = 0.30
R2 = 0.11 P = 0.13
R2 = 0.02 P = 0.55
R2 < 0.01 P < 0.01
R2 = 0.05 P = 0.33
R2 < 0.01 P = 0.90
R2 < 0.01 P = 1.0
R2 < 0.01 P = 0.88
R2 = 0.19 P = 0.047
R2 = 0.07 P = 0.26
R2 = 0.59 P < 0.01
R2 = 0.01 P = 0.62
R2 < 0.01 P = 0.99
C
ICU fatal
ICU non-fatal
229E Spike
HKU1 Spike
OC43 Spike
SARS-CoV-1 Spike
SARS-CoV-2 Nucleocapsid
SARS-CoV-2 NTD
SARS-CoV-2 Spike
SARS-CoV-2 RBD
OC43 Nucleocapsid
OC43 S2
OC43 S1
OC43 NTD
ρ
1.0
0.5
0.0
-0.5
-1.0

Figure 5. ***Antibody responses are directed against the S2 subunit of the HCoV-OC43 spike protein.*** (**A**)**. Fold-change in responses to various domains/subunits in the HCoV-OC43 spike protein and nucleocapsid.** Indirect ELISAs were used to analyse responses to the N-terminal domain (NTD), S1 subunit and S2 subunit of the HCoV-OC43 spike protein, in addition to the HCoV-OC43 nucleocapsid. Fold-change via ELISA was determined relative to the average value in the SARS-CoV-2 antibody-negative blood donor cohort as indicated by the grey division in the figure. Antibody levels are increased against all antigens apart from the nucleocapsid, with the largest increase in antibody response to the S2 subunit of the spike protein. (**B**)**. Correlation in responses between SARS-CoV-2 antigens and HCoV-OC43 spike protein domains and nucleocapsid.** The log-scale OD_{405} values from the HCoV-OC43 spike and nucleocapsid ELISAs (along the rows) is compared to the MSD V-PLEX SARS-CoV-2 results (columns). A linear model fit on the log-scale is annotated with the associated 95% confidence intervals, R^2 and p-value. Values and model fits for the non-fatal COVID-19 outcomes group is given in purple, while red is used for the fatal outcome group. The HCoV-OC43 S2 subunit ELISA result is only correlated with the concentration of SARS-CoV-2 antibodies in the fatal group. (**C**) **Correlations between ELISAs and MSD V-PLEX SARS-CoV-2 assay responses.** Responses to the S2 subunit of HCoV-OC43 are strongly correlated with the MSD concentration of SARS-CoV-2 antibodies in those who died from coronavirus, but not those who survived. Notably, there is a positive correlation between the S2 subunit response and the HCoV-OC43 and HCoV-HKU1 spike responses in the fatal COVID-19 outcome group. t tests were used to assess significance, and the reported p-values were adjusted for multiple comparisons using the Holm-Bonferroni method, in A. Spearman's rank correlations (ρ) are shown for each pair of antigens in B, C.

970

Tables

Table 1. *Features of sample cohorts analysed*

Cohort	N	Identification	Sampling stage	Clinical features	Sex (Female, n, *%*)	Age (Median, yrs, *IQR*)
Scottish Blood Donors (background HCoV exposure samples)	50	Absence of anti-SARS-CoV-2 neutralising antibodies	During routine blood donation	No SARS-CoV-2 infection. Collected between March and May 2020.	24, *48%*	44, *28*
Scottish blood donors (SARS-2 seropositive samples)	109	Detection of anti-SARS-CoV-2 neutralising antibodies (in-house SARS-CoV-2 spike pseudotype neutralisation assay)	During routine blood donation	Evidence of previous SARS-CoV-2 infection, disease course assumed mild-asymptomatic.	50, *45.9%*	49, *25*
Antenatal samples (SARS-2 seropositive samples)	56	Detection of anti-SARS-CoV-2 neutralising antibodies (n=39) ELISA positive for anti-SARS-2 antibodies (n=53)	During routine antenatal screening in the first trimester, generally at 8-12 weeks	Evidence of previous SARS-CoV-2 infection, disease course assumed mild-asymptomatic.	56, *100%*	NA
Asymptomatic samples	22	PCR-positive for SARS-CoV-2	Sampled after confirmation of PCR-positive result	Confirmed SARS-CoV-2 infection, absence of clinical symptoms (from the ISARIC4C and PITCH studies).	NA	NA
Intensive Care Unit (ICU) samples	42	PCR-positive for SARS-CoV-2	Sampled after admission to ICU	Confirmed SARS-CoV-2 infection, patients were admitted to ICU with severe COVD-19. A fatal outcome was recorded in 21/42 cases (from the AspiFlu study).	9, *42.9%* (fatal) 6, *28.6%* (non-fatal)	62, *15* (fatal) 50, *16* (non-fatal)

975