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COVID-19 in an immunocompromised host: persistent shedding of viable SARS-CoV-2 and emergence of multiple mutations: a case report

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ABSTRACT

This article reports a case of a 21-year-old woman with refractory B-cell acute lymphocytic leukaemia who presented with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). She remained positive for SARS-CoV-2 by viral culture for 78 days and by polymerase chain reaction (PCR) for 97 days. Sequencing of repeat samples over time demonstrated an increasing and dynamic repertoire of mutations.

Introduction

Throughout the severe acute respiratory syndrome coronavirus-2 (SARS CoV-2) pandemic, clinicians have faced the challenge of interpreting the results of SARS-CoV-2 to distinguish infectious virus particles from non-infectious virus particles. Previously, immunocompromised patients have remained SARS-CoV-2 polymerase chain reaction (PCR) positive for up to 151 days (Choi et al., 2020). Although detection of viral genomic material does not confirm the presence of infectious SARS-CoV-2, continued viral shedding raises questions regarding the potential for disease transmission and viral mutation, particularly among the severely immunocompromised, in whom SARS-CoV-2 is detected weeks after symptom onset. In this setting, viral culture may be used to assess whether the virus retains infective potential. A study of 129 immunocompetent and immunocompromised patients with SARS-CoV-2 demonstrated that the virus could be cultured for a median of 8 days and a maximum of 20 days after symptom onset (van Kampen et al., 2021).

Concern is growing around accumulation of SARS-CoV-2 genome mutations that may confer increased infectivity, pathogenicity or immune escape. In particular, variants of concern such as P.1, B.1.351, B.1.617.2 and B.1.1.7 (O'Toole et al., 2021) have mutations identified as gamma, beta, delta and alpha in the spike (S) protein receptor-binding domain (Rambaut et al., 2020), a site critical for initial binding of the virus to angiotensin-converting enzyme 2 (ACE2) receptors in the upper respiratory tract. Estimates suggest that SARS-CoV-2 lineages acquire one to two mutations per month as they transit through successive hosts (Duchene et al., 2020). However, case reports have documented accelerated viral evolution in immunocompromised hosts who are susceptible to prolonged SARS-CoV-2 infection and viral replication (Avanzato et al., 2020; Choi et al., 2020; Kemp et al., 2021), which is thought to have led to emergence of the alpha variant. In one such case, a patient with chronic lymphocytic leukaemia and acquired hypogammaglobulinaemia had SARS-CoV-2 cultured at 70 days, while viral RNA was detected 105 days after infection

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(Avanzato et al., 2020). Continuous viral variant turnover demonstrated new, previously undocumented variants (Avanzato et al., 2020). Another individual with marginal B-cell lymphoma and previous chemotherapy received convalescent plasma treatment, and genomic analysis over 101 days demonstrated the emergence of S protein mutations (Kemp et al., 2021). Finally, in a case by Choi et al. (2020), phylogenetic analyses in consecutive samples of a patient on cyclophosphamide, glucocorticoids, rituximab and eculizumab demonstrated S gene and receptor-binding domain mutations, while viral culture confirmed infectious virus at day 143.

This article reports a case of an immunocompromised 21-year-old woman in whom SARS-CoV-2 RNA was detected via reverse transcription-quantitative PCR (RT-qPCR) repeatedly until day 97, prior to her death on day 98. Viability was demonstrated *in vitro* via viral culture up until day 78. SARS-CoV-2 genome sequencing performed on five samples over the course of 3 months demonstrated acquisition of seven mutations from baseline.

Case presentation

A 21-year-old woman with relapsed, refractory B-cell acute lymphocytic leukaemia (ALL) presented to hospital in Surrey, British Columbia, Canada in November 2020 with fever and dyspnoea. She was diagnosed with B-cell ALL in 2014, had an allogeneic stem cell transplant in 2017 for her first relapse, received blinatumomab for her second relapse in February 2020, and experienced a third relapse in September 2020. She received weekly doses of palliative inotuzumab in October 2020. On 21 October 2020, 5 days after her third inotuzumab dose (day 1 of her symptoms), she experienced chills, sore throat and myalgia after community exposure to SARS-CoV-2. On day 7, an outpatient nasopharyngeal swab was collected, and SARS-CoV-2 RNA was detected by the laboratory-developed RT-qPCR (LeBlanc et al., 2020) [cycle threshold (Ct) value 16.60]. Her initial mild symptoms resolved within 2 weeks and public health deemed her to be non-contagious. On day 30, she received a fourth dose of inotuzumab, and developed fever and breathlessness 2 days later. On day 33, she was admitted to hospital with fever of 38.7°C and progressive dyspnoea. Oxygen saturation was 70–74% on room air, requiring 7 L/min of oxygen by face mask. SARS-CoV-2 RNA was detected by nasopharyngeal swab (Ct 16.35). Initial chest computed tomography (CT) scan showed extensive bilateral ground glass opacities consistent with SARS-CoV-2 pneumonia, with no evidence of pulmonary embolism. She received dexamethasone 6 mg intravenously daily for 10 days, and piperacillin-tazobactam for possible bacterial pneumonia until day 35. Remdesivir was not initiated due to mild liver enzyme elevation and time since symptom onset. She progressed to require high-flow oxygen with 70% fraction of inspired oxygen (FiO_2). Given marked hypoxaemia and lactate dehydrogenase of 568 U/L (reference <220 U/L), pneumocystis pneumonia was treated empirically with trimethoprim-sulfamethoxazole (from day 42 to day 63) and dexamethasone was continued, tapering to 3 mg daily from day 43 onwards. She was also treated with voriconazole (days 43–58) for possible coronavirus-disease-associated pulmonary aspergillosis (CAPA). Lower respiratory specimens to assess for pneumocystis or aspergillosis were never obtained given the lack of sputum production and instability for bronchoscopy with ongoing hypoxaemia without intubation.

On day 65, she deteriorated acutely with fever, neutrophil count of 0.8×10^9 /L (reference $2.0–8.0\times10^9$ /L), and an increase in oxygen requirement to 95% FiO_2. Given the palliative nature of her ALL and in keeping with her wishes, she was not transferred to an intensive care unit. Chest CT scan demonstrated new left apical consolidation with cavitation on the background of persistent disease. Dexamethasone was increased to 6 mg daily, and voriconazole was restarted in case deterioration was due to CAPA. Day 67 serum galactomannan was negative, but voriconazole continued. Oxygen requirements remained at 95% FiO_2.

Repeat nasopharyngeal swab on day 78 was again positive (Ct 23.84). Viral culture demonstrated in-vitro infective viability in T25 cell cultures as detectable cytopathic effect with confirmation by SARS-CoV-2 qPCR (E and RdRP gene, laboratory-developed). Serological assessment for total antibodies to SARS-CoV-2 S1 receptor-binding domain (Siemens ADVIA Centaur XP) from day 87 was non-reactive. Rubella anti-IgG testing (Siemens ADVIA Centaur XP) on the same day was equivocal, confirming a general lack of humoral immunity in this patient with up-to-date vaccination status. She received a trial of remdesivir (days 91–98). Other therapies including interleukin-6 inhibitors, neutralizing antibodies and convalescent plasma were not considered as they were either not in use in Canada, there was lack of adequate evidence at that time, or she did not meet the criteria for enrolment in clinical trials. SARS-CoV-2 RNA was again detected in nasopharyngeal swabs on days 91 (Ct 26.67) and 97 (Ct 28.00), although viral culture was negative on both days. She required progressively higher oxygen requirements, and after discussion with family, she transitioned to end-of-life care and passed away on day 98.

Sequencing of this patient's virus was conducted five times over 3 months (Figure 1), using previously published methods (Hogan et al., 2021). All sequencing attempts produced high-quality data spanning the near-complete genome (up to 99.4%) (Figure 2A). Initial sequencing assigned the virus to the Pangolin (Version 2.4.2, pangoLEARN 2021-05-19) (O'Toole et al., 2021) lineage B.1.1.306, a lineage most commonly found in Canada and India (O'Toole et al., 2021) and common in autumn 2020 in British Columbia. Over time, viral sequencing revealed the development of 12 mutations, of which 11 were protein coding changes and one was a silent mutation. In the final sample, there were seven mutations, of which six were protein coding changes observed within three Open Reading Frame 1ab (ORF1ab), two S (Figure 2B) and one nucleocapsid (N) protein locations (Figure 2C). Seven of the identified 12 mutations increased over time and were observed in the final sample (Figure 2C), while five mutations were observed once but not detected later in the infection. The significance of many of the mutations in the N, ORF1ab, ORF3a and S genes was unknown, while the envelope and S gene mutations are discussed below where information is available (Figure 2C).

Discussion

This article presents a case of prolonged shedding and mutation of SARS-CoV-2 in an immunocompromised host. The positive viral culture at 78 days after symptom onset was consistent with case reports of SARS-CoV-2 in other immunocompromised hosts (Avanzato et al., 2020; Choi et al., 2020; Kemp et al., 2021), and longer than reported early in the pandemic, where infectious virus shedding occurred to day 20 (van Kampen et al., 2020).

This report demonstrates prolonged SARS-CoV-2 infection in a patient who received inotuzumab. While there have been no case reports specifically documenting associations between anti-CD22 monoclonal antibodies such as inotuzumab and prolonged SARS-CoV-2 infection, this has been reported previously in patients receiving rituximab, eculizumab (Choi et al., 2020) and vincristine (Kemp et al., 2021). The dynamic viral population with accelerated evolution over time as seen in the study case resulted in the development of 12 mutations (11 with predicted coding alterations) in 3 months; however, in the final sample, there were seven mutations, including six protein coding changes, which matched the

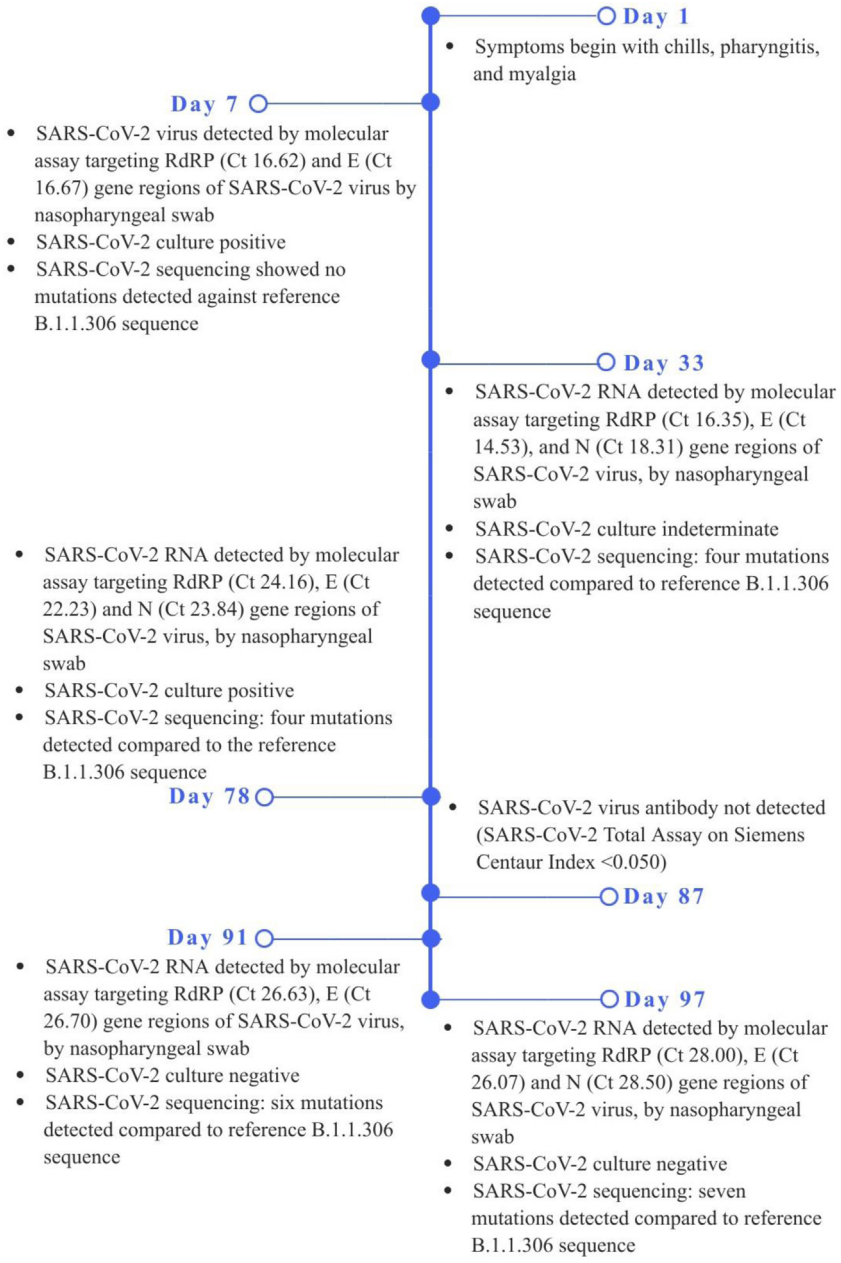


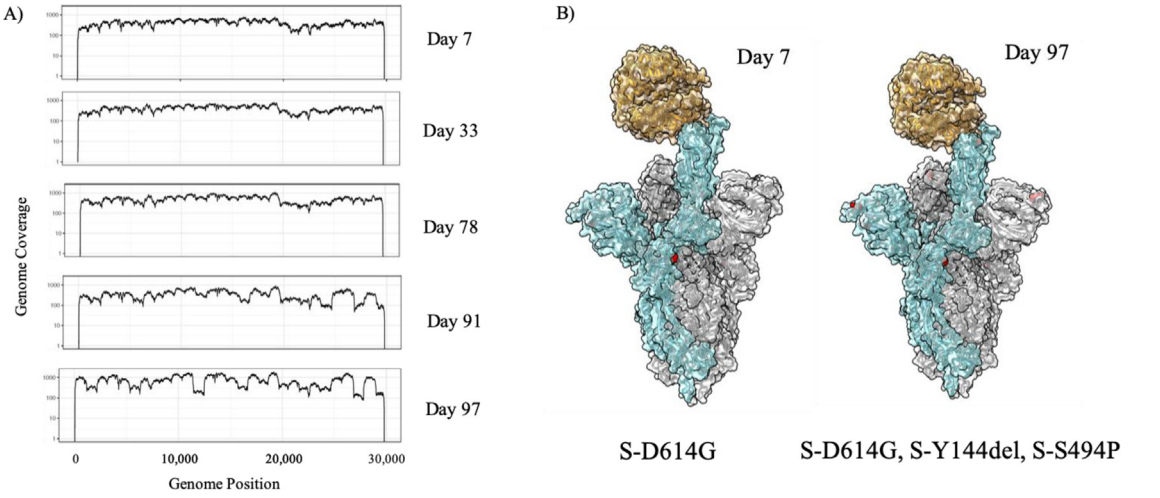
Figure 1. Timeline of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)-related investigations. Ct, cycle threshold; E, envelope; N, nucleocapsid; RdRP, RNA-dependent RNA polymerase.

overall expected mutation rate (Duchene et al., 2020). Interestingly, although 12 mutations were observed in total, only seven mutations were present by day 97, emphasizing the ongoing viral evolution and selection within a host (Figure 2C).

Of the persistent mutations, several have been described previously. The S494P surface mutation on the S protein was shown *in silico* to increase complementarity between the receptor-binding domain of SARS-CoV-2 and ACE2 (Chakraborty, 2021), possibly increasing viral virulence. This mutation also acts as an escape mutation, selected for with administration of camelid nanobodies (engineered heavy-chain-only antibodies) for SARS-CoV-2 treatment (Koenig et al., 2021). The Y144del surface mutation is found in the B.1.1.7 and B.1.351 variants, and may convey immune escape based on decreased antibody binding (Wang et al., 2021).

The prevalence of seven of 12 viral mutations increased over time, while five mutations were only seen once (Figure 2C). While fixation of several mutations did occur, others were observed to arise and then not detected, suggestive of a combination of selective pressures and a diverse viral population. The study patient developed 11 non-synonymous mutations and one synonymous mutation; the development of more non-synonymous than synonymous mutations was consistent with other reports (Choi et al., 2020; Khatamzas et al., 2021).

The mutation profile shifted markedly from day 91 to day 97 (Figure 2C), which coincided with remdesivir treatment (days 91–98). In addition to three mutations that were present in high frequencies by day 78, six new protein coding mutations were observed, four of which occurred in the S and N genes (Figure 2C). This dynamic change may warrant further investigation in relation to possible association with remdesivir. None of these mutations were found to be associated with remdesivir in the literature.



C)

Genome Position	Ref base	Alt base	Day 7	Day 33	Day 78	Day 91	Day 97	Coding change	Consequence
4233	A	C	0.%	0%	97%	97%	100%	ORF1ab-D1323A	Missense variant
4778	A	G	0%	0%	0%	0%	50%	ORF1ab-I1505V	Missense variant
5178	C	T	0%	36%	97%	98%	100%	ORF1ab-T1638I	Missense variant
5181	A	C	0%	53%	0%	0%	0%	ORF1ab-D1639A	Missense variant
21990	TTTA	T	0%	80%	98%	88%	98%	S-Y144del	Disruptive in-frame deletion
23030	T	C	0%	0%	0%	57%	0%	S-F490L	Missense variant
23042	T	C	0%	0%	0%	1%	100%	S-S494P	Missense variant
24953	G	C	0%	0%	8%	32%	0%	S-G1131R	Missense variant
25687	G	T	0%	0%	0%	61%	5%	ORF3a-A99S	Missense variant
26333	C	T	0%	67%	0%	0%	0%	E-T30I	Missense variant
28382	T	C	0%	0%	0%	0%	96%	N-S37P	Missense variant

Figure 2. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection mutation accumulation in an immunocompromised host in collected nasopharyngeal swabs. Coverage plots showing high-quality data spanning the entire viral genome (A), depiction of mutations across the SARS-CoV-2 spike (S) glycoprotein with one angiotensin-converting enzyme 2 (ACE2)-bound protein structure 7A94 (Benton et al., 2020) visualized using ChimeraX 1.1, with a single S protein subunit (blue) interaction with angiotensin-converting enzyme 2 (yellow) illustrated (B), and table showing temporal development of 11 protein coding changes over time across the SARS-CoV-2 genome with percentages of reads showing indicated mutation (C). E, envelope; N, nucleocapsid; n/a, not available; ORF1ab, open reading frame 1ab; ORF3a, open reading frame 3a. Comparison of mutational change based on first sample as baseline.

	Day 0			Day 26			Day 71			Day 84			Day 90		
Genome Position	ref	alt	aa	ref	alt	aa	ref	alt	aa	ref	alt	aa	ref	alt	aa
203													C	T	NA
241	C	T	NA	C	T	NA	C	T	NA	C	T	NA	C	T	NA
313	C	T	orf1ab-L16L	C	T	orf1ab-L16L	C	T	orf1ab-L16L	C	T	orf1ab-L16L	C	T	orf1ab-L16L
346	C	T	orf1ab-L27L	C	T	orf1ab-L27L	C	T	orf1ab-L27L	C	T	orf1ab-L27L	C	T	orf1ab-L27L
1347	C	T	orf1ab-P361L	C	T	orf1ab-P361L	C	T	orf1ab-P361L	C	T	orf1ab-P361L	C	T	orf1ab-P361L
1943	C	T	orf1ab-R560C	C	T	orf1ab-R560C	C	T	orf1ab-R560C	C	T	orf1ab-R560C	C	T	orf1ab-R560C
3037	C	T	orf1ab-F924F	C	T	orf1ab-F924F	C	T	orf1ab-F924F	C	T	orf1ab-F924F	C	T	orf1ab-F924F
4233							A	C	orf1ab-D1323A	A	C	orf1ab-D1323A	A	C	orf1ab-D1323A
4778													A	G	orf1ab-I1505V
5178				C	T	orf1ab-T1638I	C	T	orf1ab-T1638I	C	T	orf1ab-T1638I	C	T	orf1ab-T1638I
5181				A	C	orf1ab-D1639A									
5700	C	A	orf1ab-A1812D	C	A	orf1ab-A1812D	C	A	orf1ab-A1812D	C	A	orf1ab-A1812D	C	A	orf1ab-A1812D
6031	C	T	orf1ab-N1922N	C	T	orf1ab-N1922N	C	T	orf1ab-N1922N	C	T	orf1ab-N1922N	C	T	orf1ab-N1922N
7153	T	C	orf1ab-D2296D	T	C	orf1ab-D2296D	T	C	orf1ab-D2296D	T	C	orf1ab-D2296D	T	C	orf1ab-D2296D
13975	G	T	orf1ab-G4571C	G	T	orf1ab-G4571C	G	T	orf1ab-G4571C	G	T	orf1ab-G4571C	G	T	orf1ab-G4571C
14408	C	T	orf1ab-P4715L	C	T	orf1ab-P4715L	C	T	orf1ab-P4715L	C	T	orf1ab-P4715L	C	T	orf1ab-P4715L
17944	G	T	orf1ab-V5894L	G	T	orf1ab-V5894L	G	T	orf1ab-V5894L	G	T	orf1ab-V5894L	G	T	orf1ab-V5894L
21990				TTTA	T	S-Y144del	TTTA	T	S-Y144del	TTTA	T	S-Y144del	TTTA	T	S-Y144del
23030										T	C	S-F490L			
23042													T	C	S-S494P
23403	A	G	S-D614G	A	G	S-D614G	A	G	S-D614G	A	G	S-D614G	A	G	S-D614G
24953										G	C	S-G1131R			
25687										G	T	ORF3a-A99S			
26043	T	C	ORF3a-T217T	T	C	ORF3a-T217T	T	C	ORF3a-T217T	T	C	ORF3a-T217T	T	C	ORF3a-T217T
26333				C	T	E-T30I									
28382													T	C	N-S37P
28881	G	A	N-R203K	G	A	N-R203K	G	A	N-R203K	G	A	N-R203K	G	A	N-R203K
28882	G	A	N-R203R	G	A	N-R203R	G	A	N-R203R	G	A	N-R203R	G	A	N-R203R
28883	G	C	N-G204R	G	C	N-G204R	G	C	N-G204R	G	C	N-G204R	G	C	N-G204R

Figure 3. Variant information for all five sequenced samples. Genome was compared with Pangolin lineage B.1.1.306 (O'Toole et al., 2021). The following mutations are identified differences (>25% read fraction) between the sequenced viral samples and the severe acute respiratory syndrome coronavirus-2 isolate Wuhan-Hu-1, NCBI Reference Sequence: NC_045512.2 (National Centre for Biotechnology Information, 2020). Grey text indicates synonymous amino acid changes; ref, reference Wuhan-Hu-1 sequence; alt, identified sequence mutation; aa amino acid alteration due to mutation.

Conclusion

Viral viability and variable acquisition of 12 mutations over 3 months was demonstrated in this case of prolonged SARS-CoV-2 viral evolution in an immunocompromised host. Ongoing infection and viral evolution of SARS-CoV-2 in immunocompromised individuals may result in variants, which may, in turn, increase virulence and potential for immune escape. In addition, many current infection control guidelines assume that persistently PCR-positive individuals are shedding residual RNA and not infectious virus, and this case report highlights the potential to use viral culture to confirm the presence of infectious SARS-CoV-2 in select patients.

Conflict of interest statement

None declared.

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None.

Ethical approval

Not required. Consent was obtained from the patient's mother.

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