Monitoring the Immune Response to Coronaviruses in SARS-CoV-2 Exposed and Vaccinated Individuals

Mingdi Wu[1,a*], Brian J Willett [2,b], Maria Manali[3,c], Irfanulhaq Ahmadzai[4,d], Xiaoyu Dong[5,e]

1. College of Medical, Veterinary & Life Sciences, University of Glasgow, Glasgow G128QQ, United Kingdom.

2. MRC-University of Glasgow Centre for Virus Research, Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, United Kingdom.

3. MRC-University of Glasgow Centre for Virus Research, University of Glasgow, Glasgow G12 8QQ, United Kingdom.

4. College of Medical, Veterinary & Life Sciences, University of Glasgow, Glasgow G12 8QQ, United Kingdom.

5. College of Veterinary Medicine, Northwest A&F University, Shanxi 712100, China.

Abstract: Identifying factors associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) exposure and immunity is critical for quantifying mass immunity and preventing future pandemics. The current SARS-CoV-2 vaccine was losing efficacy in the face of new variants, and it is urgent to seek a broad coronavirus vaccine. We conducted a cross-sectional serosurvey of patients in Scotland from September 2021 to April 2022. The indirect enzyme-linked immunosorbent assay (ELISA) against 2135 patient serum samples showed monthly infection and vaccination rates from 24.71%-72.00% and 28.00%-61.50%. We showed that people over 65 receive higher priority vaccination, and 65–74-year-old female patients exhibited higher vaccination rates and lower probability of infection. In addition, we investigated cross-reactive antibodies of SARS-CoV-2 negative, infected, and vaccinated samples against the seasonal human coronavirus (sHCoV) spike (S) protein by ELISA. We found a 1.2-1.4-fold increase in antibody reactivity among sHCoV-229E, NL63, and OC43 against vaccinated donors compared to negative donors. These findings provided insights into exposure patterns in Scotland and support the feasibility of developing a broad coronavirus vaccine.

Keywords: SARS-CoV-2; sHCoVs; ELISA; Seroprevalence; Cross Protection

1. Author Summary

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has experienced a global pandemic in the past two years. Identifying factors associated with SARS-CoV-2 infection and vaccination is essential for controlling transmission and preventing possible future pandemics. With the continuous mutation of SARS-CoV-2 and the perennial prevalence of seasonal human coronavirus (sHCoV), it is also important to develop a vaccine that can deal with various coronaviruses. Our study conducted a cross-sectional survey of patient sera in Scotland from September 2021 to April 2022 using an enzyme-linked immunosorbent assay (ELISA). Monthly infection and vaccination rates were calculated from 24.71%-72.00% and 28.00%-61.50%. We showed that people over 65 receive higher priority vaccination, and 65–74-year-old female patients exhibited a higher vaccination rate and lower probability of infection. In addition, We

performed sHCoV-spike (S)-protein-ELISA against SARS-CoV-2 negative, infected, and vaccinated samples. Vaccinated samples exhibited significant antibody reactivity to sHCoV-229E, NL63, and OC43. This study could provide insights into the Scotland epidemic prevention strategy and confirm the feasibility of developing a broad coronavirus vaccine.

2. Introduction

The current Coronavirus disease 2019 (COVID-19) pandemic caused by Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) broke out in China and various European countries in late 2019 and 2021[1]. The infection rate of Delta and Omicron variants in Scotland has seen a significant peak in August/September 2021. SARS-CoV-2 seroprevalence in populations over 60 has exceeded 90%, and vaccination programs have been expanded to cover 12-to-17-year-old people[2]. Comparably, there are four common seasonal human coronaviruses (sHCoVs) (α-sHCoV229E, sHCoV-NL63, and β-sHCoV-HKU1, OC43) that have spread globally, accounting for about 30% of common cold cases in humans[3]. There was a study that reported that the seroprevalence of adults for sHCoV-229E, sHCoV-NL63, and sHCoV-OC43 was more than 90% and that for sHCoV-HKU1 was nearly 60% [4]. This indicated that most of the millions of individuals who may be infected with sHCoVs may have received the SARS-CoV-2 vaccine.

Spike (S) protein is the most crucial surface membrane protein of SARS-CoV-2, an important site for host neutralizing antibodies, and a key target for vaccine design. The S protein enables virus entry into human cells, consisting of S1 and S2 protein subunits, of which S1 promotes the ability of the virus to bind with host cell receptors and contains an essential C-terminal receptor binding domain [5]. Nucleocapsid (N) protein holds the RNA genome, regulates the life cycle of the virus, is highly immunogenic, and is one of the conditions for confirming infected patients [6]. SARS-CoV-2 and SARS-CoV belong to the same family. The S proteins of both have approximately 75% amino acid sequence identity, whereas the S protein of SARS-CoV-2 exhibits a lower identity with sHCoVs of only 23%-29% [7]. Despite the fact that this sequence identity was thought to be minimal from the standpoint of vaccination-mediated cross-reactive antibodies, some studies have reported cross immune-reactivity between COVID-19 sera and other sHCoVs [8]. Serological assays are necessary to detect serum antibody levels and cross-reactivity with viral pathogens [9]. SARS-CoV-2 exhibits clinical symptoms in up to 7 days, and some people are asymptomatic, whereas the sHCoVs typically develop clinical symptoms within 3 days [10, 11]. In addition, serological assays can investigate s immunoglobulin (IgG) antibodies against SARS-CoV-2 and sHCoVs and recognize viral exposures, including asymptomatic infection. Antibody levels in this study were measured by an indirect enzyme-linked immunosorbent assay (ELISA) with SARS-CoV-2 S1 protein and nucleocapsid (N)protein and sHCoVs spike (S) protein.

Therefore, in this report, we sought to build models linking serum sample information (e.g., date of sampling, sex, and age) to exposure and vaccination to SARS-CoV-2 to investigate the vaccination and infection situation in the late 2021 and 2022 by SARS-CoV-2-S1-subdomain ELISA and SARS-CoV-2-N-protein ELISA assays. In addition, we tried to identify broad sHCoVs antibody responses resulting from SARS-CoV-2 exposure and vaccinated individuals. We used sHCoVs S protein as the ELISA assay's main target of cross-reactive antibodies.

2.1 Aim of study

Herein, we aimed to study hypothesizing that vaccination rates and exposure prevalence may differ by age, sex, and region in late 2021 and 2022 and whether SARS-CoV-2 vaccines exhibit broad immunity against sHCoVs. We showed that the infected population of SARS-CoV-2 is mainly distributed among the elderly over 65 years old. Whether the increase in the vaccination rate can reduce the exposure prevalence of this part of the population requires further analysis. In addition, although the S protein of SARS-CoV-2 has a low amino acid sequence identity with that of sHCoVs, whether the SARS-CoV-2 vaccine could generate cross-immune protection with sHCoVs also requires further analysis. The efficacy of

the SARS-CoV-2 vaccine against sHCoVs is needed to be analyzed to guide the development of a broad coronavirus vaccine.

3. Materials & Methods

3.1 Serum samples

The NHS Greater Glasgow and Clyde (NHSGGC) Biorepository provided ethical support for this study and random residual biochemical serum samples from healthcare facilities from September 2021 to April 2022 (n=2135). The relevant metadata includes only the time of sample collection, gender, age, region, and time of vaccination and infection. Samples are numbered, and the patient's name and private information are kept confidential. All serum samples were cryopreserved at -20°C and inactivated in a constant temperature water bath at 56°C for 30 minutes before testing.

3.2 ELISA assay

SARS-CoV-2-S1 and N antigens and S antigens of sHCoV-229E, HKU1, NL63, and OC43 were made according to other instructions [12]. Antigen constructs were tagged with twin Strep. All proteins were transiently expressed in Expi293F cells maintained in FreeStyle medium ((Thermo Fisher Scientific). After seven days post-transfection, By centrifuging the supernatant for 30 minutes at 4000 rpm, the protein was extracted from the mixture. Twin Strep-tagged proteins were collected by filtration on a Steritop filter unit (IBA LifeSciences). Further purification was performed using size exclusion chromatography on Superdex 200 (GE Healthcare). Ultrafiltration was used to concentrate antigen to 5 mg/mL, then frozen quickly and stored at –80°C for later use.

IgG antibody detection against sHCoVs S antigen and SARS-CoV-2 S and N antigens were carried out as previously described. [13]. First, 96-well plates (Immulon 2HB 3455) were coated with 50 μL antigens at 1:100 diluted in 1 X phosphate-buffered saline (PBS), incubating overnight at 4 °C. After being washed 3 times with wash buffer (10 X PBS/0.05% Tween-20) by using the microplate washer (thermoscientific 5165050), antigens were blocked with 200 μL diluent buffer making of wash buffer with 10% casein (Vector labs. SP-5020) and serum samples were 1:100 diluted in the diluent buffer. Incubate for 1 hour at room temperature before the second wash (consistent with the above washing method). Rabbit anti-human lgG(H+L) horseradish peroxidase (HRP) conjugate (A80-118P, Bethyl labs) was diluted at 1:3000 in the diluent buffer, then added 50 μL per well and incubated for 1 hour. After the third wash, 50 μL per well of 3,3′,5,5′-tetramethylbenzidine (Sigma-Aldrich (Merck)) was added and allowed to develop in the dark for 10 minutes.

The reaction was stopped by adding 50 μL of 1-mol/L H2SO4. Read at 450nm absorbance on a plate reader immediately (Labsystems Multiskan Ascent). Since there was no negative control sample for sHCoVs, no negative and positive controls are set on each sHCoVs antigen plate, leaving one well as a blank control. One known positive and negative control was placed on each SARS-CoV-2 antigen plate. The raw optical density (OD) values were adjusted using the following formula.

$$\frac{\text{sample absorbance} - \text{negative ccontrol mean}}{\text{negative control mean}}$$

Receiver operating characteristic (ROC) analysis was performed to choose the positive and negative cutoff values from corrected absorbance values for positive and negative samples tested in the assay.

A positive control is a sample that is not exposed to the experimental treatment but is exposed to a known effect-producing treatment. As positive controls, 100 samples in total were used. All samples in this ELISA assay were tested against SARS-CoV-2-S1 and nucleocapsid (N) antigens. ROC analysis was used in the 9.4.0 version of GraphPad Prism (GraphPad). Determining whether a sample was infected or vaccinated was calculated by applying the respective cutoff

values for S1 and N antigens derived from the ROC analysis. Namely, if a sample was positive for both antigens (adjusted OD of S1 protein >7.761 & adjusted OD of N protein <3.666), it was considered as positive. Conversely, it was uninfected if a sample was positive for S1 antigen (adjusted OD of S1 protein >7.761) but negative for N antigen (adjusted OD of N protein <3.666), it was considered as negative.

3.3 Sensitivity and specificity

True positives, true negatives, false positives, and false negatives were determined using negative and positive cutoff to calculate specificity and sensitivity. There were 704 RT-PCR-confirmed SARS-CoV-2 infections. A total of 16 samples were classified as false positives. A sensitivity for SARS-CoV-2 was 98%. To confirm the specificity of the SARS-CoV-2 ELISA assays, a total of 59 SARS-CoV-2 RT-PCR negatives, of which 2 were positive by ELISA. Specificity for SARS-CoV-2 was 97%.

3.4 Statistical Analysis

The infection and vaccination rates of different gender groups and age groups and each month were shown using histograms and scatterplots. Differences in unadjusted optical density values of ELISA-determined SARS-CoV-2 exposed, negative, and vaccinated samples of the four sHCoVs were investigated using the Wilcoxon paired signed-rank test. We performed a straightforward calculation to establish the sample size for test sHCoVs. The seropositive prevalence (p) of the samples tested in this study was 91.78%, leaving the confidence level as 95%. The sample size was 116 by substituting into the equation $n=\frac{1.96^2 p（1-p）}{d^2}$ (d is precision as 0.05). All analysis and data visualisation was done by R software [14], version 4.2.1. The dplyr and ggplot2 packages were used to run the models [15].

4. Result

4.1 SARS-CoV-2

The S1 subdomain of the SARS-CoV-2 and N protein was detected by IgG antibodies in 2 ELISA assays in 2135 residual biochemical serum samples from NHSGGC patients [13]. The sample spans 8 months, starting September 22nd, 2021, and ending April 21st, 2022. Covering all NHSGGC regions and all age groups but not collecting children under the age of 15, the pediatric patient sample was not representative enough to reflect the SARS-CoV-2 infection rate, and the incidence of clinical symptoms in children was low. Because parents can avoid taking their children to medical facilities to protect them from the virus and potentially reduce the risk of COVID-19 [16].

The overall adjusted infection prevalence was 32.22% (95% CI, 30.24%-34.21%) (Figure 1A). Infection rates in men and women patients were approximately the same and consistent with the overall infection rate, as well as vaccination rates in men and women were consistent with overall (59.02%, 95% CI, 56.93%-61.10%) (Figure 1B). In the 15-44-year-old group, both men and women, the infection rate was highest (Figure 1A). It may be because this age group includes young people aged 15-18, resulting in a lower vaccination rate (50.38%, 95% CI, 46.07%-54.71%) (Figure 1B). In contrast, the 65-74 and 75+ age groups have the highest vaccination rates (66.35%, 95%CI, 61.82%-70.87% and 66.67%, 95%CI, 62.41%-70.92%) and the lowest infection rates (26.25%, 95%CI, 22.04%-30.47% and 23.35%, 95%CI, 19.95%-27.18%). Antibodies generally increased earlier in the older group than in the younger group. This reflected the prioritization of age in existing vaccination schedules [17]. For the age group 65-74 years, the infection rate was higher in men (32.18%, 95%CI, 25.73%-46.83%) (Figure 1A), possibly due to gender bias in treatment with SARS-CoV-2 [18], since the account of women being vaccinated (72.35%, 95% CI, 66.40%-78.30%) was higher than men (59.90%, 95% CI, 53.14%-66.66%) (Figure 1B).

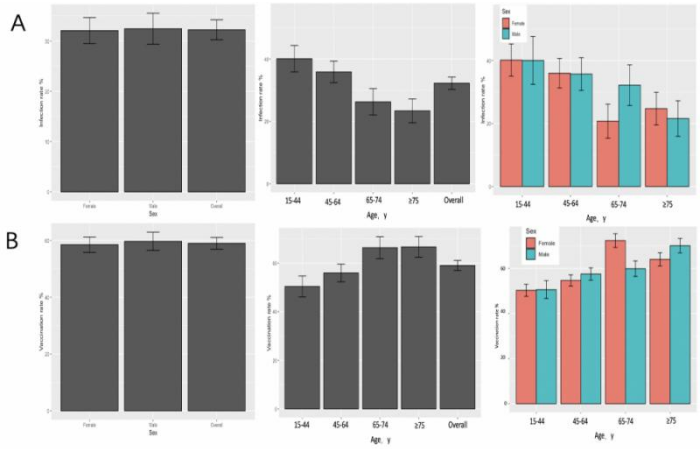

Figure 1. Glasgow, Scotland, UK, adjusted SARS-CoV-2 infection and vaccination rates in patient populations. A、B、Infection rate (A), vaccination rate (B), and 95% confidence intervals are shown across age groups and sexes.

During these 8 months, the monthly vaccination rate was roughly in line with the overall vaccination rate (59.02%, 95% CI, 56.93%-61.10%) (Figure 2A), except for January and April. There was a significant drop to 28.00% (95% CI, 19.02%-36.98%) (Figure 2A) in January, perhaps because January 2022 was the time when the rapid spread of Omicron BA.1 began, and there was no corresponding vaccine yet [2]. In April 2022, the vaccination rate decreased to 50.84% (95% CI, 43.51%- 58.16%) (Figure 2A), perhaps because reinfection by Omicron BA.2 resulted in reinfection in some vaccinated populations in the ELISA assays [19]. Meanwhile, the infection rate has continued to rise for these eight months and peaked in January (72.00%, 95%CI, 54.40%-89.60%) (Figure 2B). This was not the same as the reported weekly decline in infection rates, where more patients with secondary infections may be counted every month in this study. SARS-CoV-2 may adapt more efficiently to human transmission due to an update in immune evasion of SARS-CoV-2 [20, 21]. However, the sample size in January was too small, only 25, which may be one of the reasons for the vast difference in January.

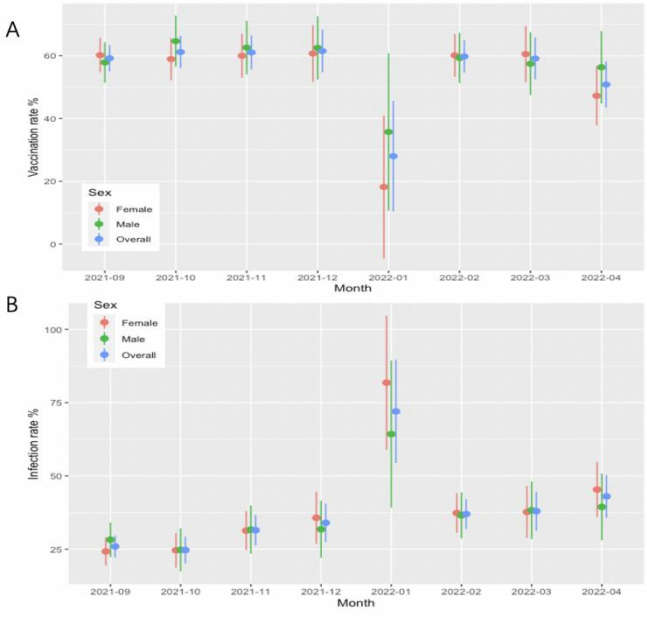

Figure 2. Glasgow, Scotland, UK, adjusted monthly SARS-CoV-2 infection and vaccination rates in patient populations. A、B、Vaccination rate (A) and infection rate (B) and 95% confidence intervals were surveyed in sequential combinations of sex

and sampling time.

In addition, the infection rate in some areas (20/106) was also calculated, where the sample size provided sufficient confidence to estimate infection rates. The estimated infection rate ranged from 11.90% (95% CI, 4.98-18.83) to 49.40% (95% CI, 38.64-60.15) (Table 1). Notably, G77, a region with a low infection rate, had the highest vaccination rate (84.52%, 95% CI, 76.79-92.26) (Table 1). Conversely, the G51 region has the lowest vaccination rate (40.96%, 95%CI, 30.38-51.54) and the highest infection rate. This suggests that vaccination may vary geographically, with different medical conditions in different regions, and areas with fewer vaccination sites may be more at risk of infection. Meanwhile, age composition in different regions may also be key to differences in infection and vaccination rates. However, the sample size was too small to do further analysis.

Postcode districts	Number of samples	Infection rate (95% CI)	Vaccination rate (95% CI)
G77	84	11.90（4.98-18.83）	84.52 (76.79-92.26)
G61	112	24.10（16.19-32.03）	71.43 (63.06-79.80)
G76	61	24.59（13.78-35.40）	63.93 (51.88-75.98)
G12	60	26.67（15.48-37.86）	63.33 (51.14-75.53)
G11	70	27.14（16.73-37.56）	58.57 (47.03-77.58)
G73	51	27.45（15.20-39.70）	66.67 (53.73-79.60)
G14	68	29.41（18.58-40.24）	55.88 (44.08-67.68)
G13	111	30.63（22.06-39.21）	60.36 (51.26-69.46)
G52	126	30.95（22.88-39.02）	60.32 (51.77-68.86)
G44	125	33.60（25.32-41.88）	62.40 (53.91-70.89)
G15	53	33.96（21.21-46.71）	58.49 (45.22-71.76)
G53	132	34.09（26.00-42.18）	58.33 (49.92-66.74)
G43	62	35.48（23.57-47.39）	58.06 (36.77-65.36)
G46	89	35.96（25.99-45.92）	57.40 (47.03-67.58)
G42	101	36.63（27.24-46.03）	54.46 (44.74-64.17)
G81	143	38.46（30.49-46.44）	52.45 (44.26-60.32)
G5	43	39.53（24.92-54.15）	48.84 (33.90-63.78)
G45	47	40.43（26.40-54.46）	51.06 (36.77-65.36)
G41	90	42.22（32.02-52.43）	46.67 (36.36-56.97)
G51	83	49.40（38.64-60.15）	40.96 (30.38-51.54)

Table 1. Infection rate, vaccination rate, and 95% CI in partial postcode districts of the Glasgow, Scotland, UK, study population with >40 samples.

4.2 sHCoVs

IgG antibodies against the spike (S) protein of sHCoV-229E, sHCoV-HKU1, sHCoV-NL63, and sHCoV-OC43 were tested in 2136 residual biochemical serum samples from NHSGGC patients using 4 ELISA assays [22]. According to the above ELISA assay for SARS-CoV-2, the samples were classified as infection (n=76), infection & vaccination (samples were vaccinated before but were infected in the ELISA assay) (n=612), vaccination (1150), and negative (n=70) (Figure 3A). Because vaccinated for the third dose had significantly higher mean corrected-absorbance (Figure 3B) and significantly increased levels of SARS-CoV-2 S1-specific IgG antibodies were seen after the third dose than the first and second doses[23]. Third-dose samples were screened from the infection & vaccination (n=520) and vaccination (n=1020) groups for further

study.

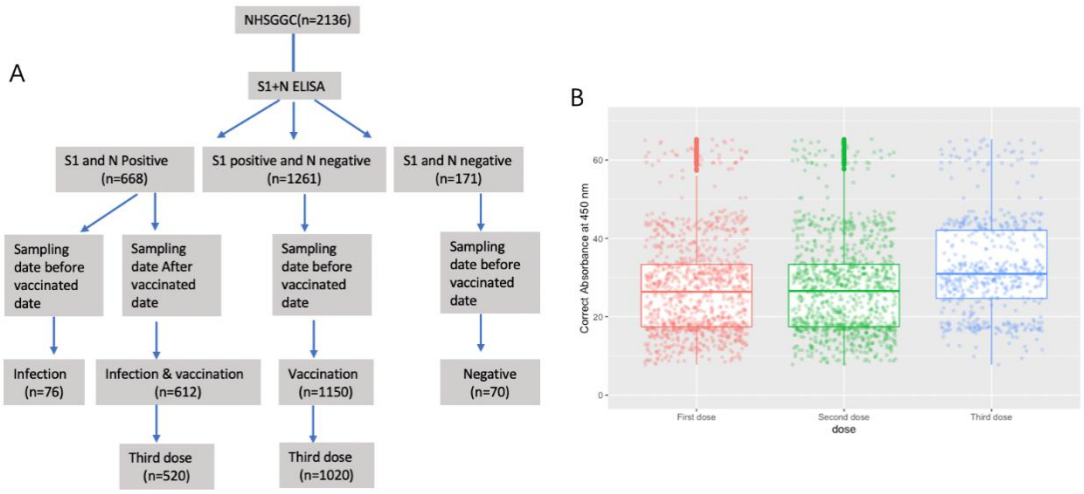

Figure 3. Sample selecting and correct absorbance of different doses. A、Diagram summarizing the sample flow used in the sHCoVs study. B、The correlation between ELISA assay corrected-absorbance and dose is shown as a scatterplot. Sample distribution between the first dose (red scatter), second dose (green scatter), and third dose (blue scatter). ELISA, enzyme-linked immunosorbent assay.

4.2.1 Infection (Aged 22-60) vs vaccination (aged 22-60)

In sHCoVs S-protein-ELISA-based assays, sHCoV-HKU1 exposure and vaccination differed significantly (R2=0.0091, P<0.01) (Figure 4A). However, it was the sera of the exposed group that showed more significant antibody reactivity (OD> 1.3-fold change) (Figure 4B); this was different from what we predicted. There were no differences detected in the sHCoVs of the other three groups. Still, it is worth noting that in the detection of sHCoV-NL63, the antibody reactivity of the exposed group was slightly higher than that of the vaccinated group. The antibody reactivity of the exposed group to sHCoV-NL63 was significantly higher than that of the other three sHCoVs (OD>1.4-fold change) (Figure 4B). Considering that the vaccine targets the S protein of SARS-CoV-2, the vaccine may have the same efficacy as the SARS-CoV-2 exposure for sHCoVs that do not differ among the three groups [8].

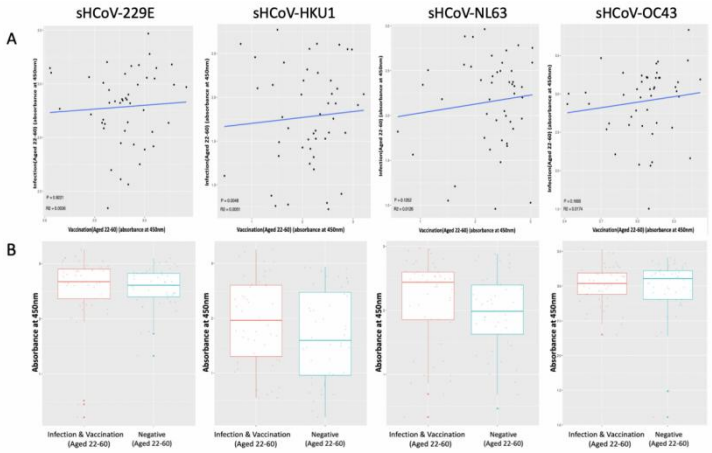

Figure 4. Difference between SARS-CoV-2 infection and vaccination samples against sHCoVs. A、The correlation between SARS-CoV-2 infection and vaccination in the 22-60 years old group was calculated by S-protein-ELISA assay absorbance at 450nm of sHCoVs. Differences were analyzed with the Wilcoxon matched-pairs signed-rank test. B、The correlation

between SARS-CoV-2 infection and vaccination aged 22-60 was calculated by S-protein-ELISA assay absorbance at 450nm of sHCoVs, shown by scatterplots. Sample distribution between infection aged 22-60 (red scatter) and vaccination aged 22-60 (blue scatter). ELISA, enzyme-linked immunosorbent assay.

4.2.2 Infection & vaccination (aged 22-60) vs negative (aged 22-60)

Different from the comparison in the previous group, in the comparison of Infection & vaccination and negative, sHCoV-NL63 infection & vaccination and negative showed a moderate significant difference (R2=0.0819, P<0.05) (Figure 5A). No differences were found among the other three sHCoVs, especially the sHCoV-229E mentioned above. In addition, the sera of Infection & vaccination of sHCoV-NL63 showed significantly higher antibody reactivity than negative (OD>1.7-fold change) (Figure 5B). There were no significant differences in sera antibody reactivity in the other three comparisons. Probably because this study focused on the S1 subdomain of SARS-CoV-2; however, some research suggested that S2-specific antibodies contribute more to cross-reactivity than S1-specific antibodies, which is consistent with the S2 subdomain's higher conservation than the S1 subdomain [24, 25]. Therefore, in addition to the SARS-CoV-2 S1 protein, other proteins may be targeted by sHCoVs.

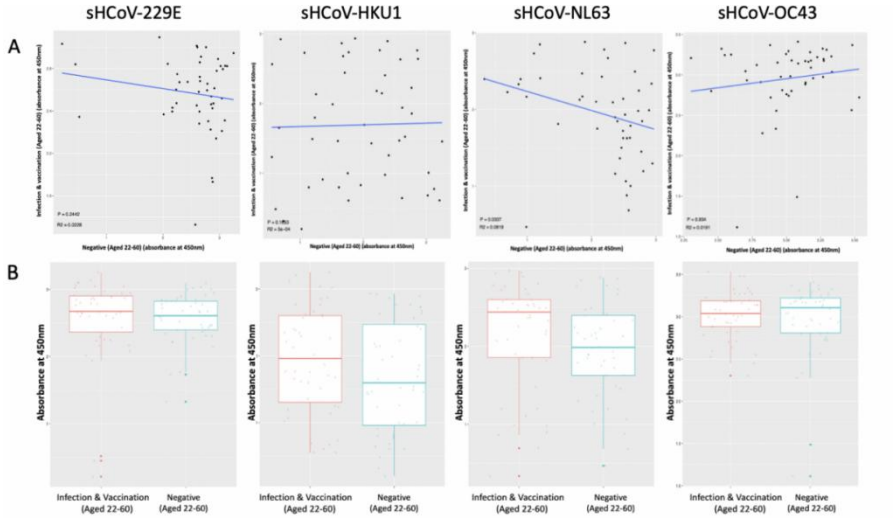

Figure 5. Difference between SARS-CoV-2 infection & vaccination and negative samples against sHCoVs. A、The correlation between SARS-CoV-2 infection & vaccination and negative in the 22-60 aged group was calculated by S-protein-ELISA assay absorbance at 450nm of sHCoVs. Differences were analyzed with the Wilcoxon matched-pairs signed-rank test. B、The correlation between SARS-CoV-2 infection & vaccination and the negative 22-60 years old group was calculated by S-protein-ELISA assay absorbance at 450nm of sHCoVs, shown by scatterplots. Sample distribution between infection & vaccination aged 22-60 (red scatter) and negative aged 22-60 (blue scatter). ELISA, enzyme-linked immunosorbent assay.

4.2.3 Vaccination (Aged>60) vs negative (Aged>60)

In the detection of sHCoV-229E and sHCoV-OC43, the sera of the SARS-CoV-2 vaccinated group and the negative group showed significant differences (R2=0.0055, R2= 0.0168, all P<0.01) (Figure 6A). Sera from the vaccinated group showed reactivity to sHCoV-OC43 in addition to significant antibody reactivity to 229E-S (>1.4 OD fold change) (Figure 6B). The difference between the sera of the vaccinated group and the negative group against NL63 showed a weak difference (P<0.05) (Figure 6A); however, the R-squared here is 0, so it has no value for statistical analysis. The OD fold rise of NL63 was slightly less than that of sHCoV-229E (>1.2 OD fold change) (Figure 6B). Like the previous group comparison, no

difference was found in the sera of the vaccinated group for sHCoV-HKU1 and the negative group. Therefore, the S1 antigen of the SARS-CoV-2 vaccine may produce cross-immune protection with sHCoV-229E and sHCoV-OC43.

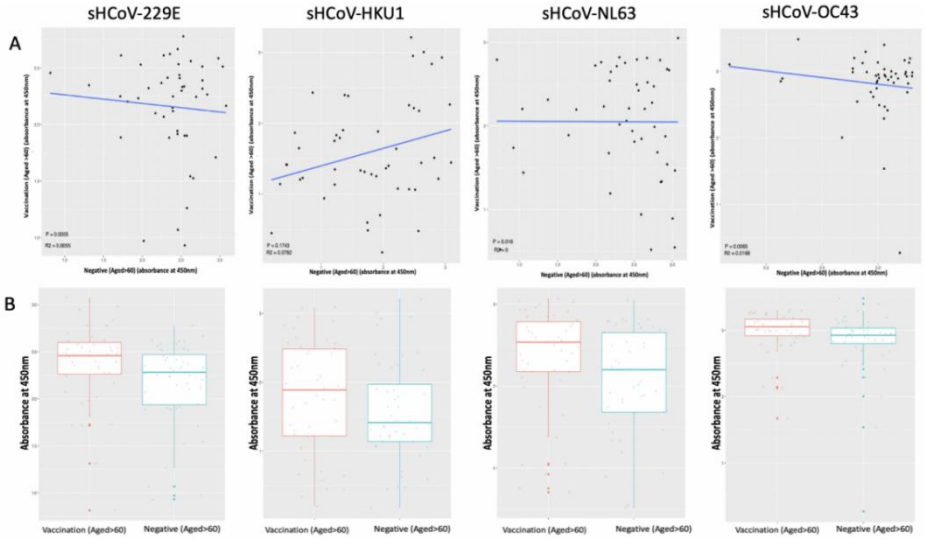

Figure 6. Difference between SARS-CoV-2 vaccination and negative samples against sHCoVs. A、 The correlation between SARS-CoV-2 vaccination and negative in more than 60 years old group was calculated by S-protein-ELISA assay absorbance at 450nm of sHCoVs. Differences were analyzed with the Wilcoxon matched-pairs signed-rank test. B、 The correlation between SARS-CoV-2 vaccination and negative aged more than 60 years old group was calculated by S-protein-ELISA assay absorbance at 450 nm of sHCoVs, shown by as scatterplot. Sample distribution between infection & vaccination aged 22-60 (red scatter) and negative aged more than 60 (blue scatter). ELISA, enzyme-linked immunosorbent assay.

5. Discussion

The start of the emergency authorization of several vaccines in late December 2020 has created a wave of vaccination campaigns around the world [26]. Determining current vaccination and infection rates is particularly important in mass vaccination. Serological surveys are a vital tool for counting vaccination and infection rates and controlling the spread of disease. Our study showed a clear gap in SARS-CoV-2 exposure over time during the Omicron pandemic and heterogeneous across different groups of patients in Glasgow. The sensitivity and specificity of our test remained above 95%, with a vaccination rate of 59.02% of the 2135 Glasgow samples tested. This was 10% lower than the 70% vaccination rate reported in Scotland [2]. This may be because the current minimum age for vaccination is 12 years old, while the minimum age of our sample was 15 years old [2]. In addition, our sample analysis relied on patients in the NHS seeking healthcare, including respiratory patients who were more likely to develop symptoms of SARS-CoV-2 infection and the general population with other diseases. Therefore, studies beyond this population analysis need to be cautious. For example, in the 65-74 age group, male patients were at significantly higher risk of infection than females, and females also have substantially higher vaccination rates than males, perhaps reflecting gender bias in COVID-19 vaccination [27] or sociocultural disparities that resulted in more exposure [28]. We still observed a vaccination rate of over 66% and an infection rate of less than 25% in the over-65 population. This means that the elderly group receives priority vaccine treatment.

It was crucial to note that in January 2022, the infection rate of SARS-CoV-2 had risen significantly to 43.02%. This was related to the sweeping of Omicron BA.1 in January, and the lack of a vaccine in time has led to high infection rates [2]. At the same time, the vaccination rate in Glasgow has also fallen to a low point (50.84%). However, it should also be considered that the sample size detected in January is too small (n=25). Hence the sample data in January may not reflect the

actual infection rate and vaccination rate.

Meanwhile, the sample in this study can be divided into primary care and secondary care patients, among which pregnant women in primary protection patients may receive exceptional care, and the exposure probability was lower than that of the general population. In addition, this study found that some people had secondary infection, so it cannot be ruled out that some patients were in the early stage of infection and had not been seroconverted at the time of sampling. Therefore, with the aforementioned qualifications, the infection rate in this study may be underestimated. But this study emphasizes the importance of vaccination, especially the third dose. Male in certain age groups with high vaccination rates was found to have lower infection rates. At the same time, compared with the first and second dose, the third injection had significant antibody reactivity (>1.3 OD-fold change).

With the relaxation of epidemic prevention measures in various countries, there is an optimism that SARS-CoV-2 will be suppressed with mass vaccination. However, the sweeping Omicron variants and the ever-present sHCoVs are still challenging. Some studies suggest that SARS-CoV-2 vaccines are insufficient to address increasingly human-adapted SARS-CoV-2 variants [29, 30]. Hence, it is vital to develop a broad SARS-CoV-2 vaccine against SARS-CoV-2 variants and sHCoVs.

In this study, infected & vaccinated samples had higher antibody reactivity to NL63 than negative donors, but we did not find this in vaccinated samples. This differs from previous reports that only NL63 binding antibodies cannot be depleted by the SARS-CoV-2 S protein [25]. This may be because mutations in SARS-CoV-2 were associated with vaccine updates. Since this study targeted the S1 subdomain of SARS-CoV-2, other subdomains may play a more influential role in the infection & vaccination population [25]. Meanwhile, studies have shown that spike protein between NL63 and SARS-CoV-2 can be recognised by T cells, and T cell responses induced or boosted by SARS-CoV-2 mRNA vaccines may be able to control SARS-CoV-2 variations and result in resistance to NL63 cross protection [31]. In addition, the sera of the vaccinated aged group (Aged > 60) had significant antibody reactivity to 229E and OC43, which was not found in the infection & vaccination (Aged 22-60) group. Therefore, we considered that the cross-protection of sHCoVs by the SARS-CoV-2 vaccine might be age-differentiated. Others demonstrated cross-reactive antibodies in SARS-CoV-2 patients due to enhanced pre-existing immunity following natural infection [32]. According to increasing age, the elderly population may have multiple innate immunities to sHCoVs, leading to enhanced vaccine cross-protection. With secondary infection, the sera of the infected and vaccinated groups may also enhance the cross-protection with NL63. Interestingly, the sera of the exposed group produced stronger antibody reactivity to HKU1 than the vaccinated group. It may be because the nucleocapsid (N) protein of SARS-CoV-2 can partially deplete the antibody bound to the sHCoVs N protein in the serum of the exposed group. The vaccine was directed against the S protein of SARS-CoV-2 to induce an immune response but not N protein, so there is no antibody against N protein in the vaccinated group. Therefore, targeting the N protein of SARS-CoV-2 may be the key to designing a broad coronavirus vaccine. Moreover, HKU1 is a β-coronavirus, which is more closely related to SARS-CoV-2 belonging to the β family than sHCoV-229E or NL63 [33], so the antibodies of the exposed group showed a stronger immune response to HKU1. Of note is that virus neutralisation tests were not applicable. It is unable to replicate sHCoV-HKU1 because there is no cell line of HKU1. The available strains of sHCoV-229E and sHCoV-OC43 cultured from 1960 and modified by the laboratory are not representative of the wild-type virus [34]. We suggested that the subsequent study can try to capture sHCoVs pseudotypes and do virus neutralisation assay. Taking SARS-CoV-2 as an example, the results of the pseudovirus neutralization assay correlate well with measurements using live SARS-CoV-2 [35].

This study provided data on the SARS-CoV-2 exposure and immunity population. The observed heterogeneity of exposure and immunity by region, sex, and age, suggests that in the absence of vaccination, SARS-CoV-2 still has the potential to become a pandemic in the future. At the same time, findings of differences in cross-reactive antibodies to other sHCoVs following SARS-CoV-2 exposure and vaccination underscore the feasibility of a broad coronavirus vaccine.

6. Acknowledgements

We thank the NHSGGC Biorepository for providing us with the serum samples required for this work. We would also like to thank Prof. Brian Willett, Nicola Logan, and Maria Manali for their guidance and assistance with this experiment and the paper.

References

[1] Majumder J, Minko T. Recent Developments on Therapeutic and Diagnostic Approaches for COVID-19. Aaps j. 2021;23(1):14.

[2] Coronavirus (COVID-19): strategic approach [cited 2022 Jul 22]. Available from: https:// www.gov. scot/collections/ coronavirus-covid-19-strategic-approach/.

[3] Lawrenz J, Xie Q, Zech F, Weil T, Seidel A, Krnavek D, et al. SARS-CoV-2 Vaccination boosts Neutralizing Activity against Seasonal Human Coronaviruses. Clin Infect Dis. 2022.

[4] Severance EG, Bossis I, Dickerson FB, Stallings CR, Origoni AE, Sullens A, et al. Development of a nucleocapsid-based human coronavirus immunoassay and estimates of individuals exposed to coronavirus in a U.S. metropolitan population. Clin Vaccine Immunol. 2008;15(12):1805-10.

[5] Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell. 2020;181(2):281-92.e6.

[6] Bai Z, Cao Y, Liu W, Li J. The SARS-CoV-2 Nucleocapsid Protein and Its Role in Viral Structure, Biological Functions, and a Potential Target for Drug or Vaccine Mitigation. Viruses. 2021;13(6).

[7] Barnes CO, West AP, Jr., Huey-Tubman KE, Hoffmann MAG, Sharaf NG, Hoffman PR, et al. Structures of Human Antibodies Bound to SARS-CoV-2 Spike Reveal Common Epitopes and Recurrent Features of Antibodies. Cell. 2020; 182(4):828-42.e16.

[8] Song G, He W, Callaghan S, Anzanello F, Huang D, Ricketts J, et al. Cross-reactive serum and memory B cell responses to spike protein in SARS-CoV-2 and endemic coronavirus infection. Nat. Commun. 12, 2938. 2021.

[9] Winter AK, Hegde ST. The important role of serology for COVID-19 control. Lancet Infect Dis. 2020;20(7):758-9.

[10] Poutanen SM. Human Coronaviruses. Principles and Practice of Pediatric Infectious Diseases. 2012:1117-20.e4.

[11] Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody Responses to SARS-CoV-2 in Patients With Novel Coronavirus Disease 2019. Clin Infect Dis. 2020;71(16):2027-34.

[12] Ng KW, Faulkner N, Cornish GH, Rosa A, Harvey R, Hussain S, et al. Preexisting and de novo humoral immunity to SARS-CoV-2 in humans. Science. 2020; 370(6522):1339-43.

[13] Amanat F, Stadlbauer D, Strohmeier S, Nguyen THO, Chromikova V, McMahon M, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. Nat Med. 2020;26(7):1033-6.

[14] Team RC. R: A language and environment for statistical computing. 2013.

[15] Wickham H. Data analysis. ggplot2: Springer; 2016. p. 189-201.

[16] Götzinger F, Santiago-García B, Noguera-Julián A, Lanaspa M, Lancella L, Calò Carducci FI, et al. COVID-19 in children and adolescents in Europe: a multinational, multicentre cohort study. Lancet Child Adolesc Health. 2020;4(9):653-61.

[17] Coronavirus (COVID-19): ONS Infection Survey–antibody data–16 September 2021 [cited 2022 Jul 26]. Available from: https://www.gov.scot/publications/ coronavirus-covid-19-ons-infection-survey-antibody-data-16-september-2021/.

[18] Docherty AB, Harrison EM, Green CA, Hardwick HE, Pius R, Norman L, et al. Features of 20 133 UK patients in hospital with covid-19 using the ISARIC WHO Clinical Characterisation Protocol: prospective observational cohort study.

Bmj. 2020; 369:m1985.
[19] Chekol Abebe E, Tiruneh GMM, Behaile TMA, Asmamaw Dejenie T, Mengie Ayele T, Tadele Admasu F, et al. Mutational Pattern, Impacts and Potential Preventive Strategies of Omicron SARS-CoV-2 Variant Infection. Infect Drug Resist. 2022; 15:1871-87.
[20] Rotondo JC, Martini F, Maritati M, Mazziotta C, Di Mauro G, Lanzillotti C, et al. SARS-CoV-2 Infection: New Molecular, Phylogenetic, and Pathogenetic Insights. Efficacy of Current Vaccines and the Potential Risk of Variants. Viruses. 2021;13(9).
[21] Coronavirus (COVID-19): ONS Infection Survey – headline results – 28 January 2022 [cited 2022 Jul 26]. Available from: https://www.gov.scot/publications/ coronavirus-covid-19-ons-infection-survey-headline-results-28-january-2022/.
[22] Dowlatshahi S, Shabani E, Abdekhodaie MJ. Serological assays and host antibody detection in coronavirus-related disease diagnosis. Arch Virol. 2021; 166(3): 715-31.
[23] Belik M, Jalkanen P, Lundberg R, Reinholm A, Laine L, Väisänen E, et al. Comparative analysis of COVID-19 vaccine responses and third booster dose-induced neutralizing antibodies against Delta and Omicron variants. Nat Commun. 2022; 13(1):2476.
[24] Nguyen-Contant P, Embong AK, Kanagaiah P, Chaves FA, Yang H, Branche AR, et al. S protein-reactive IgG and memory B cell production after human SARS-CoV-2 infection includes broad reactivity to the S2 subunit. MBio. 2020;11(5):e01991-20.
[25] Grobben M, van der Straten K, Brouwer PJM, Brinkkemper M, Maisonnasse P, Dereuddre-Bosquet N, et al. Cross-reactive antibodies after SARS-CoV-2 infection and vaccination. eLife. 2021;10:e70330.
[26] Kantarcioglu B, Iqbal O, Lewis J, Carter CA, Singh M, Lievano F, et al. An Update on the Status of Vaccine Development for SARS-CoV-2 Including Variants. Practical Considerations for COVID-19 Special Populations. Clin Appl Thromb Hemost. 2022;28:10760296211056648.
[27] Scully EP, Haverfield J, Ursin RL, Tannenbaum C, Klein SL. Considering how biological sex impacts immune responses and COVID-19 outcomes. Nat Rev Immunol. 2020;20(7):442-7.
[28] Bavel JJV, Baicker K, Boggio PS, Capraro V, Cichocka A, Cikara M, et al. Using social and behavioural science to support COVID-19 pandemic response. Nat Hum Behav. 2020;4(5):460-71.
[29] Voysey M, Clemens SAC, Madhi SA, Weckx LY, Folegatti PM, Aley PK, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet. 2021;397(10269):99-111.
[30] Xie X, Liu Y, Liu J, Zhang X, Zou J, Fontes-Garfias CR, et al. Neutralization of SARS-CoV-2 spike 69/70 deletion, E484K and N501Y variants by BNT162b2 vaccine-elicited sera. Nat Med. 2021;27(4):620-1.
[31] Woldemeskel BA, Garliss CC, Blankson JN. SARS-CoV-2 mRNA vaccines induce broad CD4+ T cell responses that recognize SARS-CoV-2 variants and HCoV-NL63. J Clin Invest. 2021;131(10).
[32] Anderson EM, Goodwin EC, Verma A, Arevalo CP, Bolton MJ, Weirick ME, et al. Seasonal human coronavirus antibodies are boosted upon SARS-CoV-2 infection but not associated with protection. Cell. 2021;184(7):1858-64.e10.
[33] Dugas M, Grote-Westrick T, Vollenberg R, Lorentzen E, Brix T, Schmidt H, et al. Less severe course of COVID-19 is associated with elevated levels of antibodies against seasonal human coronaviruses OC43 and HKU1 (HCoV OC43, HCoV HKU1). Int J Infect Dis. 2021;105:304-6.
[34] Eidge AWD, Kaczorowska J, Hoste ACR, Bakker M, Klein M, Loens K, et al. Seasonal coronavirus protective immunity is short-lasting. Nature medicine. 2020; 26(11):1691-3.
[35] Crawford KHD, Eguia R, Dingens AS, Loes AN, Malone KD, Wolf CR, et al. Protocol and Reagents for Pseudotyping Lentiviral Particles with SARS-CoV-2 Spike Protein for Neutralization Assays. Viruses. 2020;12(5).