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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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Highlights
- Ongoing SARS-CoV-2 infection may occur in immunocompromised hosts
- Sequencing of repeat samples demonstrated an increasing repertoire of mutations
- Viral culture can be used to confirm the presence of infectious SARS-CoV-2

Abstract

We report a case of a 21-year-old woman with refractory B-cell acute lymphocytic leukemia presenting with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), who 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.
Keywords: SARS-CoV-2, immunocompromised, sequencing, mutation, viral culture, case report

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 versus non-infectious virus particles. Previously, immunocompromised patients have remained SARS-CoV-2 polymerase chain reaction (PCR) positive up to 151 days (Choi et al., 2020). Although detection of viral genomic material does not confirm presence of infectious SARS-CoV-2, continued viral shedding raises questions regarding potential for disease transmission and viral mutation, particularly among the severely immunocompromised, where 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 SARS-CoV-2 patients demonstrated that virus could be cultured for a median of eight days and a maximum of 20 days after symptom onset (van Kampen et al., 2021).

Recently, 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 protein receptor-binding domain (Rambaut et al., 2020), a site critical for initial binding of virus to the angiotensin converting enzyme 2 (ACE2) receptor in the upper respiratory tract. Estimates suggest that SARS-CoV-2 lineages acquire 1-2 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 leukemia and acquired hypogammaglobulinemia had SARS-CoV-2 cultured at 70 days, while viral RNA was detected at 105 days after infection (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 over 101 days, genomic analysis demonstrated emergence of spike 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 spike gene and receptor binding domain mutations, while viral culture confirmed infectious virus at day 143.

We describe a case of an immunocompromised 21-year-old woman in whom SARS-CoV-2 RNA was detected via reverse transcription-quantitative polymerase chain reaction (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 three months demonstrated acquisition of seven mutations from baseline.

Case presentation

A 21-year-old woman with relapsed, refractory B-cell acute lymphocytic leukemia (ALL) presented to hospital in Surrey, BC, Canada in November 2020 with fevers and dyspnea. She was diagnosed with B-cell ALL in 2014, had allogeneic stem cell transplant in 2017 with first relapse, blinatumomab for second relapse in February 2020, and experienced a third relapse in September 2020. She received weekly doses of palliative inotuzumab in October 2020. On October 21st, 2020, five 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 our laboratory-developed RT-qPCR (LeBlanc et al., 2020) (cycle threshold [Ct] value 16.60). Her initial mild symptoms resolved within two weeks and public health deemed her not contagious. On day 30, she received a fourth dose of inotuzumab and two days later developed fever and breathlessness. On day 33 she was admitted to hospital with fever of 38.7°C and progressive dyspnea. 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 (FiO₂). Given marked hypoxemia and LDH of 568 U/L (reference < 220 U/L), pneumocystis pneumonia was treated empirically with trimethoprim-sulfamethoxazole (from day 42 to day 63) and dexamethasone continued, tapering to 3 mg daily from day 43 onwards. She was also treated with voriconazole (day 43-58) for possible coronavirus disease associated pulmonary aspergillosis (CAPA). Lower respiratory specimens to assess for pneumocystis or aspergillosis were never obtained given lack of sputum production and instability for bronchoscopy with ongoing hypoxemia without intubation.

On day 65, she acutely deteriorated with fever, neutrophil count of 0.8 x 10⁹/L (reference 2.0-8.0 x 10⁹/L), and oxygen requirement increase to 95% FiO₂. Given the palliative nature of her ALL and in keeping with her wishes, she was not transferred to intensive care. Chest CT
scan demonstrated new left apical consolidation with cavitation on background of persistent
disease. Dexamethasone was increased to 6 mg daily, and voriconazole restarted in case
deterioration was due to CAPA. Day 67 serum galactomannan was negative, but voriconazole
continued. Oxygen requirements remained at 95% FiO2.

Repeat nasopharyngeal swab (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). Serology
assessing for total antibodies to SARS-CoV-2 spike 1 receptor binding domain (Siemens
ADVIA Centaur XP) from day 87 was non-reactive. Rubella anti-IgG testing (Siemens ADVIA
Centaur XP) on 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 (day 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 or there was lack of adequate
evidence at that time, or she did not meet criteria for enrolment in clinical trials. SARS-CoV-2
RNA was again detected in nasopharyngeal swabs from day 91 (Ct 26.67) and 97 (Ct 28.00),
though viral culture was negative in both day 91 and 97. 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 three months (Table 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 BC. Over time, viral sequencing revealed development of twelve mutations, of which eleven 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 (spike) (Figure 2B), and one N (nucleocapsid) protein locations (Figure 2C). Seven of the identified twelve mutations increased over time and were observed in 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 N, ORF1ab, ORF3a, and S genes were unknown, while E and S gene mutations are discussed below where information is available (Figure 2C).

Discussion

Herein we present a case of prolonged shedding and mutation of SARS-CoV-2 in an immunocompromised host. 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).

Our report demonstrates a 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 infections, this has been previously reported 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 our case resulted in development of twelve mutations (eleven with
predicted coding alterations) in three months; however, in the final sample there were seven mutations including six protein coding changes, which matched the overall expected mutation rate (Duchene et al., 2020). Interestingly, though twelve mutations were observed in total, only seven were present at day 97, emphasizing the ongoing viral evolution and selection within a host (Figure 2C).

Of the persistent mutations, several are previously described. 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 B.1.1.7 and B.1.351 variants, and may convey immune escape based on decreased antibody binding (Wang et al., 2021).

Prevalence of 7/12 viral mutations increased over time, while five mutations were seen only 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. Our patient developed eleven non-synonymous and one synonymous mutations; 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 97 (Figure 2C) which coincided with remdesivir treatment (day 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 remdesivir association. None of these mutations were found in literature to be remdesivir associated mutations.

**Conclusion**

In this case of prolonged SARS-CoV-2 viral evolution in an immunocompromised host, we demonstrated viral viability and variable acquisition of 12 mutations over three months. Ongoing infection and viral evolution of SARS-CoV-2 in immunocompromised individuals may result in variants, which may in turn have increased 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 of using viral culture to confirm the presence of infectious SARS-CoV-2 in select patients.

**Conflict of Interest**
The authors have no conflicts of interest to disclose.

**Funding Source**
Not applicable.

**Ethical Approval**
Consent was obtained from the patient’s mother. Ethics approval not applicable.

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**Figure 1.** Timeline of SARS-CoV-2 related investigations. Abbreviations: Ct, Cycle threshold; E, Envelope; N, Nucleocapsid; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; RdRP, RNA-dependent RNA polymerase.

**Figure 2.** SARS-CoV-2 infection mutation accumulation in an immunocompromised host longitudinally 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 Glycoprotein with 1 ACE2 bound protein structure 7A94 (Benton et al., 2020) visualised using ChimeraX 1.1, with a single spike protein subunit (blue) interaction with ACE2 (yellow) illustrated (B), and table showing temporal development of eleven protein coding changes over time across the SARS-CoV-2 genome with percentages of reads showing indicated mutation (C).
Abbreviations: E, Envelope; N, Nucleocapsid; n/a, Not available; ORF1ab, Open reading frame 1ab; ORF3a, Open reading frame 3a; S, Spike; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2. Comparison of mutational change based on first sample as baseline.

| Genome Position | Day 0 | Day 26 | Day 71 | Day 84 | Day 90 |
|-----------------|-------|--------|--------|--------|--------|
|                 | ref   | alt    | ref    | alt    | ref    |
| 203             | C     | T      | T      | NA     | C      |
| 241             | C     | T      | T      | NA     | C      |
| 333             | C     | T      | C      | T      | C      |
| 366             | C     | T      | C      | T      | C      |
| 1347            | C     | T      | C      | T      | C      |
| 1943            | C     | T      | C      | T      | C      |
| 3037            | C     | T      | C      | T      | C      |
| 4233            | C     | T      | A      | C      | A      |
| 5178            | C     | T      | A      | C      | A      |
| 5811            | C     | A      | C      | A      | C      |
| 6031            | C     | T      | C      | T      | C      |
| 7163            | T     | C      | T      | C      | T      |
| 13975           | G     | T      | G      | T      | G      |
| 14408           | C     | T      | C      | T      | C      |
| 17944           | G     | T      | G      | T      | G      |
| 21990           | T     | T      | T      | T      | T      |
| 23830           | T     | C      | T      | C      | T      |
| 23842           | A     | G      | A      | G      | A      |
| 23843           | A     | G      | A      | G      | A      |
| 24953           | G     | C      | G      | C      | G      |
| 25687           | T     | G      | T      | G      | T      |
| 26463           | T     | G      | T      | G      | T      |
| 26833           | C     | T      | C      | T      | C      |
| 28382           | G     | A      | G      | A      | G      |
| 28881           | G     | A      | G      | A      | G      |
| 28882           | G     | A      | G      | A      | G      |
| 28883           | G     | C      | G      | C      | G      |

Figure 3. Variant information for all five sequenced samples. Genome was compared to 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 and (aa) amino acid alteration due to mutation.