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References

1. Zambrano LD, Ellington S, Strid P, Galang RR, Oduyebo T, Tong VT, et al.; CDC COVID-19 Response Pregnancy and Infant Linked Outcomes Team. Update: characteristics of symptomatic women of reproductive age with laboratory-confirmed SARS-CoV-2 infection by pregnancy status — United States, January 22–October 3, 2020. MMWR Morb Mortal Wkly Rep. 2020;69:1641–7. https://doi.org/10.15585/mmwr.mm6944e3
2. Nyawanda BO, Otieno NA, Otieno MO, Emukule GO, Bigogo G, Onyango CO, et al. The impact of maternal human immunodeficiency virus infection on the burden of respiratory syncytial virus among pregnant women and their infants, Western Kenya. J Infect Dis. 2020 Aug 10 [Epub ahead of print].
3. Centers for Disease Control and Prevention. National Notifiable Diseases Surveillance System: surveillance case definitions. Coronavirus disease 2019 (COVID-19) 2020 interim case definition, approved April 5, 2020 [cited 2021 Feb 24]. https://wwwn.cdc.gov/nndss/conditions/coronavirus-disease-2019-covid-19/case-definition/2020
4. Olsen SJ, Azziz-Baumgartner E, Budd AP, Brammer L, Sullivan S, Pineda RF, et al. Decreased influenza activity during the COVID-19 pandemic—United States, Australia, Chile, and South Africa, 2020. MMWR Morb Mortal Wkly Rep. 2020;69:1305–9. https://doi.org/10.15585/mmwr.mm6937a6
5. Flannery DD, Puopolo KM. Perinatal COVID-19: guideline development, implementation, and challenges. Curr Opin Pediatr. 2021;33:188–94. https://doi.org/10.1097/MOP.0000000000000997

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Genomic Evolution of SARS-CoV-2 Virus in Immunocompromised Patient, Ireland

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We examined virus genomic evolution in an immunocompromised patient with prolonged severe acute respiratory syndrome coronavirus 2 infection. Genomic sequencing revealed genetic variation during infection: 3 intrahost mutations and possible superinfection with a second strain of the virus. Prolonged infection in immunocompromised patients may lead to emergence of new virus variants.

The coronavirus disease (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to substantial illness and death in immunocompromised patients (*1*). Outcomes for patients with hematologic malignancies can be poor because of immune suppression associated with cancer itself and chemoimmunotherapy regimens used to treat these cancers (*2*).

Persistent shedding of SARS-CoV-2 RNA has been described since early in the pandemic; quantitative reverse transcription PCR (qRT-PCR) results have remained positive for 63 days (*3*). Recent studies of immunocompromised patients have detected infectious virus until 143 days after diagnosis (*4*–*6*). Phylogenetic analysis showed that single-nucleotide polymorphisms (SNPs) could be used to elucidate the transmission routes of SARS-CoV-2 in communities (*7*). Moreover, it has been demonstrated that intrahost single-nucleotide variants are restricted to specific lineages (*8*); however, no clear evidence supports a link between prolonged infection and intra-evolutionary dynamics (*9*).

We report a case of a prolonged clinical infection with persistent virus shedding in a patient with functional B-cell deficiency, hypogammaglobulinemia,

[1]These authors contributed equally to this article.

and COVID-19. We describe the sequence polymorphisms over time among the 9 whole-virus genome sequences obtained by following the ARTIC tiling-amplicon approach (https://artic.network/resources/ncov/ncov-amplicon-v3.pdf) and using the Illumina MiSeq platform as described (*7*).

In April 2020, a 52-year-old woman in Dublin, Ireland, sought emergency care for a 5-day history of fever, diarrhea, and fatigue. Five months earlier, she had received a diagnosis of stage 4, grade 1 follicular lymphoma and had since completed 3 cycles of chemotherapy with cyclophosphamide, vincristine, doxorubicin, prednisolone, and obinutuzumab (B-cell monoclonal antibody); the last therapy cycle had been completed 7 days before the emergency department visit. During the emergency department visit, SARS-CoV-2 was detected on a nasopharyngeal swab sample by qRT-PCR (Roche FLOW Flex, https://diagnostics.roche.com) with a cycle threshold (C_t) value of 25.04. Chest radiographs showed a typical pattern for COVID-19 infection. The patient received hydroxychloroquine and azithromycin for 5 days. At the time of admission, she had hypogammaglobulinemia and received intravenous immunoglobulin every 4 weeks as supportive therapy.

During her 100-day hospital stay, the patient's clinical course of illness was protracted, with fevers and oxygen requirements, requiring a 17-day stay in a critical care unit (Appendix, https://wwwnc.cdc.gov/EID/article/27/9/21-1159-App1.pdf). In the hospital, the patient was in a single room with transmission-based air-handling precautions.

During her entire hospital stay, SARS-CoV-2 was detected at varying C_t values in nasopharyngeal swab samples, except for days 31 and 85 when SARS-CoV-2 was not detected. Bronchoalveolar lavage (BAL) performed on day 95 to exclude other pathogens detected SARS-CoV-2 (C_t 30). Serologic testing did not detect antibodies to SARS-CoV-2 (Roche anti-SARS-CoV-2) on days 30, 84, and 103.

The patient was tested 17 times, and we sequenced all samples that were positive by qRT-PCR with C_t <32.8. All 9 samples that underwent whole-virus genome sequencing (Appendix Figure) belonged to clade 20B, lineage B.1.1. SNP analysis clustered these genomes into 3 groups. Genomes sequenced from the positive samples taken on days 5, 19, and 26 were indistinguishable at the sequence level (Figure). A sample taken on day 47 showed the first mutation event; 3 point mutations were identified in the whole-virus genome sequence data until day 76 after diagnosis. On day 82, genome analysis detected a new SNP (second mutation event). Sequencing of the BAL sample taken on day 95 detected a different set of sequence polymorphisms that most likely originated from a new infection event. SNP analysis indicated 11 point mutations (Appendix Table 1) giving rise to 3 amino acid substitutions in the gene coding for the spike protein (S:S50L, S:A653V, and S:L1186F).

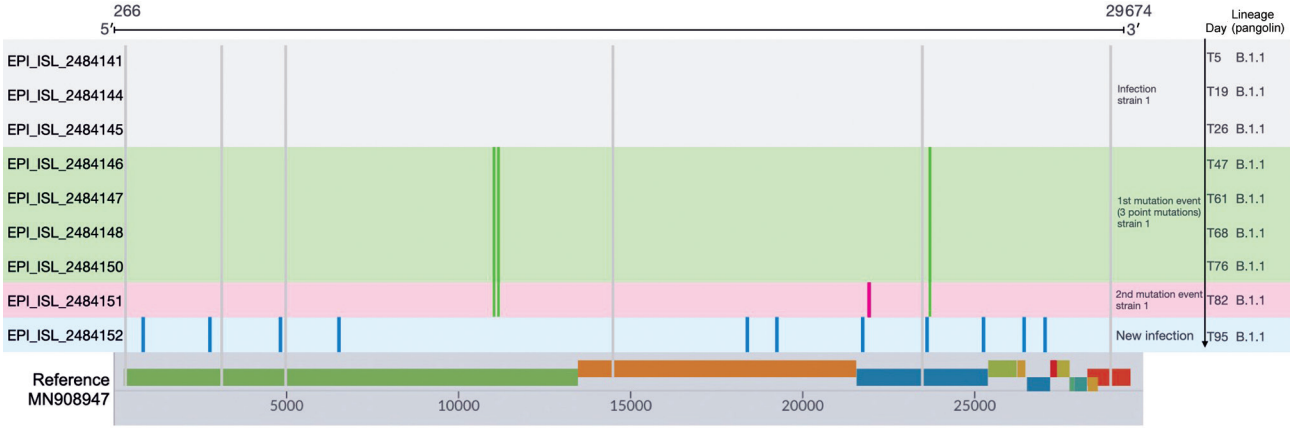

Figure. Sequence polymorphisms detected over time among the 9 whole-virus genome sequences from an immunocompromised patient with prolonged severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, Ireland. The mutations are represented by different colors; gray lines indicate the polymorphisms common to the 9 whole-virus genome sequences compared with the reference whole-virus genome (GenBank accession no. MN908947, SARS-CoV-2 isolate Wuhan-Hu-1). The infection was confirmed on day 5 of infection (at admission to the emergency department), and the sequencing demonstrated stability of the virus genome sequence on days 19 (T19) and 26 (T26) after the first detection. Green indicates mutations detected in the sample at 47 days after first the emergency department admission (T47), T61, T68, and T76. At sample time T82, the strain exhibited a fourth mutation (pink) corresponding to the second mutation event. On day 95, a bronchoalveolar lavage sample from the patient was positive for SARS-CoV-2 and the whole-virus genome had a different set sequence polymorphism that probably originated from a new infection event. GISAID (https://www.gisaid.org) identification numbers are provided.

SARS-CoV-2 shedding in this patient with lymphoma, ongoing fevers, and oxygen requirements for 6 months was prolonged. The antibody-mediated ablation of B-cell precursors by B-cell directed monoclonal antibody therapy was most likely responsible for the prolonged virus shedding. This effect, combined with hypogammaglobulinemia, explains the lack of seroconversion and the protracted clinical course.

Sequential sequencing demonstrated intrahost mutations of ≥2 events (Figure) and accumulation of 4 SNPs. Analysis of a BAL sample taken on day 95 showed 11 point mutations giving rise to 3 aa substitutions in the gene coding for the spike protein. This observation is in accordance with findings of a recent study that detected 7 new mutations in a second virus strain in an immunocompromised patient (*10*). The BAL findings, along with ongoing symptoms, are suggestive of probable superinfection with cohabitation of 2 virus strains. However, considering that this was the only BAL sampled, we cannot exclude the possibility that the origin of this strain is the result of a different evolutionary path of the original population responsible for the first infection.

The superinfection that we describe was probably a nosocomial infection despite the transmission-based precautions taken in the patient's single room during her hospital stay. However, no sequence data from other patients or healthcare workers on the ward could be explored to identify the source of infection.

Our report highlights the complex clinical course of SARS-CoV-2 in immunocompromised patients. This genomic analysis identified the ability of the virus to mutate and possibly coexist with another strain, resulting in superinfection in this immunocompromised patient.

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References

1. Fung M, Babik JM. COVID-19 in immunocompromised hosts: what we know so far. Clin Infect Dis. 2021;72:340–50. https://doi.org/10.1093/cid/ciaa863
2. Rubinstein SM, Warner JL. COVID-19 and haematological malignancy: navigating a narrow strait. Lancet Haematol. 2020;7:e701–3. https://doi.org/10.1016/S2352-3026(20)30252-0
3. Li J, Zhang L, Liu B, Song D. Case report: viral shedding for 60 days in a woman with COVID-19. Am J Trop Med Hyg. 2020;102:1210–3. https://doi.org/10.4269/ajtmh.20-0275
4. Avanzato VA, Matson MJ, Seifert SN, Pryce R, Williamson BN, Anzick SL, et al. Case study: prolonged infectious SARS-CoV-2 shedding from an asymptomatic immunocompromised individual with cancer. Cell. 2020;183:1901–1912.e9. https://doi.org/10.1016/j.cell.2020.10.049
5. Baang JH, Smith C, Mirabelli C, Valesano AL, Manthei DM, Bachman MA, et al. Prolonged severe acute respiratory syndrome coronavirus 2 replication in an immunocompromised patient. J Infect Dis. 2021;223:23–7. https://doi.org/10.1093/infdis/jiaa666
6. Choi B, Choudhary MC, Regan J, Sparks JA, Padera RF, Qiu X, et al. Persistence and evolution of SARS-CoV-2 in an immunocompromised host. N Engl J Med. 2020;383:2291–3. https://doi.org/10.1056/NEJMc2031364
7. Lucey M, Macori G, Mullane N, Sutton-Fitzpatrick U, Gonzalez G, Coughlan S, et al. Whole-genome sequencing to track severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) transmission in nosocomial outbreaks. Clin Infect Dis. 2021;72:e727-e735. https://doi.org/10.1093/cid/ciaa1433
8. Armero A, Berthet N, Avarre JC. Intra-host diversity of SARS-Cov-2 should not be neglected: case of the state of Victoria, Australia. Viruses. 2021;13:133. https://doi.org/10.3390/v13010133
9. Wang Y, Wang D, Zhang L, Sun W, Zhang Z, Chen W, et al. Intra-host variation and evolutionary dynamics of SARS-CoV-2 populations in COVID-19 patients. Genome Med. 2021;13:30. https://doi.org/10.1186/s13073-021-00847-5
10. Tarhini H, Recoing A, Bridier-Nahmias AB, Rahi M, Lambert C, Martres P, et al. Long term SARS-CoV-2 infectiousness among three immunocompromised patients: from prolonged viral shedding to SARS-CoV-2 superinfection. J Infect Dis,2021;223:1522–7.

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Appendix

Patient's Clinical Course

A 52-year-old female presented to the Emergency Department (ED) in April 2020, with a five-day history of fever, diarrhea and fatigue. The patient was diagnosed with stage 4, grade 1 follicular lymphoma 5 months earlier, and had completed 3 cycles of chemotherapy with cyclophosphamide, vincristine, doxorubicin, prednisolone, and obinutuzamab (B-cell monoclonal antibody), the last cycle being completed 7 days before presentation to the ED. SARS-CoV-2 was detected by RT-qPCR on a nasopharyngeal swab, with a cycle threshold (C_T) value of 25.04 using the Roche flowflex platform. Chest X-ray on admission showed bilateral peripheral airspace opacities in the mid- to lower-zones with a typical pattern for COVID-19 pneumonia. The patient was treated with hydroxychloroquine and azithromycin for 5 days. On admission, the patient had hypogammaglobulinemia, and received intravenous immunoglobulin (IVIG) every 4 weeks as supportive therapy, during the hospital admission.

The patient received ward-based care for 21 days, with ongoing fevers, and was transferred to the Critical Care Unit with increasing oxygen requirements. Computed tomography (CT) of the thorax reported progressing covid pneumonitis. The patient was managed with non-invasive ventilation and self-proning. Following 17 days in Critical Care, the patient was transferred to the ward, where she remained in a single room, with transmission based precautions for the hospital stay.

In all the patient remained in hospital for 100 days, with intermittent fevers, and oxygen requirements. SARS-CoV-2 was detected in NP swabs taken during the entire admission with varying C_T values, except for days-31 and −85, when SARS-CoV-2 was not detected. A bronchoalveolar lavage (BAL) was performed on day 95, to exclude other viruses, bacteria, and fungi. SARS-CoV-2 was detected (C_T value 30) in the BAL. Serological testing did not detect

antibodies to SARS-CoV-2 (Roche Anti-SARS-CoV-2) on days 30, 84, and 103. Following discharge, the patient remained on home oxygen, with intermittent fevers until 6 months after diagnosis. She continued to receive IVIG every 4 weeks. A PET CT scan for disease staging reported good partial remission of lymphoma, and further doses of immunochemotherapy were withheld.

The decision to prolong transmission-based precautions during the hospital stay, beyond the recommended 20 days post symptom onset, was based on persistent fevers, and oxygen requirements. Repeated detection of SARS-CoV-2 in respiratory samples meant a test-based strategy could not be used, hence transmission-based precautions remained for the duration of hospital admission (*1*).

Sample preparation for sequencing bioinformatics and statistical analysis

Samples were prepared for sequencing starting from cDNA synthesis (reverse transcription) using LunaScript RT SuperMix (New England Biolabs, Ipswich, UK). The sequencing library were obtained following ARTIC tiling-amplicon approach, prepared using the NEBNext Ultra II kit (New England Biolabs, Ipswich, UK) and sequenced on an Illumina MiSeq using 300-cycle v2 reagent kits (Illumina, Cambridge, UK). Raw reads were mapped to reference sequence using Bowtie 2 (*2*) and SAMtools (*3*) was used for variants calling and generate the consensus sequences (GenBank number, MN908947.3). The read coverage was estimated using samtools flagstat tool from the SAMtools software package (*3*). The libraries generated from the nine samples yield at least 337,274 (lowest value among the 9 samples) mapped reads (primer-trimmed sequences) and the genome coverage (completion) was on average 98.34% to the reference strain (MN908947.3) for the nine samples, with values between 97.64 and 99.18% (Appendix Table 2). The average coverage for the nine samples was 4,883, with values ranging between 3,778 and 5,454 (Appendix Table 2). Comparative sequence analysis was conducted by phylogenetic inference maximum likelihood (ML) method with RAxML (*4*) using the multiple sequences aligned by MAFFT (*5*). Clades and variants were assigned respectively using Nextstrain (*6*) and Pangolin COVID-19 lineage assigner (http://pangolin.cog- uk.io/). All the sequences generated were submitted to GISAID (*7*) and the ID (gisaid_epi_isl) are presented in Appendix Table 2.

References

1. Centers for Disease Control and Prevention. Duration of isolation and precautions for adults with COVID-19. 2021 [cited 2021 May 20]. https://www.cdc.gov/coronavirus/2019-ncov/hcp/duration-isolation.html

2. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9:357–9. PubMed https://doi.org/10.1038/nmeth.1923

3. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al.; 1000 Genome Project Data Processing Subgroup. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009;25:2078–9. PubMed https://doi.org/10.1093/bioinformatics/btp352

4. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 2014;30:1312–3. PubMed https://doi.org/10.1093/bioinformatics/btu033

5. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30:772–80. PubMed https://doi.org/10.1093/molbev/mst010

6. Hadfield J, Megill C, Bell SM, Huddleston J, Potter B, Callender C, et al. Nextstrain: real-time tracking of pathogen evolution. Bioinformatics. 2018;34:4121–3. PubMed https://doi.org/10.1093/bioinformatics/bty407

7. Shu Y, McCauley J. GISAID: Global initiative on sharing all influenza data - from vision to reality. Euro Surveill. 2017;22:30494. PubMed https://doi.org/10.2807/1560-7917.ES.2017.22.13.30494

Appendix Table 1. Nucleotide mutation and amino acid substitutions in SARS-CoV-2 from immuncompromised patient with prolonged clinical infection*

	Nucleotide mutation†														
Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
EPI_ISL_2484141	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
EPI_ISL_2484144	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
EPI_ISL_2484145	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
EPI_ISL_2484146	ND	ND	ND	ND	A11054G	G11083T	ND	ND	ND	ND	ND	G23608T	ND	ND	ND
EPI_ISL_2484147	ND	ND	ND	ND	A11054G	G11083T	ND	ND	ND	ND	ND	G23608T	ND	ND	ND
EPI_ISL_2484148	ND	ND	ND	ND	A11054G	G11083T	ND	ND	ND	ND	ND	G23608T	ND	ND	ND
EPI_ISL_2484150	ND	ND	ND	ND	A11054G	G11083T	ND	ND	ND	ND	ND	G23608T	ND	ND	ND
EPI_ISL_2484151	ND	ND	ND	ND	A11054G	G11083T	ND	ND	ND	G21778A	ND	G23608T	ND	ND	ND
EPI_ISL_2484152	C678T	G2626A	G4866T	C6402T	Not present	ND	C18252T	C19264T	C21711T	ND	C23520T	ND	C25118T	C26333T	C26895T
AA Substitution‡	ORF1a: A138V	ND	ND	ORF1a: P2046L	ORF1a: S3597G	ORF1a: L3606F	ND	ORF1b: L1933F	S:S50L	ND	S:A653V	ND	S:L1186F	E:T30I	M:H125Y

*Virus isolates are denoted by a the GISAID (https://www.gisaid.org)–assigned identification number, and nucleotide polymorphisms and their positions, across the SARS-CoV-2 genome are shown. E, envelope protein; ND, not detected; M, membrane protein; ORF, open reading frame; S, spike protein; SARS-CoV-2, severe acute respiratory coronavirus 2.

†Nucleotide mutations are shown in the following format: nucleotides in the reference strain (A, adenine; C, cytosine; G, guanine; T, thymine); genomic position in the sequence; and code of the nucleotide detected in the genome of the virus sequenced.

‡Amino acid substitution at protein level are shown in the following the format: protein code (ORF1a, ORF1b, S, E and M); code of the amino acid in the reference strain (A, alanine; V, valine; P, proline; L, leucine; S, serine; G, glycine, F, phenylalanine; T, threonine; H, histidine; Y ,tyrosine); and code of the amino acid detected in the genome of the virus sequenced.

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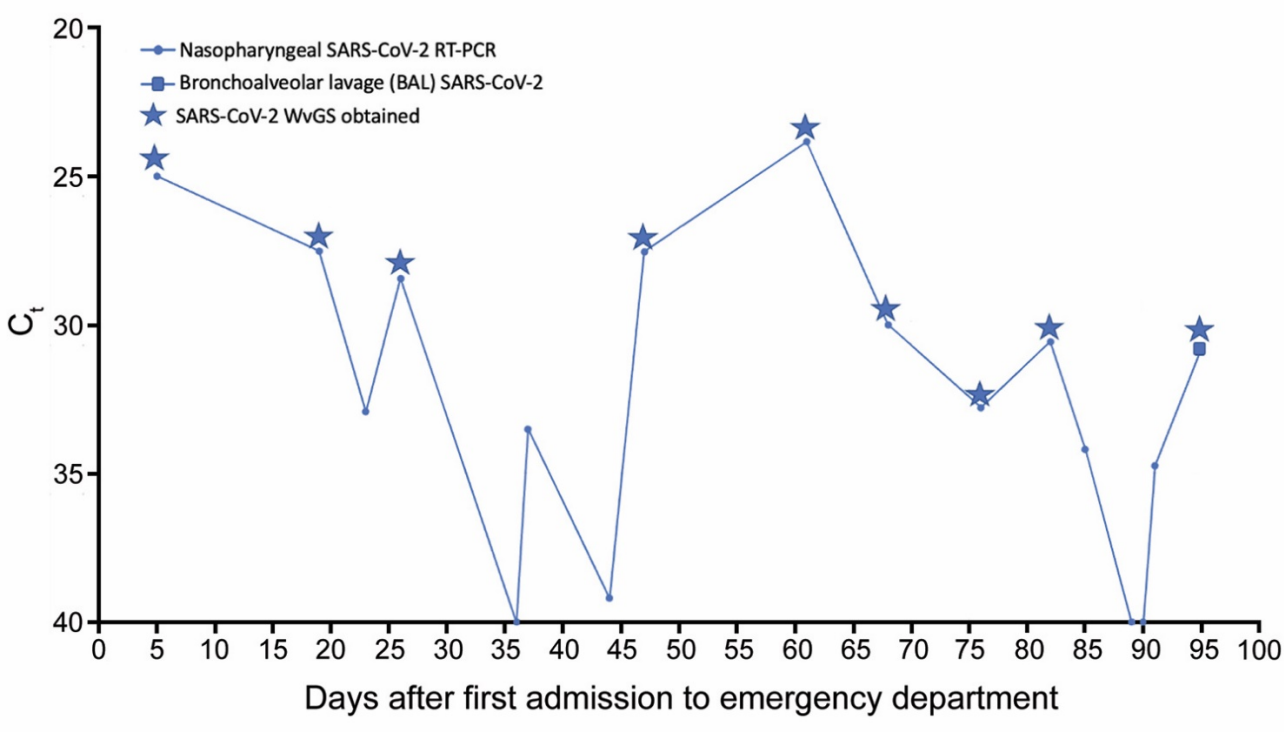
Appendix Table 2. The assigned identification numbers are presented along with the date of sampling the lineage as assigned by Pangolin and quality values (average coverage and % of coverage). The average coverage is calculated at the level of the single base pair and the % of coverage is the percentage of completion of the consensus sequences to the reference strain (MN908947.3)

ID*	Date sampling	Average coverage	% Coverage	Pangolin lineage
EPI_ISL_2484141	09/04/2020	5,454	99.12	B.1.1
EPI_ISL_2484144	23/04/2020	4,875	97.76	B.1.1
EPI_ISL_2484145	27/04/2020	5,446	98.31	B.1.1
EPI_ISL_2484146	21/05/2020	5,319	98.73	B.1.1
EPI_ISL_2484147	04/06/2020	5,219	99.18	B.1.1
EPI_ISL_2484148	11/06/2020	4,319	98.21	B.1.1
EPI_ISL_2484150	19/06/2020	4,570	97.74	B.1.1
EPI_ISL_2484151	25/06/2020	3,778	97.64	B.1.1
EPI_ISL_2484152	08/07/2020	4,967	98.36	B.1.1

*GISAID (https://www.gisaid.org) ID numbers.



Appendix Figure. Trend of the C_T values after the first admission (T_0) to the emergency department of the patient. The points highlight the SARS-CoV-2 Whole virus Genome Sequences (WvGS) that were obtained for this study.