bioRxiv preprint doi: https://doi.org/10.1101/2020.10.08.332544; this version posted October 9, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Observation submitted to mBio

Neutralizing antibodies from early cases of SARS-CoV-2 infection offer cross-protection against the SARS-CoV-2 D614G variant

Cheryl Yi-Pin Lee[1,2], Siti Naqiah Amrun[1,2], Rhonda Sin-Ling Chee[1,2], Yun Shan Goh[1,2], Tze-Minn Mak[5,10], Sophie Octavia[5,10], Nicholas Kim-Wah Yeo[1,2], Zi Wei Chang[1,2], Matthew Zirui Tay[1,2], Anthony Torres-Ruesta[1,2,3], Guillaume Carissimo[1,2], Chek Meng Poh[1,2], Siew-Wai Fong[1,2,4], Wang Bei[2], Sandy Lee[2], Barnaby Edward Young[5,6,7], Seow-Yen Tan[8], Yee-Sin Leo[5,6,7,9], David C. Lye[5,6,7,9], Raymond T. P. Lin[10,11], Sebastien Maurer-Stroh[1,4,5,10,12] Bernett Lee[2], Wang Cheng-I[2], Laurent Renia[1,2*], Lisa F.P. Ng[1,2,3,13,14*]

[1]Infectious Diseases Horizontal Technology Centre (ID HTC), Agency for Science, Technology and Research (A*STAR), Immunos, Biopolis, Singapore 138648, Singapore.

[2]Singapore Immunology Network, Agency for Science, Technology and Research (A*STAR), Immunos, Biopolis, Singapore 138648, Singapore.

[3]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 119228, Singapore.

[4]Department of Biological Sciences, National University of Singapore, Singapore 117558, Singapore.

[5]National Centre for Infectious Diseases, 16 Jalan Tan Tock Seng, Singapore 308442, Singapore.

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.08.332544; this version posted October 9, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

[6]Department of Infectious Diseases, Tan Tock Seng Hospital, 11 Jalan Tan Tock Seng, Singapore 308433, Singapore.

[7]Lee Kong Chian School of Medicine, Nanyang Technological University, 11 Mandalay Road, Singapore 308232, Singapore.

[8]Department of Infectious Diseases, Changi General Hospital, 2 Simei Street 3, Singapore 529889, Singapore.

[9]Yong Loo Lin School of Medicine, National University of Singapore and National University Health System, 10 Medical Drive, Singapore 117597, Singapore.

[10]National Public Health Laboratory, National Centre for Infectious Diseases, Singapore

[11]Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore

[12]Bioinformatics Institute, Agency for Science Technology and Research (A*STAR), Matrix, Biopolis, Singapore 138671, Singapore.

[13]National Institute of Health Research, Health Protection Research Unit in Emerging and Zoonotic Infections, University of Liverpool, Liverpool, United Kingdom.

[14]Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom.

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

***Corresponding author:** Lisa F.P. Ng: Infectious Diseases Horizontal Technology Centre, A*STAR, 8A Biomedical Grove, Immunos #04-06, Singapore 138648. Phone: (+65)-64070028. Email: lisa_ng@immunol.a-star.edu.sg;

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.08.332544; this version posted October 9, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Laurent Renia: Infectious Diseases Horizontal Technology Centre, A*STAR, 8A Biomedical Grove, Immunos #04-06, Singapore 138648. Phone: (+65)-64070007. Email: renia_laurent@immunol.a-star.edu.sg

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.08.332544; this version posted October 9, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

ABSTRACT (93 words)

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-neutralize against the G614 variant. In this report, profiling of the anti-SARS-CoV-2 humoral immunity reveals similar neutralization profiles against both S protein variants, albeit waning neutralizing antibody capacity at the later phase of infection. These findings provide further insights towards the validity of current immune-based interventions.

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.08.332544; this version posted October 9, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

IMPORTANCE

Random mutations in the viral genome is a naturally occurring event that may lead to enhanced viral fitness and immunological resistance, while heavily impacting the validity of licensed therapeutics. A single point mutation from aspartic acid (D) to glycine (G) at position 614 of the SARS-CoV-2 spike (S) protein, termed D614G, has garnered global attention due to the observed increase in transmissibility and infection rate. Given that a majority of the developing antibody-mediated therapies and serological assays are based on the S antigen of the original Wuhan reference sequence, it is crucial to determine if humoral immunity acquired from the original SARS-CoV-2 isolate is able to induce cross-detection and cross-protection against the novel prevailing D614G variant.

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.08.332544; this version posted October 9, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

OBSERVATION

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 36 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, 9). Notably, single point mutations have been shown to induce resistance to neutralizing antibodies in other coronaviruses, including SARS-CoV and Middle East respiratory syndrome (MERS-CoV) (10, 11). More importantly, mutations in the S protein of SARS-CoV-2 have been shown to induce conformational modifications that alter antigenicity (12, 13). Hence, determining any cross-neutralizing capability of antibodies developed against the earlier G614 variant is of paramount importance to validate the therapeutic efficacy of developing immune-based interventions.

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.08.332544; this version posted October 9, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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 1B). 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 neutralization 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, the D614G mutation only appeared in February 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 neutralizing 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 (14). The neutralization EC_{50} values of each patient were interpolated from the respective dose-response neutralization titration curves (Table 2, Figure 1D and 1E, Supplementary figure 1). Notably, these antibodies were able to neutralize both SARS-CoV-2 D614 and G614 pseudoviruses at similar levels, despite having a significantly lower neutralization capacity at median 98 days pio in all COVID-19 patients (Figure 1D and 1E, Supplementary figures 1 and 2). Corroborating other studies, severe patients have a higher and persisting level of neutralizing antibodies as compared with both mild and moderate patients (Table 2, Supplementary figure 2) (15, 16). Of clinical importance, all the patients infected with either the D614 or G614 clade elicited a similar degree of

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.08.332544; this version posted October 9, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

neutralization against both D614 and G614 pseudoviruses (Figure 1F), suggesting that the D614G mutation does not impact the neutralization 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, 17).

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 (18, 19). 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 (20, 21). Fortunately, a recent pre-print reported no observable difference in IgM, IgG and IgA profiles against either S variant in an antigen-based serological assay (22), 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 neutralizing antibodies or epitopes (23). Here, we confirm that cross-reactivity occurs at the functional level of the humoral response on both the S protein variants. Our results, together with the recent serological evaluation (22), strongly suggest that existing serological assays will be able to detect both D614 and G614 clades of SARS-CoV-2 with a similar sensitivity. However, it is of clinical relevance to assess if cross-reactivity between the variants may enhance viral infection when neutralizing antibodies are present at suboptimal concentrations (24). More importantly, further studies using monoclonal

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.08.332544; this version posted October 9, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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 neutralization 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.

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.08.332544; this version posted October 9, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Acknowledgements

The authors would like to 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. The authors would also like to thank Professor Yee-Joo Tan (Department of Microbiology, NUS; Institute of Molecular and Cell Biology (IMCB), A*STAR) for kindly providing the CHO-ACE2 cells, and Dr. Brendon John Hanson (DSO National Laboratories, Singapore) for kindly providing the SARS-CoV-2 wildtype S protein.

Funding Sources

This study was supported by core and COVID-19 (H20/04/g1/006) research grants from Biomedical Research Council (BMRC), and the A*ccelerate GAP-funded project (ACCL/20-GAP001-C20H-E) from Agency for Science, Technology and Research (A*STAR), and National Medical Research Council (NMRC) COVID-19 Research fund (COVID19RF-001, COVID19RF-007 and COVID19RF-060). ATR is supported by the Singapore International Graduate Award (SINGA), A*STAR. The funding sources had no role in the study design; collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Declaration of Interests

All authors declare no conflicts.

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.08.332544; this version posted October 9, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Author Contributions

CYPL, SNA, RSLC, YSG, TMM, and SO designed the experiments, acquired and analyzed the data, and wrote the manuscript. NKWY, ZWC, MZT, ATR and CMP acquired and analyzed the data, and wrote the manuscript. GC, SWF, WB, SL, SMS, BL and WCI analyzed the data and wrote the manuscript. BEY, SYT, YSL, DCL and RTPL designed and supervised sample collection. LR and LFPN conceptualized the study, analyzed the data and wrote the manuscript. All authors revised and approved the final version of the manuscript.

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.08.332544; this version posted October 9, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

References

1. Cohen J, Normile D. New SARS-like virus in China triggers alarm. Science. 2020;367(6475):234-5.
2. Worldometer. Dover, Delaware, U.S.A. 2020 [Available from: https://www.worldometers.info/coronavirus/?
3. Sevajol M, Subissi L, Decroly E, Canard B, Imbert I. Insights into RNA synthesis, capping, and proofreading mechanisms of SARS-coronavirus. Virus Research. 2014;194:90-9.
4. Grubaugh ND, Hanage WP, Rasmussen AL. Making Sense of Mutation: What D614G Means for the COVID-19 Pandemic Remains Unclear. Cell. 2020.
5. Ren L, Zhang Y, Li J, Xiao Y, Zhang J, Wang Y, et al. Genetic drift of human coronavirus OC43 spike gene during adaptive evolution. Scientific Reports. 2015;5(1):11451.
6. Chibo D, Birch C. Analysis of human coronavirus 229E spike and nucleoprotein genes demonstrates genetic drift between chronologically distinct strains. Journal of General Virology. 2006;87(5):1203-8.
7. Song H-D, Tu C-C, Zhang G-W, Wang S-Y, Zheng K, Lei L-C, et al. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proceedings of the National Academy of Sciences of the United States of America. 2005;102(7):2430.
8. Zhang L, Jackson CB, Mou H, Ojha A, Rangarajan ES, Izard T, et al. The D614G mutation in the SARS-CoV-2 spike protein reduces S1 shedding and increases infectivity. bioRxiv : the preprint server for biology. 2020:2020.06.12.148726.
9. Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, et al. Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. Cell. 2020.
10. Sui J, Aird DR, Tamin A, Murakami A, Yan M, Yammanuru A, et al. Broadening of Neutralization Activity to Directly Block a Dominant Antibody-Driven SARS-Coronavirus Evolution Pathway. PLOS Pathogens. 2008;4(11):e1000197.
11. Tang X-C, Agnihothram SS, Jiao Y, Stanhope J, Graham RL, Peterson EC, et al. Identification of human neutralizing antibodies against MERS-CoV and their role in virus adaptive evolution. Proceedings of the National Academy of Sciences. 2014;111(19):E2018.
12. Eaaswarkhanth M, Al Madhoun A, Al-Mulla F. Could the D614G substitution in the SARS-CoV-2 spike (S) protein be associated with higher COVID-19 mortality? International Journal of Infectious Diseases. 2020;96:459-60.
13. Phan T. Genetic diversity and evolution of SARS-CoV-2. Infection, Genetics and Evolution. 2020;81:104260.
14. Poh CM, Carissimo G, Wang B, Amrun SN, Lee CY-P, Chee RS-L, et al. Two linear epitopes on the SARS-CoV-2 spike protein that elicit neutralising antibodies in COVID-19 patients. Nature Communications. 2020;11(1):2806.
15. Wang X, Guo X, Xin Q, Pan Y, Hu Y, Li J, et al. Neutralizing Antibody Responses to Severe Acute Respiratory Syndrome Coronavirus 2 in Coronavirus Disease 2019 Inpatients and Convalescent Patients. Clinical Infectious Diseases. 2020.
16. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clinical Infectious Diseases. 2020.

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.08.332544; this version posted October 9, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

17. Barnes CO, West AP, 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.

18. Lee CY-P, Lin RTP, Renia L, Ng LFP. Serological Approaches for COVID-19: Epidemiologic Perspective on Surveillance and Control. Frontiers in immunology. 2020;11:879-.

19. Wang C, Gao Z, Shen K, Cao J, Shen Z, Jiang K, et al. Safety and efficiency of endoscopic resection versus laparoscopic resection in gastric gastrointestinal stromal tumours: A systematic review and meta-analysis. European Journal of Surgical Oncology. 2020;46(4, Part A):667-74.

20. Sanjuán R, Nebot MR, Chirico N, Mansky LM, Belshaw R. Viral Mutation Rates. Journal of Virology. 2010;84(19):9733.

21. Ojosnegros S, Beerenwinkel N. Models of RNA virus evolution and their roles in vaccine design. Immunome research. 2010;6 Suppl 2(Suppl 2):S5-S.

22. Klumpp-Thomas C, Kalish H, Hicks J, Mehalko J, Drew M, Memoli MJ, et al. D614G Spike Variant Does Not Alter IgG, IgM, or IgA Spike Seroassay Performance. medRxiv. 2020:2020.07.08.20147371.

23. Hicks J, Klumpp-Thomas C, Kalish H, Shunmugavel A, Mehalko J, Denson J-P, et al. Serologic cross-reactivity of SARS-CoV-2 with endemic and seasonal Betacoronaviruses. medRxiv : the preprint server for health sciences. 2020:2020.06.22.20137695.

24. Arvin AM, Fink K, Schmid MA, Cathcart A, Spreafico R, Havenar-Daughton C, et al. A perspective on potential antibody-dependent enhancement of SARS-CoV-2. Nature. 2020;584(7821):353-63.

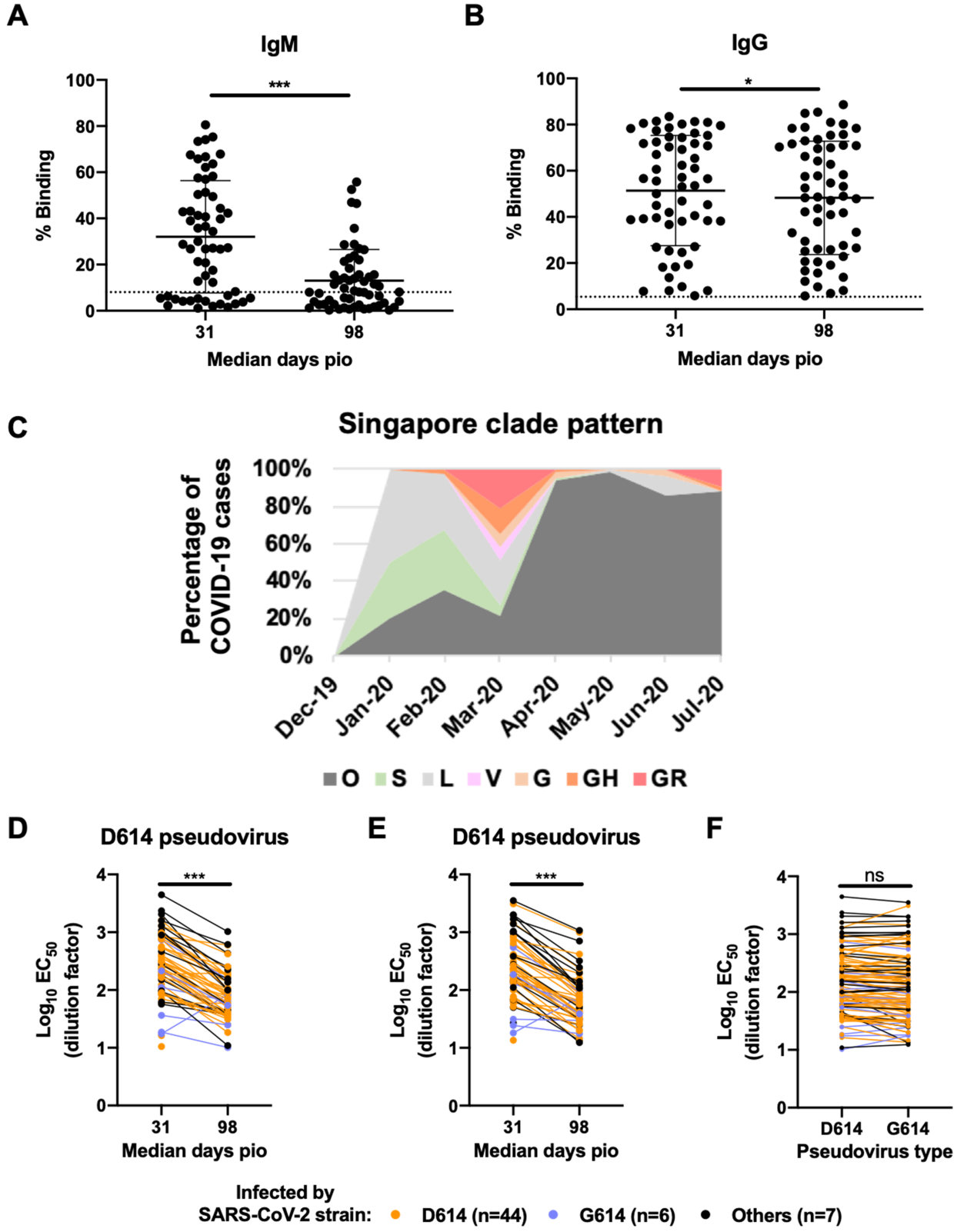

Figure 1. Timeline of events during the SARS-CoV-2 outbreak in Singapore, and the antibody profiles of COVID-19 patients and their neutralizing capacity against both D614 and G614 variants of SARS-CoV-2. Plasma samples of COVID-19 patients (n=57) at median 31 and median 98 days post-illness onset (pio) 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. 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 Wilcoxon signed-rank test (*P<0.05, ***P<0.001). **(C)** Percentage of COVID-19 cases 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 neutralizing 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} neutralization 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 paired t test (***P<0.001). **(F)** Comparison of log_{10} neutralization 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 paired t test. All data point are non-significant (ns).

Figure 1 Lee *et al.*, 2020

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.08.332544; this version posted October 9, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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%)
Hyperlipidemia	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	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 represented as Mean (SD) or n (%). COVID-19: Coronavirus Disease 2019.
Others: represent O, S, L, V, G, GH or GR clades.

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.08.332544; this version posted October 9, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Table 2. Neutralization EC50 values of COVID-19 patients

Patient	Recovery phase	D614 (EC50) Dilution factor	D614 (Log 10 EC50) Dilution factor	G614 (EC50) Dilution factor	G614 (Log 10 EC50) Dilution factor
Mild (No pneumonia)					
#1	Early	93.821	1.972300058	27.088	1.432776941
	Late	36.481	1.562066734	ND	ND
#2	Early	59.67	1.775756038	59.527	1.774713996
	Late	59.156	1.7719988	46.489	1.667350204
#3	Early	84.26	1.925621455	100.33	2.001430812
	Late	36.216	1.558900481	20.109	1.303390474
#4	Early	264.7	2.422753941	371.63	2.570110765
	Late	85.178	1.930327439	101.03	2.004450353
#5	Early	401.03	2.603176862	229.98	2.36169007
	Late	93.083	1.968870372	42.272	1.626052796
#6	Early	56.708	1.753644331	49.807	1.697290384
	Late	37.541	1.574505837	24.87	1.395675785
#7	Early	182.16	2.260453018	179.26	2.253483392
	Late	37.299	1.571697188	31.102	1.492788317
#8	Early	70.715	1.849511546	64.52	1.809694359
	Late	38.049	1.580343247	32.853	1.516575034
#9	Early	61.803	1.791009557	67.785	1.8311336
	Late	45.326	1.656347394	13.3	1.123851641
#10	Early	123.21	2.090645958	72.937	1.862947896
	Late	18.353	1.263707065	ND	ND
#11	Early	312.72	2.495155657	135.08	2.130591052
	Late	103.42	2.014604533	60.652	1.782845126
#12	Early	365.85	2.563303059	233.92	2.369067355
	Late	79.832	1.90217701	35.665	1.552242228
#13	Early	110.63	2.043872912	127.51	2.105544246
	Late	65.001	1.812920038	63.342	1.801691772
#14	Early	151.32	2.179896333	143.27	2.156155261
	Late	39.825	1.600155784	31.445	1.497551599
#15	Early	242.06	2.383923029	241.44	2.382809222
	Late	58.31	1.765743041	52.821	1.722806619
#16	Early	169.39	2.228887768	134.4	2.128399269
	Late	78.702	1.895985769	78.239	1.893423291
#17	Early	89.4	1.951337519	77.364	1.888538916
	Late	25.104	1.399742926	14.494	1.161188257
#18	Early	16.219	1.210024074	13.513	1.130751777
	Late	ND	ND	ND	ND
#19	Early	18.721	1.272329043	24.532	1.389732956
	Late	10.11	1.004751156	17.581	1.245043574
#20	Early	941.37	2.973760354	856.37	2.932661445
	Late	171	2.23299611	97.95	1.99100444
#21	Early	312.28	2.494544171	150.83	2.178487731
	Late	38.602	1.586609806	19.899	1.298831252

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.08.332544; this version posted October 9, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

#22	Early	17.385	1.240174695	18.098	1.257630584
	Late	83.448	1.921415932	74.848	1.8741802
#23	Early	36.553	1.562923026	31.281	1.495280628
	Late	24.869	1.395658322	29.766	1.473720477
#24	Early	10.477	1.020236944	ND	ND
	Late	ND	ND	ND	ND
#25	Early	849.23	2.929025328	ND	ND
	Late	601.69	2.779372794	ND	ND
Moderate (Pneumonia, without hypoxia)					
#1	Early	325.6	2.512684396	311.41	2.493332555
	Late	50.013	1.699082906	40.54	1.607883744
#2	Early	280.08	2.447282098	279.51	2.44639735
	Late	55.82	1.746789832	49.937	1.698422448
#3	Early	565.39	2.752348123	412.73	2.615666037
	Late	176.37	2.246424715	192.41	2.28422764
#4	Early	406.93	2.609519708	394.6	2.596157081
	Late	58.04	1.763727404	70.882	1.850535963
#5	Early	188.21	2.274642695	172.03	2.235604189
	Late	197.85	2.296336055	157.28	2.1966735
#6	Early	2349.4	3.370956964	2000.3	3.301095135
	Late	432.12	2.635604367	319.05	2.503858749
#7	Early	96.242	1.983364639	110.53	2.04348017
	Late	10.932	1.038699623	12.366	1.092229242
#8	Early	227	2.356025857	215.24	2.332922983
	Late	41.09	1.613736141	28.984	1.462158321
#9	Early	792.61	2.899059547	601.93	2.779545989
	Late	182.48	2.261215272	132.86	2.123394248
#10	Early	541.77	2.733814953	399.85	2.6018971
	Late	136.61	2.135482491	121.88	2.085932446
#11	Early	164.37	2.215822555	152.3	2.182699903
	Late	34.63	1.539452492	41.678	1.61990687
#12	Early	241.37	2.38268329	267.15	2.426755179
	Late	35.053	1.544725193	39.4	1.595496222
#13	Early	84.158	1.925095406	51.315	1.710244333
	Late	34.56	1.538573734	25.507	1.406659382
#14	Early	220.86	2.344117068	171.07	2.233173855
	Late	31.918	1.50403567	33.142	1.520378713
#15	Early	200.82	2.302806963	156.64	2.194902674
	Late	70.748	1.849714167	65.35	1.815245592
#16	Early	308.07	2.488649409	201.4	2.304059466
	Late	90.322	1.955793546	56.963	1.755592854
#17	Early	1079.6	3.033262876	1039.5	3.016824494
	Late	100.36	2.001560653	119.98	2.079108858
#18	Early	89.823	1.953387556	69.059	1.839220285
	Late	31.172	1.493764668	31.425	1.497275286
#19	Early	214.79	2.332014058	186.07	2.269676358
	Late	54.362	1.735295426	38.613	1.586733545
Severe (Pneumonia, with hypoxia)					
#1	Early	740.24	2.869372549	548.74	2.739366619

bioRxiv preprint doi: https://doi.org/10.1101/2020.10.08.332544; this version posted October 9, 2020. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

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