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SHORT COMMUNICATION

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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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.[1] 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.[2] 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.[3,4] For instance, antigenic drift was previously reported in other common cold coronaviruses, OC43 and 229E, as well as in SARS-CoV.[5–7]

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.[8] 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.[8] *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.[8–12] 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).[13,14] More importantly, mutations in the S protein of SARS-CoV-2 have been shown to induce conformational modifications that alter antigenicity.[15,16] 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.[9] 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

Table 1. Demographic and clinical information of COVID-19 patients

	Patients (n = 57)
Demographics	
Age, years	45 (13)
Sex	
Male	38 (66.7%)
Female	19 (33.3%)
Ethnicity	
Chinese	42 (73.7%)
Others	15 (26.3%)
Comorbidities	29 (50.9%)
Hyperlipidaemia	14 (24.6%)
Hypertension	13 (22.8%)
Diabetes	7 (12.3%)
Myocardial infection (history)	5 (8.8%)
Others	10 (17.5%)
D614G infection status	
D614	44 (77.2%)
G614	6 (10.5%)
Others[a]	7 (12.3%)
Clinical outcome (clinical severity; group)	
No pneumonia (0; mild)	25 (43.9%)
Pneumonia, without hypoxia (1; moderate)	19 (33.3%)
Pneumonia, with hypoxia (2; severe)	13 (22.8%)

Data are presented as Mean (SD) or *n* (%). COVID-19: Coronavirus Disease 2019.
[a]Others: O, S, L, V, G, GH or GR clades.

of live virus.[17] The neutralisation EC_{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).[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.[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.[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.[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,[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.[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.[27] While IgA has also been reported to mediate neutralising activities during SARS-CoV-2 infection at a lower potency,[27] investigations on the IgA levels and neutralising capacity in patients infected by the G614 clade would be needed to confirm earlier findings. Interestingly, although there was no significant difference between the neutralising capacity against both D614 and G614 pseudoviruses, individuals infected by the G614 clade, albeit small patient numbers, appear to have a lower log10 EC50 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,[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

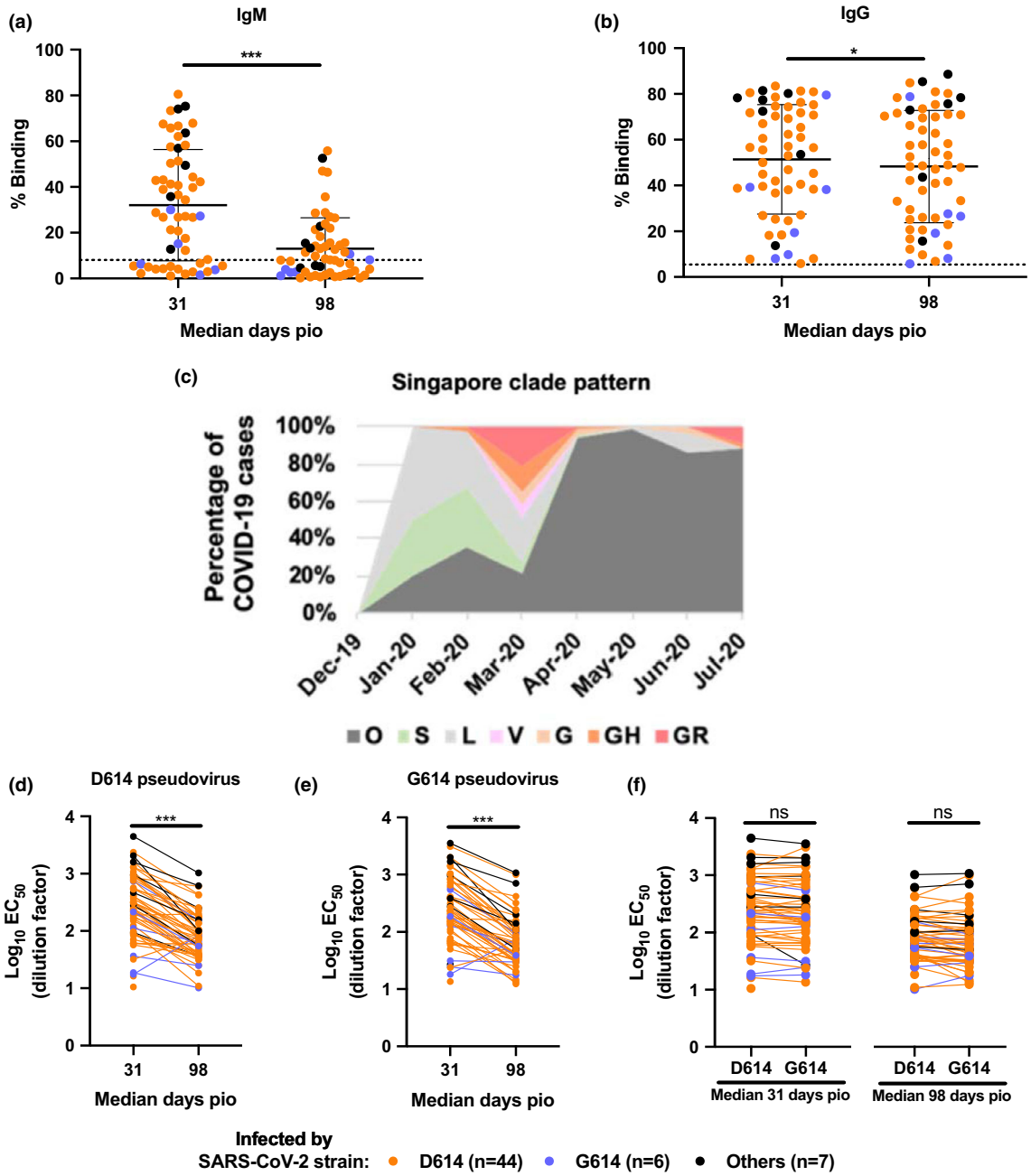

Figure 1. Timeline of events during the SARS-CoV-2 outbreak in Singapore, and the antibody profiles of COVID-19 patients and their neutralising capacity against both D614 and G614 variants of SARS-CoV-2. Plasma samples of COVID-19 patients (n = 57) at median 31 and median 98 days post-illness onset (pio) 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. Log_{10} neutralisation EC_{50} profiles against **(d)** D614 and **(e)** 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 log_{10} neutralisation EC_{50} 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.[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.[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. Neutralisation EC50 values of COVID-19 patients

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
Mild (No pneumonia)							
#1	39	Early	Others	93.821	1.972300058	27.088	1.432776941
	95	Late		36.481	1.562066734	ND	ND
#2	34	Early	D614	59.67	1.775756038	59.527	1.774713996
	152	Late		59.156	1.7719988	46.489	1.667350204
#3	30	Early	D614	84.26	1.925621455	100.33	2.001430812
	111	Late		36.216	1.558900481	20.109	1.303390474
#4	29	Early	D614	264.7	2.422753941	371.63	2.570110765
	92	Late		85.178	1.930327439	101.03	2.004450353
#5	30	Early	D614	401.03	2.603176862	229.98	2.36169007
	100	Late		93.083	1.968870372	42.272	1.626052796
#6	32	Early	D614	56.708	1.753644331	49.807	1.697290384
	96	Late		37.541	1.574505837	24.87	1.395675785
#7	30	Early	D614	182.16	2.260453018	179.26	2.253483392
	107	Late		37.299	1.571697188	31.102	1.492788317
#8	30	Early	D614	70.715	1.849511546	64.52	1.809694359
	88	Late		38.049	1.580343247	32.853	1.516575034
#9	25	Early	D614	61.803	1.791009557	67.785	1.8311336
	101	Late		45.326	1.656347394	13.3	1.123851641
#10	32	Early	D614	123.21	2.090645958	72.937	1.862947896
	110	Late		18.353	1.263707065	ND	ND
#11	33	Early	D614	312.72	2.495155657	135.08	2.130591052
	91	Late		103.42	2.014604533	60.652	1.782845126
#12	33	Early	D614	365.85	2.563303059	233.92	2.369067355
	96	Late		79.832	1.90217701	35.665	1.552242228
#13	31	Early	G614	110.63	2.043872912	127.51	2.105544246
	94	Late		65.001	1.812920038	63.342	1.801691772
#14	24	Early	D614	151.32	2.179896333	143.27	2.156155261
	100	Late		39.825	1.600155784	31.445	1.497551599
#15	28	Early	D614	242.06	2.383923029	241.44	2.382809222
	98	Late		58.31	1.765743041	52.821	1.722806619
#16	31	Early	D614	169.39	2.228887768	134.4	2.128399269
	92	Late		78.702	1.895985769	78.239	1.893423291
#17	39	Early	D614	89.4	1.951337519	77.364	1.888538916
	97	Late		25.104	1.399742926	14.494	1.161188257
#18	26	Early	D614	16.219	1.210024074	13.513	1.130751777
	99	Late		ND	ND	ND	ND

(Continued)

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
#19	39	Early	G614	18.721	1.272329043	24.532	1.389732956
	99	Late		10.11	1.004751156	17.581	1.245043574
#20	35	Early	D614	941.37	2.973760354	856.37	2.932661445
	99	Late		171	2.23299611	97.95	1.99100444
#21	35	Early	D614	312.28	2.494544171	150.83	2.178487731
	99	Late		38.602	1.586609806	19.899	1.298831252
#22	32	Early	G614	17.385	1.240174695	18.098	1.257630584
	98	Late		83.448	1.921415932	74.848	1.8741802
#23	62	Early	G614	36.553	1.562923026	31.281	1.495280628
	104	Late		24.869	1.395658322	29.766	1.473720477
#24	38	Early	D614	10.477	1.020236944	ND	ND
	99	Late		ND	ND	ND	ND
#25	18	Early	D614	849.23	2.929025328	ND	ND
	105	Late		601.69	2.779372794	ND	ND
Moderate (Pneumonia, without hypoxia)							
#1	29	Early	D614	325.6	2.512684396	311.41	2.493332555
	99	Late		50.013	1.699082906	40.54	1.607883744
#2	29	Early	Others	280.08	2.447282098	279.51	2.44639735
	91	Late		55.82	1.746789832	49.937	1.698422448
#3	37	Early	D614	565.39	2.752348123	412.73	2.615666037
	99	Late		176.37	2.246424715	192.41	2.28422764
#4	29	Early	D614	406.93	2.609519708	394.6	2.596157081
	92	Late		58.04	1.763727404	70.882	1.850535963
#5	29	Early	D614	188.21	2.274642695	172.03	2.235604189
	106	Late		197.85	2.296336055	157.28	2.1966735
#6	25	Early	D614	2349.4	3.370956964	2000.3	3.301095135
	96	Late		432.12	2.635604367	319.05	2.503858749
#7	34	Early	D614	96.242	1.983364639	110.53	2.04348017
	104	Late		10.932	1.038699623	12.366	1.092229242
#8	28	Early	D614	227	2.356025857	215.24	2.332922983
	113	Late		41.09	1.613736141	28.984	1.462158321
#9	31	Early	D614	792.61	2.899059547	601.93	2.779545989
	96	Late		182.48	2.261215272	132.86	2.123394248
#10	32	Early	D614	541.77	2.733814953	399.85	2.6018971
	99	Late		136.61	2.135482491	121.88	2.085932446
#11	29	Early	D614	164.37	2.215822555	152.3	2.182699903
	90	Late		34.63	1.539452492	41.678	1.61990687
#12	32	Early	D614	241.37	2.38268329	267.15	2.426755179
	89	Late		35.053	1.544725193	39.4	1.595496222
#13	58	Early	D614	84.158	1.925095406	51.315	1.710244333
	101	Late		34.56	1.538573734	25.507	1.406659382
#14	25	Early	D614	220.86	2.344117068	171.07	2.233173855
	106	Late		31.918	1.50403567	33.142	1.520378713
#15	36	Early	D614	200.82	2.302806963	156.64	2.194902674
	87	Late		70.748	1.849714167	65.35	1.815245592
#16	27	Early	D614	308.07	2.488649409	201.4	2.304059466
	106	Late		90.322	1.955793546	56.963	1.755592854
#17	34	Early	D614	1079.6	3.033262876	1039.5	3.016824494
	115	Late		100.36	2.001560653	119.98	2.079108858
#18	42	Early	D614	89.823	1.953387556	69.059	1.839220285
	107	Late		31.172	1.493764668	31.425	1.497275286
#19	30	Early	G614	214.79	2.332014058	186.07	2.269676358
	99	Late		54.362	1.735295426	38.613	1.586733545

(Continued)

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
	92	Late		154.05	2.187661703	92.754	1.967332648
#2	33	Early	Others	940.91	2.973548084	967.53	2.98566444
	97	Late		250.17	2.398235229	199.92	2.300856243
#3	29	Early	D614	1597.5	3.203440867	1443.9	3.159537116
	96	Late		173.92	2.240349527	236.97	2.374693369
#4	29	Early	D614	970.61	2.987044761	651.53	2.813934418
	104	Late		106.39	2.026900809	86.982	1.939429389
#5	34	Early	D614	755.31	2.878125235	822.44	2.915104224
	113	Late		71.959	1.857085119	74.804	1.873924822
#6	33	Early	Others	2042.2	3.310098272	2007.9	3.30274208
	110	Late		100.71	2.003072596	108.06	2.033664963
#7	30	Early	D614	1291.7	3.11116166	3109.8	3.492732459
	87	Late		420.78	2.624055089	996.85	2.998629813
#8	28	Early	D614	1298.1	3.11330815	1391.8	3.143576832
	109	Late		224.08	2.350403096	246.4	2.391640703
#9	37	Early	Others	466.49	2.668842338	383.24	2.583470831
	92	Late		156.93	2.195705975	140.67	2.148201487
#10	39	Early	Others	4453.3	3.648681953	3528.8	3.547627045
	116	Late		1024.2	3.010384771	1072.7	3.030478281
#11	40	Early	D614	529.25	2.723660867	730.88	2.863846078
	60	Late		253.5	2.403977964	419.99	2.62323895
#12	31	Early	D614	891.98	2.950355117	1016.9	3.007278247
	93	Late		136.02	2.133602771	108.15	2.034026524
#13	40	Early	Others	1595.2	3.202815141	1691.3	3.228220649
	60	Late		612.24	2.7869217	702.75	2.846800854

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.

Board (DSRB) under study number 2012/00917. Specimens from healthy donors were collected under study numbers 2017/2806 and NUS IRB 04-140.

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[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® CPT™ tubes (BD Biosciences, Franklin Lakes, NJ, USA) and centrifuged at 1700 *g* for 20 min to obtain plasma fractions. Plasma samples were either heat-inactivated at 56°C for 30 min,[17] or treated with Triton™ X-100 (Thermo Fisher Scientific, Waltham, MA, USA) to a final concentration of 1% for 2 h at room temperature (RT) for virus inactivation.[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.[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[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

10% FBS, 1% MEM non-essential amino acid solution (Thermo Fisher Scientific), and 0.5 mg mL^{-1} of Geneticin 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% CO_2.

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.[35] Cells were seeded at 1.5×10^5 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.[3] 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 3×10^7 of HEK293T cells with 12 μg pMDLg/PRRE (Addgene, Watertown, Massachusetts, USA), 6 μg pRSV-Rev (Addgene), 12 μg pTT5LnX-coV-SP (SARS-CoV-2 wildtype S, a kind gift from Dr Brendon John Hanson, DSO National Laboratories, Singapore) or pTT5Lnx-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.[3] CHO-ACE2 cells were seeded at 3.2 x 10^4 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 μL^{-1} of 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.

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CONFLICT OF INTEREST

All authors declare no conflicts.

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