Human neutralising antibodies elicited by SARS-CoV-2 non-D614G variants offer cross-protection against the SARS-CoV-2 D614G variant

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Received 20 November 2020; Revised 21 December 2020; Accepted 23 December 2020

doi: 10.1002/cti2.1241

Clinical & Translational Immunology 2021; 10: e1241

Abstract

Objectives. The emergence of a SARS-CoV-2 variant with a point mutation in the spike (S) protein, D614G, has taken precedence over the original Wuhan isolate by May 2020. With an increased infection and transmission rate, it is imperative to determine whether antibodies induced against the D614 isolate may cross-neutralise against the G614 variant. Methods. Antibody profiling against the SARS-CoV-2 S protein of the D614 variant by flow cytometry and assessment of neutralising antibody titres using pseudotyped lentiviruses expressing the SARS-CoV-2 S protein of either the D614 or G614 variant tagged with a luciferase reporter were performed on plasma samples from COVID-19 patients with known D614G status (n = 44 infected with D614, n = 6 infected with G614, n = 7 containing all other clades: O, S, L, V, G, GH or GR). Results. Profiling of the anti-SARS-CoV-2 humoral immunity reveals similar neutralisation profiles against both S protein variants, albeit waning neutralising antibody capacity at the later phase of infection. Of clinical importance, patients infected with
either the D614 or G614 clade elicited a similar degree of neutralisation against both pseudoviruses, suggesting that the D614G mutation does not impact the neutralisation capacity of the elicited antibodies. **Conclusions.** Cross-reactivity occurs at the functional level of the humoral response on both the S protein variants, which suggest, that existing serological assays will be able to detect both D614 and G614 clades of SARS-CoV-2. More importantly, there should be negligible impact towards the efficacy of antibody-based therapies and vaccines that are currently being developed.

**Keywords:** clade, COVID-19, cross-reactivity, D614G variant, neutralising antibodies, SARS-CoV-2

**INTRODUCTION**

Coronavirus disease 2019 (COVID-19) is the consequence of an infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which emerged in Wuhan, China, in December 2019. The rapid expansion of the COVID-19 pandemic has affected 213 countries and territories, with a global count of more than 80 million laboratory-confirmed human infection cases to date. An inevitable impact of this pandemic is the accumulation of immunologically relevant mutations among the viral populations due to natural selection or random genetic drift, resulting in enhanced viral fitness and immunological resistance. For instance, antigenic drift was previously reported in other common cold coronaviruses, OC43 and 229E, as well as in SARS-CoV. In early March 2020, a non-synonymous mutation from aspartic acid (D) to glycine (G) at position 614 of SARS-CoV-2 spike (S) protein was identified. This variant, G614, rapidly became the dominant SARS-CoV-2 clade in Europe by May 2020, suggesting a higher transmission rate over the original isolate, D614. In vitro and animal studies have also indicated that the G614 variant may have an increased infectivity and may be associated with higher viral loads and more severe infections. Notably, single point mutations have been shown to induce resistance to neutralising antibodies in other coronaviruses, including SARS-CoV and Middle East respiratory syndrome (MERS-CoV). More importantly, mutations in the S protein of SARS-CoV-2 have been shown to induce conformational modifications that alter antigenicity. Hence, determining any cross-neutralising capability of antibodies developed against the earlier G614 variant is of paramount importance to validate the therapeutic efficacy of developing immune-based interventions.

**RESULTS**

Antibody profiling against the SARS-CoV-2 S protein was first assessed using plasma samples collected from COVID-19 patients (n = 57) during the Singapore outbreak between January and April 2020, across the early recovery phase [median 31 days post-illness onset (pio)] and a later post-recovery time point (median 98 days pio) (Table 1, Figure 1a and b). All patients showed a decrease in IgM response (Figure 1a), and a prolonged IgG response over time (Figure 1b). Although one recent study has demonstrated similar neutralisation profiles against both D614 and G614 SARS-CoV-2 pseudoviruses, the virus clade by which the six individuals were infected with was not identified. According to Singapore’s SARS-CoV-2 clade pattern from December 2019 till July 2020 based on n = 736 cases with genome availability, the D614G mutation, indicated as G clade following the GISAID clade nomenclature, only appeared in March 2020 (Figure 1c). Hence, with knowledge on the D614G status of a subset of COVID-19 patients (n = 44 infected with D614, n = 6 infected with G614, n = 7 containing all other clades: O, S, L, V, G, GH or GR; Table 1, Figure 1c), the neutralising capacity of these anti-SARS-CoV-2 antibodies was assessed using pseudotyped lentiviruses expressing the SARS-CoV-2 S protein tagged with a luciferase reporter as a surrogate...
of live virus.\textsuperscript{17} The neutralisation EC\textsubscript{50} values of each patient were interpolated from the respective dose–response neutralisation titration curves (Table 2, Figure 1d and e, Supplementary figure 1). Notably, these antibodies were able to neutralise both SARS-CoV-2 D614 and G614 pseudoviruses at similar levels, despite having a significantly lower neutralisation capacity at median 98 days pio in all COVID-19 patients (Figure 1d and e, Supplementary figures 1 and 2). Corroborating other studies, severe patients have a higher and persisting level of neutralising antibodies as compared with both mild and moderate patients (Table 2, Supplementary figure 2).\textsuperscript{18,19} Of clinical importance, all the patients infected with either the D614 or G614 clade elicited a similar degree of neutralisation against both D614 and G614 pseudoviruses (Figure 1f), suggesting that the D614G mutation does not impact the neutralisation capacity of the elicited antibodies. Our results support the notion that the locus where the point mutation occurred is not critical for antibody-mediated immunity and may not have an impact on virus resistance towards antibody-based interventions.\textsuperscript{4,20}

**DISCUSSION**

The emergence of a new virus clade due to random mutations could heavily deter the therapeutic outcome of treatments and vaccines. Majority of the current immunoassays developed against SARS-CoV-2 are based on the S antigen of the original Wuhan reference sequence.\textsuperscript{21,22} Moreover, pioneer batches of therapeutics and candidate vaccines were mostly designed based on earlier infections. As a result, mutations in the dominant variant sequence could potentially alter the viral phenotype and virulence, thereby rendering current immune-based therapies less efficient and effective.\textsuperscript{23,24} Fortunately, a recent pre-print reported no observable difference in IgM, IgG and IgA profiles against either the D614 or G614 S variant in an antigen-based serological assay,\textsuperscript{25} providing preliminary findings on the effectiveness of current diagnostic approaches to detect SARS-CoV-2 G614 infections.

In addition, determining the level of cross-reactivity is essential for immunosurveillance, as well as to identify broadly neutralising antibodies or epitopes.\textsuperscript{26} Here, we confirm that cross-reactivity occurs at the functional level of the humoral response on both the S protein variants. Of note, the stronger neutralising capacity observed during the early recovery phase may be due to the higher level of IgM response at median 31 days pio, as plasma IgM has been shown in a recent pre-print to contribute towards SARS-CoV-2 neutralisation.\textsuperscript{27} While IgA has also been reported to mediate neutralising activities during SARS-CoV-2 infection at a lower potency,\textsuperscript{27} investigations on the IgA levels and neutralising capacity in patients infected by the G614 clade would be needed to confirm earlier findings.

Regarding clinical outcomes, it appears that patients infected with the G614 clade, albeit small patient numbers, appear to have a lower log\textsubscript{10} EC\textsubscript{50} value (Figure 1d–f). While it remains elusive, this observation may be associated to the lower IgM and IgG levels in these patients. Nonetheless, our results, together with the recent serological evaluation,\textsuperscript{25} strongly suggest that existing serological assays will be able to detect both D614 and G614 clades of SARS-CoV-2 with a similar sensitivity. Recent studies have also demonstrated an overall equivalent sensitivity against both the D614 and G614 pseudotyped
Figure 1. Timeline of events during the SARS-CoV-2 outbreak in Singapore, and the antibody profiles of COVID-19 patients and their neutralising capacity against both D614 and G614 variants of SARS-CoV-2. Plasma samples of COVID-19 patients \( (n = 57) \) at median 31 and median 98 days post-illness onset (pio) were assessed for anti-SARS-CoV-2 IgM and IgG antibody response. Plasma samples (1:100 dilution) were incubated with transduced HEK293T cells expressing SARS-CoV-2 spike protein, and (a) anti-IgM and (b) anti-IgG levels were quantified by flow cytometry. Percentage binding indicates the percentage of cells with antibody binding. Data are shown as mean ± SD of two independent experiments. Dotted line indicates mean + 3SD of healthy controls \( (n = 22) \). Statistical analysis was carried out with the Wilcoxon signed-rank test \( (*) P < 0.05, ***P < 0.001 \). (c) Percentage of COVID-19 cases with genome available \( (n = 736) \) during the Singapore outbreak from December 2019 to July 2020, segregated by the clade with which the patients were infected following GISAID clade nomenclature. (d–f) Anti-SARS-CoV-2 neutralising antibodies were assessed using luciferase expressing lentiviruses pseudotyped with SARS-CoV-2 spike (S) protein of either the original strain, D614, or the mutant variant, G614. Log10 neutralisation EC50 profiles against D614 and G614 pseudoviruses across both time points. Data represent the mean of two independent experiments, and statistical analysis was carried out using the paired t-test \( (***) P < 0.001 \). (f) Comparison of log10 neutralisation EC50 values between D614 and G614 pseudoviruses during both time points. Data represent the mean of two independent experiments, and statistical analysis was carried out using the paired t-test. All data points are non-significant (ns).
viruses, suggesting that the D614G mutation is not expected to hinder current vaccine development.\textsuperscript{10–12,28} However, it is of clinical relevance to assess if cross-reactivity between the variants may enhance viral infection when neutralising antibodies are present at suboptimal concentrations.\textsuperscript{29} More importantly, further studies using monoclonal antibodies are necessary to validate the cross-reactivity profiles between both SARS-CoV-2 S variants.

Overall, our study shows that the D614G mutation on the S protein does not impact SARS-CoV-2 neutralisation by the host antibody response, nor confer viral resistance against the humoral immunity. Hence, there should be negligible impact towards the efficacy of antibody-based therapies and vaccines that are currently being developed.

**METHODS**

**Ethical approval**

Written informed consent was obtained from participants in accordance with the tenets of the Declaration of Helsinki. The study design protocol was approved by National Healthcare Group (NHG) Domain Specific Review Table 2.

| Patient | Days post-illness onset (pio) | Recovery phase | Infection by SARS-CoV-2 strain* | D614 (EC50) |
|---------|-------------------------------|----------------|---------------------------------|-------------|
| #1      | 39 Early Others                | 93.821         | 1.97230058                      | 27.088      |
| #2      | 95 Late                        | 36.481         | 1.562066734                     | ND          |
| #3      | 152 Early D614                 | 59.67          | 1.77575038                      | 59.527      |
| #4      | 111 Early D614                 | 84.26          | 1.925621455                     | 100.33      |
| #5      | 92 Late                        | 36.216         | 1.558900481                     | 20.109      |
| #6      | 100 Early D614                 | 264.7          | 2.422759341                     | 371.63      |
| #7      | 107 Late                       | 85.178         | 1.930327439                     | 101.03      |
| #8      | 100 Early D614                 | 401.03         | 2.603176862                     | 229.98      |
| #9      | 88 Late                        | 93.083         | 1.968870372                     | 42.272      |
| #10     | 88 Late                        | 59.156         | 1.7719988                       | 46.489      |
| #11     | 96 Late                        | 59.156         | 1.7719988                       | 46.489      |
| #12     | 92 Late                        | 84.26          | 1.925621455                     | 100.33      |
| #13     | 96 Late                        | 36.216         | 1.558900481                     | 20.109      |
| #14     | 92 Late                        | 264.7          | 2.422759341                     | 371.63      |
| #15     | 100 Early D614                 | 85.178         | 1.930327439                     | 101.03      |
| #16     | 100 Early D614                 | 401.03         | 2.603176862                     | 229.98      |
| #17     | 96 Late                        | 93.083         | 1.968870372                     | 42.272      |
| #18     | 92 Late                        | 59.156         | 1.7719988                       | 46.489      |
| #19     | 96 Late                        | 84.26          | 1.925621455                     | 100.33      |
| #20     | 92 Late                        | 36.216         | 1.558900481                     | 20.109      |

(Continued)
| Patient | Days post-illness onset (pio) | Recovery phase | Infection by SARS-CoV-2 strain<sup>a</sup> | D614 (EC50) Dilution factor | D614 (Log 10 EC50) Dilution factor | G614 (EC50) Dilution factor | G614 (Log 10 EC50) Dilution factor |
|---------|------------------------------|----------------|--------------------------------------|-------------------------------|-----------------------------------|------------------------------|-----------------------------------|
| #19     | 39 Early                      | G614           | 18.721                               | 1.272329043                   | 24.532                            | 1.389732956                   |                                   |
| #20     | 35 Early                      | D614           | 941.37                               | 2.973760354                   | 856.37                            | 2.932661445                   |                                   |
| #21     | 35 Early                      | D614           | 312.28                               | 2.494544171                   | 150.83                            | 2.17847731                    |                                   |
| #22     | 32 Early                      | D614           | 17.385                               | 1.921415932                   | 74.848                            | 1.8741802                     |                                   |
| #23     | 62 Early                      | G614           | 36.553                               | 1.562912306                   | 31.281                            | 1.495280628                   |                                   |
| #24     | 38 Early                      | D614           | 10.477                               | 1.020236944                   | ND                                | ND                            |                                   |
| #25     | 18 Early                      | D614           | 849.23                               | 2.92052328                    | ND                                | ND                            |                                   |
| #26     | 105 Late                      | D614           | 601.69                               | 2.779372794                   | ND                                | ND                            |                                   |
| Moderately (Pneumonia, without hypoxia) | | | | | | | |
| #1      | 29 Early                      | D614           | 325.6                                | 2.512684396                   | 311.41                            | 2.49332555                    |                                   |
| #2      | 29 Early                      | Others         | 50.013                               | 1.699082906                   | 40.54                            | 1.60783744                    |                                   |
| #3      | 37 Early                      | D614           | 17.385                               | 1.921415932                   | 74.848                            | 1.8741802                     |                                   |
| #4      | 29 Early                      | D614           | 406.93                               | 2.609519708                   | 394.6                             | 2.596157081                   |                                   |
| #5      | 29 Early                      | D614           | 188.21                               | 2.753248123                   | 412.73                            | 2.61566037                    |                                   |
| #6      | 25 Early                      | D614           | 2349.4                               | 3.209519708                   | 2000.3                            | 3.01051353                    |                                   |
| #7      | 34 Early                      | D614           | 96.242                               | 1.983664369                   | 110.53                            | 2.04348017                    |                                   |
| #8      | 28 Early                      | D614           | 227                                  | 2.356025857                   | 215.24                            | 2.33292983                    |                                   |
| #9      | 31 Early                      | D614           | 792.61                               | 2.899059547                   | 601.93                            | 2.77954589                    |                                   |
| #10     | 32 Early                      | D614           | 541.77                               | 2.733814953                   | 399.85                            | 2.6018971                     |                                   |
| #11     | 29 Early                      | D614           | 164.37                               | 2.215822555                   | 152.3                             | 2.18269903                    |                                   |
| #12     | 32 Early                      | D614           | 241.37                               | 2.38268329                    | 267.15                            | 2.42675519                    |                                   |
| #13     | 58 Early                      | D614           | 84.158                               | 1.925095406                   | 51.315                            | 1.71042433                    |                                   |
| #14     | 25 Early                      | D614           | 220.86                               | 2.344117068                   | 171.07                            | 2.23317385                    |                                   |
| #15     | 36 Early                      | D614           | 200.82                               | 2.344117068                   | 171.07                            | 2.23317385                    |                                   |
| #16     | 27 Early                      | D614           | 308.07                               | 2.488649409                   | 201.4                             | 2.30405946                    |                                   |
| #17     | 34 Early                      | D614           | 1079.6                               | 3.033262876                   | 1039.5                            | 3.01682449                    |                                   |
| #18     | 42 Early                      | D614           | 90.322                               | 1.955793546                   | 56.963                            | 1.75592854                    |                                   |
| #19     | 30 Early                      | G614           | 214.79                               | 2.332014058                   | 186.07                            | 2.26967658                    |                                   |
| #20     | 104 Late                      | D614           | 24.869                               | 1.395638322                   | 29.766                            | 1.47372047                    |                                   |
| #21     | 99 Late                       | D614           | 10.11                                | 1.04751156                    | 17.581                            | 1.245043754                   |                                   |
| #22     | 32 Early                      | G614           | 38.602                               | 1.586609806                   | 19.899                            | 1.29831252                    |                                   |
| #23     | 99 Late                       | D614           | 17.385                               | 1.240174695                   | 18.098                            | 1.257630584                   |                                   |
| #24     | 35 Early                      | D614           | 82.448                               | 1.921415932                   | 74.848                            | 1.8741802                     |                                   |
| #25     | 99 Early                      | G614           | 18.721                               | 1.272329043                   | 24.532                            | 1.389732956                   |                                   |
| #26     | 99 Early                      | G614           | 38.602                               | 1.586609806                   | 19.899                            | 1.29831252                    |                                   |

(Continued)
COVID-19 patients and sample collection

Fifty-seven patients who tested PCR-positive for SARS-CoV-2 in nasopharyngeal swabs in Singapore were recruited into the study from January to March 2020\(^\text{30,31}\) (Table 1). Patients were categorised into three groups based on clinical severity during hospitalisation: mild (no pneumonia on chest radiographs (CXR), \(n = 25\)), moderate (pneumonia on CXR without hypoxia, \(n = 19\)) and severe (pneumonia on CXR with hypoxia (desaturation to \(\leq 94\%)\), \(n = 13\)). Whole blood of patients was collected in BD Vacutainer\textsuperscript{TM}CPT\textsuperscript{TM} tubes (BD Biosciences, Franklin Lakes, NJ, USA) and centrifuged at 1700 \(\text{g}\) for 20 min to obtain plasma fractions. Plasma samples were either heat-inactivated at 56°C for 30 min,\(^\text{17}\) or treated with Triton\textsuperscript{TM} X-100 (Thermo Fisher Scientific, Waltham, MA, USA) to a final concentration of 1% for 2 h at room temperature (RT) for virus inactivation.\(^\text{31,32}\)

Determining D614G mutation status of COVID-19 patients

Residual clinical RNA was subjected to tiled amplicon PCR using ARTIC nCoV-2019 version 3 panel.\(^\text{33}\) Sequencing libraries were prepared using the Nextera XT and sequenced on MiSeq (Illumina, San Diego, California, USA) to generate 300 bp paired-end reads. The reads were subjected to a hard-trim of 50 bp on each side to remove primer artefacts using BBMap\(^\text{34}\) prior to consensus sequence generation by Burrows-Wheeler Aligner-MEM v0.7.17. Sequences with nucleotide mutation A23403G were assigned as D614G.

Cells

Human embryonic kidney (HEK) 293T (ATCC, Manassas, VA, USA) cells were maintained in DMEM (Cytiva Life Sciences, Marlborough, MA USA) with 10% heat-inactivated foetal bovine serum (FBS; Cytiva Life Sciences). CHO cells expressing human ACE2 (CHO-ACE2; kindly gifted by Professor Yee-Joo Tan, Department of Microbiology, NUS & IMCB, A*STAR, Singapore) were cultured in DMEM with

#### Table 2. Continued.

| Patient | Days post-illness onset (pio) | Recovery phase | Infection by SARS-CoV-2 strain\(^a\) | D614 (EC50) Dilution factor | D614 (Log 10 EC50) Dilution factor | G614 (EC50) Dilution factor | G614 (Log 10 EC50) Dilution factor |
|---------|-------------------------------|---------------|----------------------------------|----------------------------|----------------------------------|----------------------------|----------------------------------|
| Severe (Pneumonia, with hypoxia) |
| #1      | 31 Early G614                 | 740.24        | 2.869372549                     | 548.74                     | 2.739366619                     |
| #2      | 92 Late                       | 154.05        | 2.187661703                     | 92.754                     | 1.967332648                     |
| #3      | 33 Early Others               | 940.91        | 2.973548084                     | 967.53                     | 2.98566444                     |
| #4      | 97 Late                       | 250.17        | 2.398235229                     | 199.92                     | 2.300856243                     |
| #5      | 29 Early D614                 | 1597.5        | 3.20440867                     | 1443.9                     | 3.159537116                     |
| #6      | 96 Late                       | 173.92        | 2.40349527                     | 236.97                     | 2.374693369                     |
| #7      | 29 Early D614                 | 970.61        | 2.987044761                     | 651.53                     | 2.81394418                      |
| #8      | 34 Early D614                 | 755.31        | 2.878125235                     | 822.44                     | 2.915104224                     |
| #9      | 113 Late                      | 71.959        | 1.857085119                     | 74.804                     | 1.873924822                     |
| #10     | 33 Early Others               | 2042.2        | 3.10098272                     | 2007.9                     | 3.30274208                     |
| #11     | 110 Late                      | 100.71        | 2.003072596                     | 108.06                     | 2.033664963                     |
| #12     | 30 Early D614                 | 1291.7        | 3.111116166                     | 3109.8                     | 3.492732459                     |
| #13     | 87 Late                       | 420.78        | 2.624050589                     | 996.85                     | 2.99862981                     |
| #14     | 28 Early D614                 | 1298.1        | 3.11330815                     | 1391.8                     | 3.143576832                     |
| #15     | 109 Late                      | 224.08        | 2.350403096                     | 246.4                      | 2.391640703                     |
| #16     | 37 Early Others               | 466.49        | 2.668842338                     | 383.24                     | 2.583470831                     |
| #17     | 92 Late                       | 156.93        | 2.195705795                     | 140.67                     | 2.148201487                     |
| #18     | 39 Early Others               | 4453.3        | 3.648681953                     | 3528.8                     | 3.547627045                     |
| #19     | 116 Late                      | 1024.2        | 3.010384771                     | 1072.7                     | 3.03478281                     |
| #20     | 40 Early D614                 | 529.25        | 2.723660867                     | 730.88                     | 2.863846078                     |
| #21     | 60 Late                       | 253.5         | 2.403977964                     | 419.99                     | 2.62323895                     |
| #22     | 31 Early D614                 | 891.98        | 2.950355117                     | 1016.9                     | 3.007278247                     |
| #23     | 93 Late                       | 136.02        | 2.133602771                     | 108.15                     | 2.03406524                     |
| #24     | 40 Early Others               | 1595.2        | 3.202815141                     | 1691.3                     | 3.228220649                     |
| #25     | 60 Late                       | 612.24        | 2.7869217                      | 702.75                     | 2.84800854                     |

COVID-19: Coronavirus Disease 2019; Early: median 31 days post-illness onset (pio); Late: median 98 days pio; ND: not determined.

\(^a\)Others: O, S, L, V, G, GH or GR clades.

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Cross-neutralising antibodies against the SARS-CoV-2 D614G mutant
10% FBS, 1% MEM non-essential amino acid solution (Thermo Fisher Scientific), and 0.5 mg mL⁻¹ of Genetin selective antibiotic (Thermo Fisher Scientific). Surface expression of ACE2 on CHO-ACE2 cells was confirmed using anti-human ACE2 Alexa Fluor 647 (Santa Cruz Biotechnology, Dallas, TX, USA). All cells were maintained at 37°C with 5% CO₂.

**S-flow assay**

Full-length SARS-CoV-2 Spike (S) protein of the D614 variant-expressing HEK293T cells was produced by transduction with lentiviral particles. Cells were seeded at 1.5 × 10⁵ per well in 96-well plates and incubated with Triton X-100 inactivated plasma samples (1:100 dilution) in 10% FBS in PBS (FACS blocking buffer), followed by a secondary incubation of Alexa Fluor 647-conjugated anti-human IgM or IgG (1:500 dilution; Thermo Fisher Scientific) and propidium iodide (1:2500 dilution; Sigma-Aldrich, St. Louis, MO, USA). Cells were acquired on BD™ LSR II laser (BD Biosciences), and results were analysed with FlowJo (version 10, Tree Star Inc. Becton Dickinson, Ashland, OR). Results are presented as percentage of binding, which indicates the percentage of cells with antibody binding.

**SARS-CoV-2 pseudovirus production**

The pseudotyped lentiviruses were produced as previously described. Briefly, using the third-generation lentivirus system, pseudotyped viral particles expressing SARS-CoV-2 D614 strain or G614 variant S proteins were generated by reverse transfection of 3 × 10⁵ of HEK293T cells with 12 µg pMDLg/PRRE (Addgene, Watertown, Massachusetts, USA), 6 µg pRSV-Rev (Addgene), 12 µg pPTSLnX-cov-SP (SARS-CoV-2 wildtype S), a kind gift from Dr Brendon John Hanson, DSO National Laboratories, Singapore) or pPTSLnX-cov-SP-D614G (SARS-CoV-2 mutant D614G S), and 24 µg pHIV-Luc-ZsGreen (Addgen) using Lipofectamine 2000 transfection (Invitrogen, Carlsbad, California, USA). Cells were cultured for 3 days, before viral supernatant was harvested by centrifugation to remove cell debris and filtered through a 0.45 µm filter unit (Sartorius, Gottingen, Germany). Viral titres were quantified with Lenti-X™ p24 Rapid Titre Kit (Takara Bio, Kusatsu, Shiga, Japan).

**Pseudovirus neutralisation assay**

The pseudotyped lentivirus neutralisation assay was performed as previously described, with slight modifications. CHO-ACE2 cells were seeded at 3.2 × 10⁴ per well in a 96-well black microplate (Corning, New York, NY) in culture medium without Geneticin. Serially diluted heat-inactivated plasma samples (1:10 to 1:31 250 dilutions) were incubated with equal volume of pseudovirus expressing SARS-CoV-2 S proteins of either original wildtype or D614G mutant strain (0.4 ng p24) at 37°C for 1 h, before being added to pre-seeded CHO-ACE2 cells. Cells were refreshed with culture media after 1 h incubation. After 48 h, cells were washed with PBS and lysed with 1× Passive Lysis Buffer (Promega, Madison, Wisconsin, USA) with gentle shaking at 125 rpm for 30 min at 37°C. Luciferase activity was subsequently quantified with Luciferase Assay System (Promega) on a GloMax Luminometer (Promega).

**Data and statistical analysis**

Data were analysed using GraphPad Prism (version 8.4.3; GraphPad Software, San Diego, CA) and Microsoft Excel (version 16.39; Microsoft). The Wilcoxon signed-rank test and the paired t-test were carried out to compare the antibody and neutralisation profiles of COVID-19 patients at median of 31 and 98 days’ post-illness onset (pio). P-values less than 0.05 are considered to be statistically significant.

**ACKNOWLEDGMENTS**

The authors thank the study participants who donated their blood samples to this project and the healthcare workers caring for the COVID-19 patients. The authors also wish to thank Ding Ying and the Singapore Infectious Disease Clinical Research Network (SCRN) for their help in patient recruitment and the staffs at the National Centre for Infectious Diseases (NCID) who assisted with data analysis on viral sequences and determination of the D614G status.

All authors declare no conflicts.

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Supporting Information

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