

Poor antibody responses to SARS-CoV-2 infection or vaccination are associated with high re-infection rates in haemodialysis and renal transplant patients

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Research in context

Evidence before this study

We searched PubMed for research articles and pre-prints available between database inception and September 29th, 2021, using the search terms “SARS-CoV-2” OR “COVID-19”, AND (“breakthrough Infection” OR “re-infection”) AND (“transplant” OR “hemodialysis” OR “ESRD” OR “antibody”) with no language restrictions. Article type was set to journal article. We found 317 articles of interest. After title and abstract screening, 29 articles were obtained in full. 2 studies of vaccine-naïve haemodialysis patients (HD) reported reduced relative risk of SARS-CoV-2 infection in participants with pre-existing seropositivity for SARS-CoV-2, when compared to seronegative participants. 1 study reported lower levels of SARS-CoV-2-specific antibody prior to re-infection (RI), when compared to HD patients with a single infection. 3 observational studies measured serological responses post 2-dose vaccination and reported breakthrough infections (BT). 1 reported 2.6% BT rate at mean of 39 days post 2nd dose of BNT162b2 vaccination in a healthcare worker cohort. Mean NAb titres taken on the day of BT diagnosis (n=19 participants) were lower than those of the remaining cohort. 1 reported 1% BT rate (n=9 patients) at a median 94 days post 2nd dose of BNT162b2 vaccination in a cohort of patients with haematological malignancy, with undetectable seroconversion following vaccination in 6/9 BT patients. 1 reported 5% BT rate (n=9 patients) at a median 18 days post 2nd dose of BNT162b2 vaccination in a renal transplant patient cohort, with undetectable seroconversion following vaccination in BT patients.

Added value of this study

This study describes high rates of RI in end-stage renal disease (ESRD) patients, both prior to vaccination (10/46 patients, 22%) and after 2-dose vaccination with AZD1222 at 6-month follow-up (3/61 patients, 5%). ESRD patients who received 2-dose vaccination with BNT162b2 had not developed SARS-CoV-2 infection at 6-month follow-up (n=16). RI prior to vaccination was associated with poor Spike and RBD IgG responses, rather than impaired T cell responses. However, Spike and RBD IgG titres increased following RI. SARS-CoV-2-naïve haemodialysis and renal transplant patients who received 2-dose vaccination with AZD1222 mounted poor surrogate NAb responses in comparison to those who received 2-dose vaccination with BNT162b2 (n=16). However surrogate NAb titres in the AZD1222 cohort increased with infection experience. ESRD patients with repeated infection also developed higher surrogate NAb titres following 2-dose AZD1222 vaccination than those who had a single infection.

Implications of all the available evidence

Patients with ESRD are particularly vulnerable to RI with SARS-Cov-2 despite 2-dose vaccination. High RI rates are associated with poor SARS-CoV-2-specific antibody responses rather than cellular responses. However, SARS-CoV-2-specific IgG and surrogate NAb responses increase with repeated exposure (infection experience and/or vaccination) in those patients who survive infection. Our findings support the case for specific booster regimens in such immune-incompetent patients.

Abstract

Background

Patients with end-stage renal disease (ESRD) are vulnerable to SARS-CoV-2 infection and mount poor antibody responses to standard vaccines. We addressed whether ESRD patients could mount immune responses that protected against re-infection following natural SARS-CoV-2 infection or 2-dose vaccination.

Methods

Haemodialysis (HD and renal transplant patients were recruited following SARS-CoV-2 infection (n=46) or before SARS-CoV-2 vaccination (n=94). SARS-CoV-2 IgG responses, surrogate neutralising antibody (NAb) titres to wildtype and VOCs, T cell responses and viral sequencing in the vaccine-naïve convalescent cohort were serially assessed following infection. Surrogate NAb titres were measured pre-vaccination and 33 days after 2nd vaccine. Incidence of breakthrough infection was assessed 180 days following 1st vaccination.

Findings

22% of vaccine-naïve HD (n=9/36) and transplant patients (n=1/10) demonstrated PCR-positive re-infection (RI) at median 212 days (IQR 140-239) post 1st infection. Prior to RI episodes, RI patients demonstrated poor IgG Spike and RBD responses which were equivalent to levels in pre-pandemic sera (median RI titres: Spike 187 AU/ml, IQR 143-3432, p=0.96; RBD 145 AU/ml, IQR 85-938, p>0.99), unlike patients who developed a single infection only (SI) when compared to pre-pandemic sera (median SI titres: Spike 22826 AU/ml, IQR 1255-63811, p<0.0001; RBD 9588 AU/ml, IQR 270-21616, p=0.001). IgG Spike and RBD titres increased following RI compared to pre-pandemic sera (median RI titres: Spike 22611 AU/ml, IQR 4488-75509, p=0.0006; RBD 6354 AU/ml, IQR 1671-20962, p=0.01). T cell analysis revealed no differences between RI and SI cohorts. Following 2-dose vaccination, 5% of the HD cohort who received AZD1222 (n=3/61) developed breakthrough infection at 6 months following 1st vaccination, unlike those who received BNT162b2 (n=0/16). AZD1222-vaccinated, infection-naïve (I-N) HD patients (n=32) and immunosuppressed transplant recipients (n=17) made poor NAb responses to wildtype, alpha, beta and gamma when compared to infection-experienced (I-E) HD patients (n=29) (I-N vs I-E HD wildtype p<0.0001, alpha p=0.0007, beta p<0.0001, gamma p=0.002). NAb responses improved with BNT162b2 vaccination (n=16); RI patients mounted larger NAb responses to AZD1222 vaccination than SI patients (wildtype p=0.01, alpha p=0.02, beta p<0.02).

Interpretation

ESRD patients are highly susceptible to SARS-CoV-2 re-infection, or breakthrough infection following vaccination, associated with poor protective antibody responses. SARS-CoV-2-specific IgG and surrogate NAb responses increase with repeated exposure (infection experience and/or vaccination) in patients who survive infections. Our findings support the case for specific booster regimens in such immune-incompetent patients.

Funding

Oxford Transplant Foundation, Oxfordshire Health Services Research Committee, UK Department of Health and Social Care, Huo Family Foundation, NIHR (COV19-RECPLAS), UK Coronavirus Immunology Consortium, NIHR Oxford Biomedical Research Centre, WT109965MA.

1 **Introduction**

2
3 There are almost 66 000 patients in the UK with end-stage renal disease (ESRD) who require
4 life sustaining renal replacement therapy (RRT) and who are vulnerable to infection due to
5 complex co-morbidities and profound immunosuppression. The UK Renal Registry and NHS
6 Blood & Transplant have reported case fatality rates of up to 29% in haemodialysis (HD)
7 patients, and over 25% in renal transplant patients hospitalised with COVID-19 (1-4). Poor
8 seroconversion is well-documented following standard vaccination amongst RRT patients
9 (5). Whilst being classified as ‘clinically extremely vulnerable,’ HD patients cannot easily
10 socially distance when required to attend dialysis units several times per week for life-
11 sustaining treatment (6, 7). Moreover, RRT patients are often excluded from population-
12 based immunological studies and vaccine trials (8-10).

13
14 Whilst multiple reports have emerged which describe sustained seroconversion with
15 detectable anti-Spike antibody levels following natural infection or vaccination in HD and
16 transplant patients (11-17), the majority have not examined the more consistent correlates of
17 protection from SARS-CoV-2: neutralising or surrogate neutralising antibody responses to
18 wildtype and emerging variants of concern (VOCs) (18-20). To explore this, we assessed
19 levels of protection in ESRD patients following infection or vaccination. ACE2 inhibition
20 assays were used as a surrogate to measure neutralising antibody responses (NAb) (19, 21),
21 and proliferation assays to assess SARS-CoV-2-specific T cell responses (22). We found that
22 high incidence of re-infection (22%) within the vaccine-naïve convalescent cohort and
23 breakthrough infections (5%) within the AZD1222-vaccinated HD cohort were associated
24 with poor functional antibody responses. Boosted serological responses were observed
25 following both repeated infection and vaccination.

26 **Methods**

27 **Study design and participants**

28
29 Haemodialysis (HD) and transplant cohorts: In this prospective, observational cohort study,
30 HD and transplant patients within Oxford University Hospitals NHS Foundation Trust
31 (OUH) were recruited under Oxford Radcliffe Biobank approved studies, “Biomarkers to
32 stratify risk in Renal Transplant Recipients and Dialysis Patients with Covid-19” (ref: ORB
33 20/A056), and “Immunological responses to COVID-19 vaccines in transplant and
34 haemodialysis patients” (ref: ORB 21/A014). The Oxford Radcliffe Biobank has a favorable
35 ethics opinion from the South Central Oxford Committee C (REC: 19/SC/0173). Patient
36 recruitment is outlined in Supplementary Figures 1a, 1b and 8 (Appendix p17, 30). Patient
37 demographics are summarised in Supplementary Tables 1, 2, 8, 9 and 10 (Appendix p6-7, 13-
38 15).

39
40 Healthcare Worker cohort (HC, PITCH study): PITCH is a sub-study of the SIREN study
41 which was approved by the Berkshire Research Ethics Committee, Health Research 250

Authority (IRAS ID 284460, REC reference 20/SC/0230), with PITCH recognised as a sub-study on 2 December 2020. SIREN is registered with ISRCTN (Trial ID:252 ISRCTN11041050). Some participants were recruited under aligned study protocols. Full details of ethical approvals are included in the Supplementary Information (Appendix, p4). Full details of recruitment and sample collection are described elsewhere (23). Demographics of participants included in the current study are summarised in Supplementary Tables 5, 7 and 11 (Appendix p10, 12, 16).

Details of ESRD patient sample collection are included in the Supplementary Methods (Appendix, p4). Individuals consenting to participate were recruited from hospital-based screening programmes for SARS-CoV-2 and by word of mouth. Individuals were defined as SARS-CoV-2 naïve (infection-naïve) or previously infected (infection-experienced) based on documented PCR and/or serology results within OUH Trust since the study commenced (16/03/2020). If these results were not available for the vaccine cohorts, infection-experienced patients were defined as those who were seropositive at baseline prior to vaccination (baseline samples collected within 6 weeks prior to 1st vaccine; Wildtype Spike titres >0.1 units/ml or positive IgG NP Abbott test). Infection-experienced but vaccine-naïve patients were recruited between 16/03/2020-16/11/2020 and were followed-up until 16/02/2021. Vaccinated patients were recruited between 04/01/2021-28/02/2021 and were followed-up until 31/08/2021. The study was conducted in compliance with all relevant ethical regulations for work with human participants, and according to the principles of the Declaration of Helsinki (2008) and the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines. Written informed consent was obtained for all patients enrolled in the study.

Mesoscale Discovery (MSD) binding assays

IgG responses to SARS-CoV-2, SARS-CoV-1, MERS-CoV and seasonal coronaviruses were measured using a multiplexed MSD immunoassay: The V-PLEX COVID-19 Coronavirus Panel 3 (IgG) Kit (cat. no. K15399U) from Meso Scale Diagnostics, Rockville, MD USA. A multiplexed MSD immunoassay (MSD, Rockville, MD) was also used to measure the ability of human sera to inhibit ACE2 binding to SARS-CoV-2 spike (wildtype, alpha, beta, gamma). Multiplex MSD Assays were performed as per manufacturer's instructions. Full details are in the Supplementary Methods (Appendix, p5).

T Cell proliferation assays

T cell proliferation assays were conducted as previously described (22). Data was analysed on FlowJo v10 and all datapoints are presented as background subtracted data. Positivity threshold of 1% was established from historical data using mean responses in DMSO only wells + 3x standard deviation (SD) (22). Full details are in the Supplementary Methods (Appendix, p5).

RT-PCR

RT-PCR was performed on one of five commercial assays as per manufacturer's instructions: Abbott RealTime (targeting RdRp and N genes; Abbott, Maidenhead, UK); Altona RealStar (targeting E and S genes; Altona Diagnostics, Liverpool, UK); Cepheid Xpert® Xpress SARS-CoV-2 (targeting N2 and E; Cepheid, California, USA); BioFire® Respiratory 2.1 (RP2.1) panel with SARS-CoV-2 (targeting ORF1ab and ORF8; Biofire diagnostics, Utah, USA); Thermo Fisher TaqPath assay (targeting S and N genes, and ORF1ab; Thermo Fisher, Abingdon, UK).

SARS-CoV-2 whole genome sequencing

Viral whole genome sequencing was performed on swabs from all individuals where either a primary sample or RNA extract had been stored. Whole genome sequencing was performed using a multiplex PCR-based approach with the ARTIC LoCost protocol and v3 primers using R9.4.1 flow cells (Oxford Nanopore Technologies, Oxford, UK). Consensus sequences were generated using ARTIC fieldbioinformatics v1.2.1 All sequences underwent quality control, requiring >50% consensus genome coverage at ≥ 20 depth, epidemiological lineages were assigned with Pangolin.

Statistical analysis

Statistical analyses were performed and figures made using GraphPad Prism 9. Testing for normality was performed using D'Agostino Pearson analysis. Non-parametric paired data was analysed using Wilcoxon rank-sum test. Unpaired comparisons of non-parametric data across two groups were performed using the Mann Whitney test. Unpaired comparisons of non-parametric data across multiple groups were performed using the Kruskal-Wallis test with Dunn's post-test for multiple comparisons. Correlative analysis of non-parametric data was performed using Spearman rank correlation. For parametric data, unless otherwise stated, p values are 2-tailed t tests with Welch's correction for single level continuous variables. Fisher's exact test was undertaken for categorical data. Antibody and T cell data are presented on a log₁₀ scaled axis for visualisation with statistical comparisons carried out on untransformed data.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

SARS-CoV-2 IgG and NAb responses in ESRD patients with re-infection

SARS-CoV-2-specific serological responses in convalescent HD and renal transplant patients were compared to historic, pre-pandemic sera (collected between May 2017 to January 2020)

from the same recruited renal cohort. Antigen-specific responses were detectable to Spike, RBD and Nucleocapsid (NP) proteins following SARS-CoV-2 infection in both HD and transplant patients, with minimal cross-reactivity to other seasonal coronaviruses (Figure 1a; Supplementary Figure 2a, Appendix p18). Cross-reactivity was noted, however, with SARS-CoV-1 and MERS-CoV. Spike and RBD IgG titres remained stable over 58-191 days following a single SARS-CoV-2 infection in the HD cohort, whilst NP IgG titres decreased over time (Supplementary Figure 2b-e; Appendix p18). There were no differences in Spike, RBD or NP IgG titres when comparing HD to renal transplant patients (Figure 1a). However, Spike IgG responses were lower in ESRD patients in comparison to those of a healthy Healthcare Worker convalescent cohort (HC) from the PITCH study (23) (Figure 1a; Supplementary Table 5, Appendix p10).

22% of recruited patients developed a PCR-confirmed re-infection (RI) within 47-264 days of the first infection (9/36 in the HD cohort and 1/10 in the renal transplant cohort, median 212 days, IQR 140-239) (Supplementary Table 6; Appendix p11). RIs were diagnosed following either an initial positive PCR (n=8) or positive SARS-CoV-2 anti-nucleoprotein IgG (n=2). Detailed clinical description and sequencing data of the RI cohort are included in the Supplementary Materials (Supplementary Information, Appendix p3; Supplementary Tables 2, 3, 4, 6, Appendix p7, 8, 9, 11). To determine whether RI was associated with lower serological responses in this cohort when compared to the single infection cohort who did not develop repeat infection within the same follow-up period (SI), the latest timepoint available prior to RI (63-188 days post 1st infection; median 141 days, IQR 110-177) and those of SI patients (58-191 days post SI, median 122 days, IQR 67-159) were compared to pre-pandemic sera. RBD and Spike IgG titres post 1st infection within the RI cohort did not increase in relation to pre-pandemic sera. However, significant responses were mounted by the SI cohort (Figure 1b). Stratification across smaller time-ranges (30-90 days, 91-150 days, 151-210 days) following diagnosis of SARS-CoV-2 infection, further illustrated a trend of reduced Spike, S1 RBD and Nucleocapsid IgG titres post 1st infection in the RI cohort throughout the convalescent period when compared to the SI cohort (Supplementary Figure 4a; Appendix p22). These observations suggested that patients who subsequently developed an RI, may have mounted poor SARS-CoV-2-specific serological responses post 1st infection. Longitudinal analysis of SARS-CoV-2-specific IgG responses and of other coronaviruses from all available samples, before and after 1st and 2nd SARS-CoV-2 infections in relation to clinical PCR testing for each RI patient is shown in Supplementary Figure 3 (Appendix p20).

Following the 2nd infection, RBD and Spike IgG titres within the RI cohort (28-48 days post 2nd infection; median 30 days, IQR 29-39) were significantly higher when compared to pre-pandemic sera (Figure 1b). Longitudinal analysis of available paired sera after both 1st and 2nd infections (n=5 patients) suggested a boost in RBD IgG titres in 4 patients following a 2nd infection (Supplementary Figure 4b; Appendix p22). This potential boost following repeat infection was antigen-specific, as no such increase was observed in titres of seasonal coronaviruses following a 2nd infection (Supplementary Figure 4c; Appendix p22). Similar to SI HD patients, cross-reactivity was observed with boosted responses to SARS-CoV-1 following a 2nd infection in the RI cohort (Supplementary Figure 4c; Appendix p22).

Comparison of mean post 1st infection titres across available timepoints per RI patient to those of the SI cohort also demonstrated the same trend of lower SARS-CoV-2-specific IgG responses in the RI cohort, with a boosted response post 2nd infection (Supplementary Figure 4d; Appendix p22).

Assessment of NAb responses, using an ACE2 inhibition assay, to wildtype, alpha and beta VOCs demonstrated that whilst median titres post 1st infection in the RI cohort were lower than those in the SI cohort, these differences were not statistically significant (Figure 1c). A trend of increasing Wildtype and VOC NAb titres were observed post 2nd infection when compared to post 1st infection within RI patients.

SARS-CoV-2-specific T cell responses were also assessed within the SI and RI cohorts and compared to those of a cohort of unvaccinated, convalescent healthcare workers (HC) (Supplementary Table 7; Appendix p12) using a T cell proliferation assay as previously described (Supplementary Figure 5a-b; Appendix p24) (22). Detailed description of T cell responses in these cohorts are in the Supplementary Information (Appendix p3). Whilst the magnitude of the T cell response may be reduced in ESRD patients compared to healthy controls infected with SARS-CoV-2 (Supplementary Figure 6a-b; Appendix p26), there were no discernible differences between the HD and transplant cohorts, nor the SI and RI cohorts (Supplementary Figure 6c, 7a-d; Appendix p26, 28). Taken together, these data suggest that patients who develop poor SARS-CoV-2-specific IgG responses and surrogate NAb titres following infection are more vulnerable to repeat infection. Patients who subsequently survive re-infection may mount an enhanced protective serological response.

NAb responses following 2-dose SARS-CoV-2 vaccination in ESRD patients

Given the high rate of re-infection in unvaccinated ESRD patients, we prospectively recruited 94 HD and renal transplant patients to assess protective serological responses following 2-dose vaccination to SARS-CoV-2 (Supplementary Figure 8; Supplementary Tables 7, 8, 9; Appendix p13-15, 30). Surrogate NAb responses to wildtype SARS-CoV-2 and VOCs alpha, beta and gamma were assessed at a median of 33 days (IQR 31-38) after a 2nd dose.

Infection-naïve HD patients who received AZD1222 (n=32) developed poor NAb responses to wildtype and all VOCs tested when compared to the infection-experienced HD cohort (n=29) (Figure 2a). 31% (n=10) of the infection-naïve cohort demonstrated NAb responses to wildtype that were below the quantitative levels of detection (0.01 units/ml). Comparison of baseline and post-vaccine responses within the infection-experienced cohort confirmed that detectable NAb responses to wildtype, alpha and beta VOCs increased from baseline following 2-dose vaccination with AZD1222 (Supplementary Figure 9a-b; Appendix p31). However, no such increase was observed to gamma. Infection-naïve renal transplant patients made similarly poor NAb responses to wildtype and all VOCs tested following 2-dose AZD1222 vaccination (Figure 2a). There were no differences in demographics of these two cohorts other than immunosuppression exposure (Supplementary Table 8, 10; Appendix p13, 15). Given that only 15.6% of the HD infection-naïve cohort were taking immunosuppression

when compared to 100% of the renal transplant ($p<0.0001$), these observations illustrate the poor efficacy of AZD1222 vaccination in infection-naïve patients with ESRD.

In contrast, NAb responses to wildtype, alpha and beta in infection-naïve HD patients who received BNT162b2 ($n=16$) were markedly increased when compared to the infection-naïve AZD1222 HD cohort ($n=32$) (Figure 2b). Comparison to vaccine-naïve HD SI patients ($n=18$) demonstrated that NAb titres after natural infection were equivalent to those of the infection-naïve BNT162b2 cohort, but higher than those of the infection-naïve AZD1222 cohort. When compared to infection-naïve healthy participants ($n=111$) reported in the PITCH study (23), infection-naïve HD patients demonstrated attenuated NAb responses following BNT162b2 vaccination (Supplementary Figure 9c & Supplementary Table 11; Appendix p16, 31).

Correlative assessment of responses to VOCs in relation to wildtype within each vaccine cohort illustrated that whilst the BNT162b2 vaccine induced comparable responses to each VOC when compared to wildtype (Spearman rank correlation coefficients: alpha 0.96, beta 0.87, gamma 0.82), these correlations decreased within the infection-naïve AZD1222 HD cohort (Spearman rank correlation coefficients: alpha 0.57, beta 0.49, gamma 0.27) (Supplementary Figure 10a-b; Appendix p32). However, these correlations improved within the AZD1222 cohort when in the context of infection-experience (Spearman rank correlation coefficients: alpha 0.93, beta 0.89, gamma 0.33) (Supplementary Figure 10c; Appendix p32).

Of the 10 ESRD patients with RI, 1 had died during the re-infection, 2 withdrew from the study prior to vaccination, 1 was lost to follow-up and 3 died between vaccine doses. The remaining 3 RI patients received 2 doses of AZD1222 vaccine. Comparison of vaccine responses of RI patients ($n=3$) to those of SI patients ($n=26$) within the AZD1222 cohort, illustrated significantly higher protective immune responses in the RI patients to wildtype, alpha and beta (Figure 2c). Evaluation of post-vaccine and post-2nd infection NAb responses in the RI cohort illustrated that vaccination did induce an increase in NAb titres in these patients (Supplementary Figure 11a; Appendix p33). Thus those HD patients who survived repeated infection, were able to mount superior post-vaccine responses to AZD1222 than those who experienced a single infection only.

7% of the HD AZD1222 vaccinated, infection-experienced cohort (2/29 patients) and 3% of the HD AZD1222 vaccinated, infection-naïve cohort (1/32 patients) developed PCR positive breakthrough infections at 6 months following 1st vaccination. Median NAb titres 33 days after 2-dose vaccination in infection-experienced patients with subsequent breakthrough infection were below the median titres for this cohort, although this did not reach statistical significance (Figure 2d; Supplementary Figure 11b, Appendix p33). All 3 patients developed mild symptoms and have not required hospitalisation. No breakthrough infections have yet been identified in the HD BNT162b2 cohort.

Taken together, these observations indicate infection-naïve HD patients and immunosuppressed renal transplant patients who received 2 doses of AZD1222 vaccination,

made equally poor protective serological responses. Two doses of BNT162b2 induced enhanced NAb across wildtype and all VOCs tested in infection-naïve HD patients, when compared to AZD1222. These responses improved in the AZD1222 cohort in patients who had recovered from previous infection. Moreover, repeated infection experience was associated with a boost in protective serological titres. Breakthrough infections were seen in the AZD1222 HD cohort at 6-months, consistent with low Nab titres following vaccination in this cohort.

Discussion

Whilst re-infection rates from SARS-CoV-2 within the general population are generally low (8), higher incidences of potential re-infection (1.6-3.9%) have been reported in UK-based renal cohorts (24, 25). However, detailed immunological analyses of such re-infection cohorts to better understand the mechanisms underpinning these observations, have yet to be described. We report a high rate of re-infection with SARS-CoV-2 (22%) prior to vaccination within patients who had ESRD, the majority of whom could not effectively shield during the pandemic due to the requirement of in-hospital haemodialysis, 2-3 visits/week. Our higher estimate for re-infection rate may reflect early adoption of asymptomatic PCR screening in the hospital haemodialysis cohort in our region (26). Whilst all ESRD patients who survived SARS-CoV-2 infection demonstrated detectable levels of IgG titres to Spike and S1 RBD, and/or mounted antigen-specific CD4⁺ and CD8⁺ T cell responses, some of these responses were lower than those of healthy albeit younger control cohorts. There were no differences in cellular or humoral responses between HD and pharmacologically immunosuppressed transplant patients, emphasising the poor qualitative immune responses of the HD cohort.

Patients who developed RIs demonstrated persistently lower levels of IgG titres to Spike, RBD and NP proteins when compared to SI controls. Whilst there was no significant difference in surrogate Nab titres between the SI and RI cohorts, the median Nab titres to wildtype Spike and VOCs were lower in the RI cohort. Cellular responses were more heterogeneous within the RI cohort, with 2 of 4 patients demonstrating almost non-existent T cell responses after the 1st infection. We did not measure the quality of the T cell response, which could also be a contributing factor. It is likely that poor protective immune responses post 1st infection, together with repeated exposure to emerging VOCs during regular hospital visits and frequent PCR-screening, have culminated in a high rate of RI in ESRD patients when compared to that reported in the general population.

In the majority of RI patients who survived a 2nd infection, a boosted response was observed in IgG titres to Spike, S1 RBD, and surrogate Nab responses following this repeated infection experience. With such small patient numbers, it is difficult to confidently attribute statistical significance to these observations. However, the finding that repeated antigen experience can result in an increased protective immunological response supports the prioritisation of boosters during vaccination regimens in these patients.

1 Surrogate neutralising antibody responses following 2-dose AZD1222 vaccination in
2 infection-naïve ESRD patients were overwhelmingly poor when compared to those of
3 infection-experienced ESRD patients, and to those who had received 2 doses of BNT162b2.
4 Of particular concern, correlation of functional antibody responses to emerging VOCs in
5 relation to wildtype Spike, decreased significantly in the infection-naïve HD cohort who had
6 received AZD1222 vaccination. These findings are consistent with a report of live
7 neutralisation assays post-vaccination in a larger HD cohort (27). All HD and renal transplant
8 patients, regardless of infection experience or vaccine type, exhibited poor or no response to
9 the gamma VOC. Thus whilst it is likely that some protection from SARS-CoV-2 infection is
10 induced upon 2-dose vaccination with existing vaccines, effective protection against
11 emerging VOCs remains questionable in these vulnerable cohorts as the pandemic
12 progresses. Interestingly, within the infection-experienced AZD1222-vaccinated HD cohort,
13 vaccination induced significantly higher surrogate NAb responses in patients who had
14 survived re-infection, when compared to SI patients. These findings reinforce the observation
15 of a booster effect following natural re-infection and emphasise the potential benefit of a
16 specific booster vaccine regimen in these vulnerable patients.

17
18 To the best of our knowledge, this is the first comparison of functional antibody responses in
19 renal transplant recipients to HD patients, after 2 doses of adenovirus-vector-based vaccine to
20 SARS-CoV-2. These finding are consistent with a large-scale study which has recently
21 reported attenuated IgG Spike and RBD responses post-vaccination in renal transplant
22 patients (14). Recent trials of 3rd dose mRNA vaccination in solid organ transplant recipients
23 have reported that 25-44% of previous non-responders subsequently mounted IgG Spike or
24 RBD responses to this 3rd dose, but surrogate neutralising data was not provided (28, 29).
25 Given the heterogeneity in responses to wildtype and emerging VOCs described here, it will
26 be valuable to analyse functional antibody responses following future booster vaccinations in
27 both HD and transplant patients, in order to fully appreciate the levels of relevant protection
28 achieved.

29
30 There are several limitations to this study. Viral sequencing during 1st and 2nd infections were
31 not available for all RI patients. Some patients may have instead exhibited prolonged viral
32 shedding with falsely negative interim PCR tests, or a subclinical course with subsequent
33 reactivation. However, multiple negative PCR results before repeat infection would suggest
34 that this is less probable. Alternatively, the 1st infection detected may have been as a result of
35 a falsely positive PCR swab. However, detectable seroconversion and/or SARS-CoV-2-
36 specific T cell responses in RI patients make this less likely. Paired sera post 1st and post 2nd
37 infection were not available for the entire RI cohort; some patients who displayed strong
38 antibody responses post 2nd infection may not have exhibited a boost following RI, but
39 instead may have mounted a robust serological response post 1st infection that was
40 maintained. Given that study recruitment ranged between March to November 2020, and
41 vaccines were administered from January 2020, duration of follow-up of vaccine-naïve
42 convalescent patients was variable and so RI rates may have been underestimated. It is also
43 important to consider the potential survival bias of patients who survived RI and who
44 mounted boosted responses to both repeated infection and vaccination. Within the vaccine

1 analysis, limitations include small patient numbers within each cohort, which restricted sub-
2 group analyses. However, our findings are consistent with the recently reported, larger cohort
3 study in HD patients (27). Whilst we have to the best of our ability defined those patients
4 who were infection-naïve prior to vaccination, it is possible that some of these patients may
5 not have mounted an antibody response to a previously undetected SARS-CoV-2 infection
6 earlier in the pandemic.

7
8 Despite these limitations, the current study illustrates the underlying immune-incompetence
9 and resulting vulnerability of patients with ESRD, to repeated SARS-CoV-2 infection.
10 Moreover, our findings highlight the likelihood of future breakthrough infections to emerging
11 VOCs despite 2-dose vaccination in these cohorts. Similar to studies of healthy subjects,
12 previous infection appears to confer enhanced protection following vaccination in ESRD
13 patients. SARS-CoV-2-specific IgG and surrogate NAb responses increase with repeated
14 exposure, whether by repeat infection or vaccination. Our findings support the case for urgent
15 booster regimens in HD and renal transplant patients, as well as rigorous post-vaccination
16 assessment of functional antibody responses to emerging VOCs in order to facilitate the
17 interpretation of booster programmes.

Contributors

1 Conceptualised the project – SS, KB, PJF, PK, EB, FI, JH,
2 Designed, supervised and performed T cell experiments – AO, PK
3 Designed, supervised and performed antibody experiments – MC, CD, StL, TT, AH, MK
4 Designed, supervised and performed viral genome sequencing experiments – ShL, DC
5 Established the clinical cohorts and collected the clinical samples and data – SS, JB, MB,
6 FD, GR, FE, KB, AD, LT, AR, SJD, TdS, RPP, EB, PK
7 Provided critical reagents, technical and intellectual expertise – MC, PK, SJD, EB, SS, FI,
8 JH, KB, MB, PJF
9 Analysed the data – SS, JB, TT, AO, MK, CD, ShL, DC, FI, JH, PK, MC
10 Wrote the original draft – SS, KB, JH, FI, ShL, AO, PK
11 Reviewed and edited manuscript and figures – SS, AO, CD, ShL, DC, KB, MB, PJF, JH,
12 FI, EB, SJD, CD, TdS, LT, MC, PK

Data sharing

The data generated in this study are present in the article and its Supplementary Information files or will be made available from the corresponding author upon reasonable request.

Declaration of interests

We declare no competing interests

Acknowledgements

The study is funded by Oxford Transplant Foundation and Oxfordshire Health Services Research Committee, part of Oxford Hospitals Charity. The PITCH Consortium is funded by the UK Department of Health and Social Care, with contributions from UKRI/NIHR through the UK Coronavirus Immunology Consortium (UK-CIC), the Huo Family Foundation and The National Institute for Health Research (UKRIDHSC COVID-19 Rapid Response Rolling Call, Grant Reference Number COV19-RECPLAS).

SS is an NIHR Clinical Lecturer. JB is funded by the Wellcome Trust Institutional Strategic Support Fund (ISSF0007267) and NIHR Research Capability Fund (RCF20/055). FI is a Wellcome Trust CRCD Fellow (211122/Z/18/Z). JH is funded by EU Horizon 2020 Research and Innovation Programme (grant agreement 825392, RESHAPE). TT, StL, AH, LT and MC are supported by U.S. Food and Drug Administration Medical Countermeasures Initiative contract 75F40120C00085. EB and PK are NIHR Senior Investigators and PK is funded by WT109965MA. SJD is funded by an NIHR Global Research Professorship (NIHR300791). TdS is funded by a Wellcome Trust Intermediate Clinical Fellowship (110058/Z/15/Z). RPP is funded by a Career Re-entry Fellowship (204721/Z/16/Z). CJAD is funded by a Wellcome Clinical Research Career Development Fellowship (211153/Z/18/Z). LT is supported by the Wellcome Trust (grant number 205228/Z/16/Z). LT and PK are supported by the National Institute for Health Research Health Protection Research Unit (NIHR HPRU) in Emerging and Zoonotic Infections (NIHR200907) at University of Liverpool in partnership with Public Health England (PHE), in collaboration with Liverpool School of Tropical Medicine and the University of Oxford.

1 The views expressed are those of the author(s) and not necessarily those of the NHS, the
2 NIHR, the Department of Health and Social Care or Public Health England or the US Food
3 and Drug Administration.
4

5 We would like to thank all the staff supporting study delivery at the Oxford Radcliffe
6 Biobank, Oxford Renal Unit, and Oxford Transplant Centre, including those staff within the
7 OUH dialysis units: Oxford, Stoke Mandeville, High Wycombe, Milton Keynes and
8 Swindon. We would like to thank the Modernising Medical Microbiology group (Nuffield
9 Department of Medicine, University of Oxford) for critical support with viral genome
10 sequencing (Bede Constantinides, Nicholas Sanderson, Gillian Rodger, Teresa L Street,
11 Jeremy Swann, Kevin K Chau, Philippa C Matthews, David W Eyre, Nicole E Stoesser,
12 Derrick W Crook, Ali Vaughan, Sarah Hoosdally). We would like to thank Alex Hargreaves
13 for technical support of MSD and ACE2 inhibition assays (Wellcome Trust Centre for
14 Human Genetics, University of Oxford). We would like to thank all participants for their
15 commitment and contributions to this study.
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Figure 1 SARS-CoV-2 IgG and surrogate neutralising antibody responses in ESRD patients with re-infection

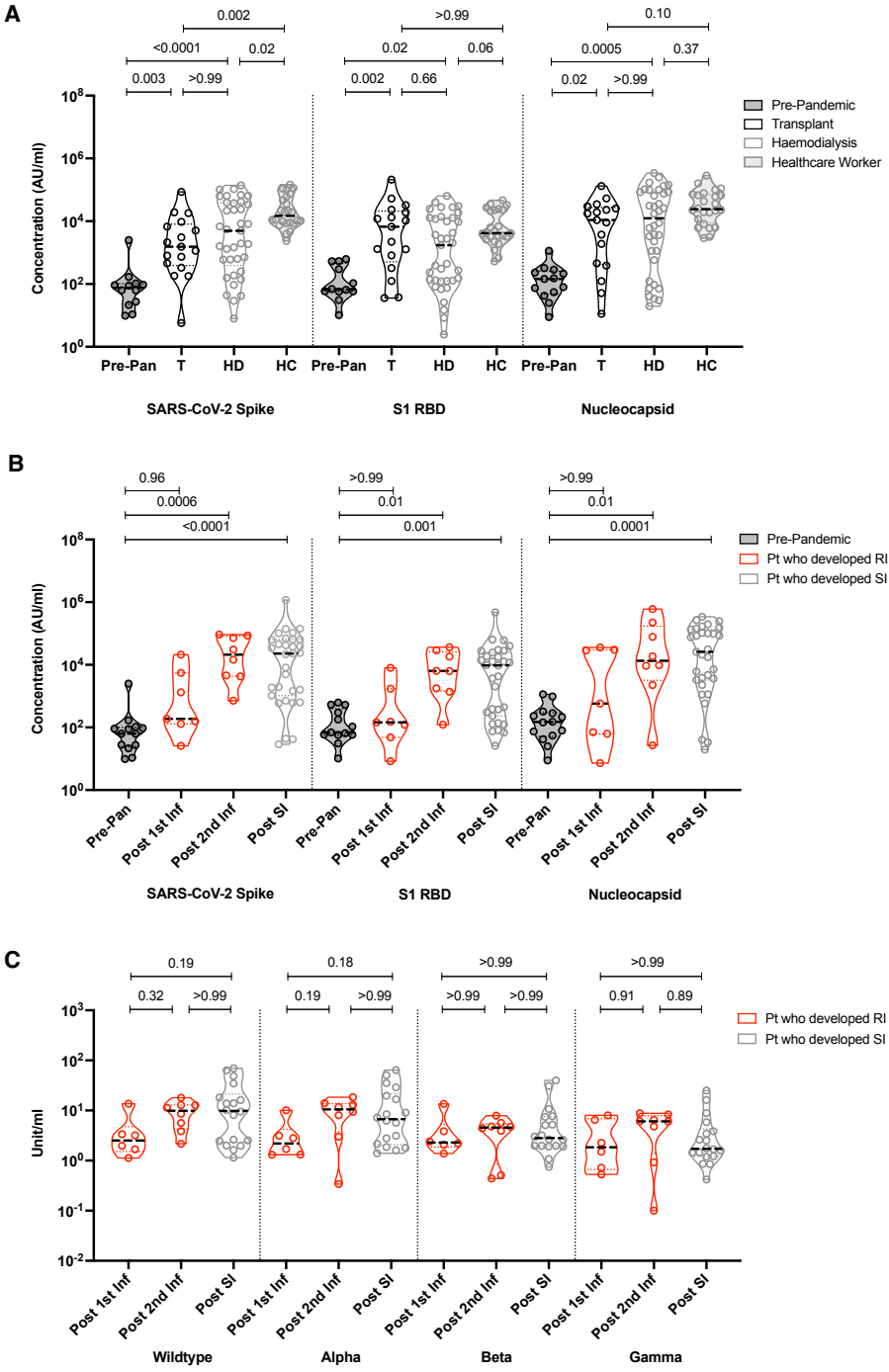


Figure 1 SARS-CoV-2 IgG and surrogate neutralising antibody responses in ESRD patients with re-infection SARS-CoV-2-specific serological responses were determined in convalescent HD and renal transplant cohorts following natural infection with SARS-CoV-2 using a high throughput multiplexed pan-Cov assay. Historic, pre-pandemic sera (collected between May 2017 to January 2020, n=10) from the same ESRD cohort provided baseline controls **a** Comparison of IgG Spike, S1-RBD and Nucleocapsid titres in transplant patients (n=10, 56-192 days post 1st infection, median timepoint post PCR +ve test 109 days, IQR 67-151) and HD patients (n=24, 58-191 days post 1st infection; median timepoint post PCR +ve test 123 days, IQR 67-159) vs Healthcare Workers (HC, PITCH Study; n=23, 78-200 days post infection) post 1st SARS-CoV-2 +ve PCR test. Each dot is an individual response at a specific timepoint post 1st infection, for some patients, multiple timepoints available; bar shows median with 95% CI. **b** Pre-pandemic sera is compared to the latest timepoint available prior to re-infection (n=7 re-infection patients, 63-188 days post 1st infection; median 141 days, IQR 110-177), the timepoint post 2nd infection (n=8 patients, 28-48 days post 2nd infection; median 30 days, IQR 29-39) and to single infection HD patients (n=17 patients, 58-191 days post single infection; median 122 days, IQR 67-159). Each dot is an individual response at a specific timepoint; bar shows median with 95% CI. **c** ACE2 inhibition assays were performed as a surrogate to assess neutralising antibody responses to SARS-CoV-2 Wildtype and VOCs alpha, beta, gamma. Titres in re-infection patients were compared at the latest timepoint prior to re-infection (n=6 patients) to those post 2nd infection (n=8 patients), and to single infection HD patients (n=17 patients). Each dot is an individual response at a specific timepoint; bar shows median with 95% CI. **a, b and c** Statistical analysis to perform unpaired comparisons across multiple groups were performed using the Kruskal-Wallis test with Dunn's post-test for multiple comparisons (adjusted p values displayed). HD = Haemodialysis; Post 1st inf = post 1st infection in an RI patient; Post 2nd inf = post 2nd infection in an RI patient; Pre-Pan = Pre-Pandemic; RI = patient who developed re-infection; SI = patient who developed a single infection only; T = renal transplant.

Figure 2 Surrogate neutralising antibody responses following 2-dose SARS-CoV-2 vaccination in ESRD patients

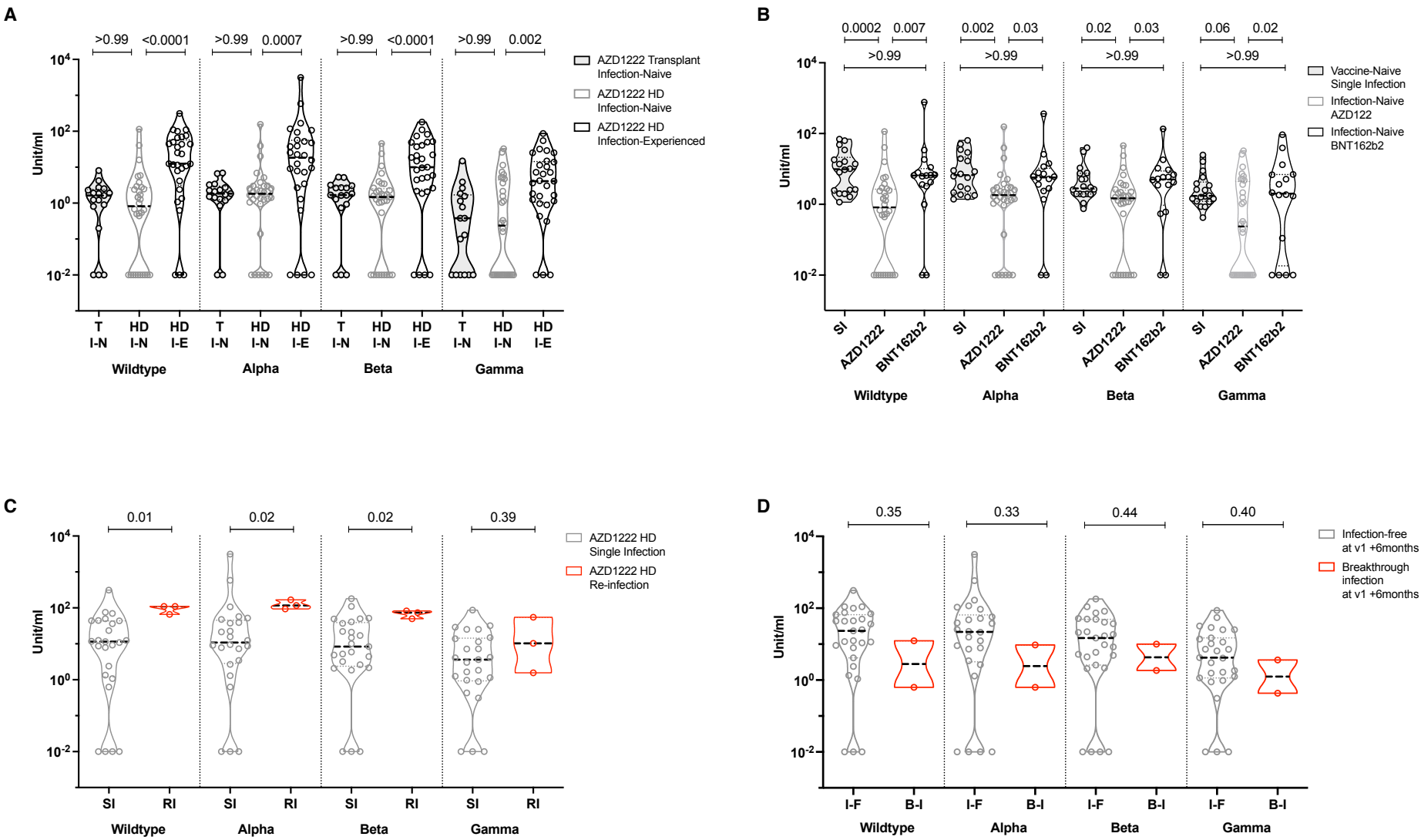


Figure 2 Surrogate neutralising antibody responses following 2-dose SARS-CoV-2 vaccination in ESRD patients. ACE2-Competition assays were performed using MSD -V-Plex serology kits to assess surrogate NAb responses 33 days (IQR 31-38) after the 2nd vaccine dose in haemodialysis (HD) and renal transplant patients, to SARS-CoV-2: wildtype, alpha, beta, gamma. Patients were stratified based on previous experience of SARS-CoV-2-infection and vaccine-type. **a** Comparison of AZD1222 HD Infection-Naïve (n=32) to AZD1222 HD Infection-Experienced (n=29) and to AZD1222 Renal Transplant Infection-Naïve (n=17) **b** Comparison of AZD1222 HD Infection-Naïve (n=32) to BNT162b2 HD Infection-Naïve (n=16) and to vaccine-naïve HD cohort who had recovered from a single infection only (n=) **c** Comparison of AZD1222 HD patients who had previously recovered from a single SARS-CoV-2 infection only (n=26) to AZD1222 HD patients who had previously recovered from repeated SARS-CoV-2 infection (n=3) **d** Comparison of AZD1222 HD Infection-Experienced patients who had remained infection-free at 6-months post 1st vaccine (n=27), to AZD1222 HD Infection-Experienced patients who developed PCR +ve breakthrough infection at 6-months post 1st vaccine (n=2). **a and b** Unpaired comparisons across multiple groups were performed using the Kruskal-Wallis test with Dunn's post-test for multiple comparisons (adjusted P values displayed); **c and d** Statistical analysis done using Mann Whitney 2-tailed T test, each dot is an individual response and bar shows median with 95% CI. B-I = Breakthrough infection at at 6-months post 1st vaccine; HD = Haemodialysis; I-E = Infection-Experienced; I=N = Infection-Naïve; I-F = Infection-free at 6-months post 1st vaccine; RI = patient who developed re-infection; SI = patient who developed a single infection only; T = renal transplant; v1 = 1st vaccine.