

Long term SARS-CoV-2 infectiousness among three immunocompromised patients: from prolonged viral shedding to SARS-CoV-2 superinfection.

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Summary: We describe three deeply immunocompromised patients presenting prolonged SARS-CoV-2 carriage and infectiousness for several months after initial diagnostic. Asymptomatic carriage, symptom resolution, or superinfection were observed. RT-PCR testing before ending isolation should be systematically performed in immunocompromised populations.

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ABSTRACT

Guidelines for stopping COVID-19 patient isolation are mainly symptom-based, with isolation for 10 to 20 days depending on their condition. Here, we describe three deeply immunocompromised patients, each with different clinical evolutions. Asymptomatic carriage, symptom resolution, or superinfection with a second SARS-CoV-2 strain were observed, all leading to prolonged infectious viral shedding several months. We followed the patients epidemiological, clinical, serological data, infectiousness using viral culture and viral mutations accumulated over time. Understanding underlying mechanisms and frequency of prolonged infectiousness is crucial to adapt current guidelines and strengthen the use of systematic PCR testing before stopping isolation in immunocompromised populations.

Keywords: COVID-19; SARS-CoV-2; Isolation; Viral Shedding; Immunocompromised patients.

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INTRODUCTION

The COVID-19 pandemic has severely disrupted healthcare systems and socioeconomic activities. The SARS CoronaVirus 2 (SARS-CoV-2), has caused large outbreaks in, bars, workplaces, households, and healthcare institutions. In the latter, patient's management and isolation is critical.

Several important questions, important to determine prevention policies, remain unanswered as the duration of infectiousness and, consequently the duration of isolation in healthcare institutions. The CDC recommends a 10-day isolation period for afebrile COVID-19 patients with mild/moderate clinical presentation and improvement of other symptoms since at least 24h. This period is extended for up to 20 days for patients with severe infection and/or severe immunosuppression. A negative SARS-CoV2 RT-PCR control is not mandatory but is encouraged in immunocompromised patients before stopping isolation [1]. For hospitalized patients in France, the isolation period should be at least 14 days after symptom onset and 48h after their resolution. This period is extended up to 24 days for severe infections or immunocompromised patients [2]. Several studies described prolonged positive RT-PCR above 15 days post symptom onset for less than 5% of hospitalized patients, but without viral culture testing [3]. However, three cases of long-term infectious shedding with high viral loads were recently reported in the literature with viral shedding for 35, 70 and 119 days [4–6].

Here, we describe three deeply immunocompromised patients, each presenting a different clinical evolution. All three lead to prolonged viral shedding with high viral load for several months. We explored their epidemiological, clinical, serological data and infectiousness using viral culture and viral mutations accumulated over time.

METHODS

Samples and patients

Respiratory samples and sera were collected from the patients as a part of their routine clinical care. The research was approved by the local ethics committee, N° CER-2020-6.

SARS-CoV-2 PCR

The respiratory samples were tested using either the Cobas® SARS-CoV-2 (Roche, Switzerland) [7] or the NeumoDX® (QIAgen, Germany) using the IP2 Institute Pasteur and the WHO E gene primers [8]. E gene cycle threshold (Ct) value was used as a proxy for viral load.

Antibody testing

Anti-SARS-CoV-2 nucleocapsid (N) and spike (S) IgG were detected using a chemiluminescent microparticle immunoassay (Architect, Abbott, USA) and an ELISA assay (EuroImmun, Lubeck, Germany), respectively.

Viral culture

Vero E6 cells (ATCC, reference R CRL-1586) were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco™) with 10% of heat-inactivated fetal bovine serum (FBS, Gibco™) at 37°C and 5% of CO₂. Briefly, 200µL of respiratory samples viral transport media mixed with 800µL of DMEM were filtered and inoculated in 12-wells plates containing 1.10⁵ cells for 1 hour before adding of 500µL of DMEM with 4% of FBS. After a 6-day incubation, cytopathogenic effect assessment and RT-PCR quantification in cells supernatant were performed to assess the production of new virions. A previously cultured SARS-CoV-2 strain was systematically added as a positive control to ensure cell sensitivity.

Viral whole-genome sequencing

Full genome viral sequencing was conducted from primary clinical samples. Reverse transcription was performed with SuperScript IV with random hexamers after MagnaPure extraction. Tiling PCR amplification was performed according to the ARTIC protocol (nCoV-2019 sequencing protocol v2) with two pools of primers (ARTIC nCoV-2019 V3 panel). Libraries were prepared with NEBNext Companion Module for Oxford Nanopore Technologies, Ligation Sequencing (SQK-LSK 109) and sequenced using MinION R9.4.1 flow cells. All sequences obtained have been deposited on GISAID (EPI_ISL_833191 to EPI_ISL_833200)

Viral genomes analysis

Reads were filtered using the Nanofilt and Nanostat python scripts [9]. They were mapped on the reference genome Wuhan Hu-1 (Genbank ID NC_045512.2) using minimap2. Alignment coverage, depth, and general quality were assessed using in-house R scripts. Variant calling was performed with bcftools suite, and the proportions of each variant in each sample were retrieved with an in-house R script. Finally, for patient 3, mutations located on the same amplicon tiles were recovered using R to assess their linkage.

RESULTS

Patient 1

A 66-years-old African male was admitted on June 4, 2020 for loss of autonomy, and confusion. He was diagnosed with HIV-1 infection (HIV-1 plasma viral load at 275,000 copies/mL and CD4 cell count at 0/mm³, CD19 cell count diminished at 60/mm³) and progressive multifocal leukoencephalopathy with positive CSF PCR for JC virus and compatible brain imagery. All other viral PCR in CSF, including SARS-CoV-2, were negative. He was diagnosed with SARS-Cov-2 infection with positive nasal PCR (Cycle threshold, Ct, value 22) and typical CT chest findings. Despite efficient multi-antiretroviral therapy, the patient had persistent CSF positive PCR for JC, no CD4 cell count increase, and progressive neurological deterioration responding only to painful stimulation after 3 months (Glasgow coma scale=8).

Throughout his hospital stay, the patient did not experience any dyspnea nor respiratory symptoms and showed increasing COVID-19 lesions. NP PCR were constantly positive (Ct from 15 to 25) until day 111. Viral cultures were positive between days 43 and 95 (Figure 1-A). The first negative SARS-CoV-2 RT-PCR was obtained at day 124. SARS-CoV-2 serology remained always negative.

Sequences, obtained at four time points up to day 75, did not show any mutations except for a transient appearance of a C23718T mutation (Figure 1). The viral strain differed from the Wuhan reference strain by 14 mutations.

Patient 2

On April 15, patient 2, a heart-transplanted 71-year-old European male patient receiving an immunosuppressive treatment (prednisone, mycophenolic acid, belatacept), was hospitalized for asthenia, dry cough, myalgia, and low-grade fever for 1-week. He also presents diabetes mellitus and chronic kidney disease (GFR 35 mL/min). He had neither dyspnea nor oxygen requirements throughout his hospital stay. NP swabs tested positive for SARS-CoV-2 at admission and day 14 with minimal COVID-19 involvement on CT scan (<10%). At day 39, he was discharged after clinical improvement, despite persistent positive PCR at day 32 (21 Ct).

On June 23, 76 days after initial symptoms' onset, the patient presented with dry cough, dyspnea, and oxygen requirement. He was admitted to the intensive care unit for cardiac decompensation due to underlying respiratory infection. CT scan showed worsened COVID-19 compatible lesions (40%). He had lymphopenia with CD4 <200/mm³ and CD19 <20/mm³. Bronchoalveolar (BAL) and NP samples collected at readmission (day 78) were positive for SARS-CoV-2 (Ct at 33 and 24, respectively). During his stay, multiple SARS-CoV-2 PCR and viral cultures were performed (Figure 1). Viral culture was positive on a NP sample collected at day 103. The last NP SARS-CoV-2 RT-PCR positive sample was collected on day 120. The patient had negative serology throughout his illness. No viral sequence could be obtained from the first episode to assess the possibility of a new infection during the second episode. Sequences from day 80, 91, and 103 show minimal evolution during this two-month period (Figure 1).

Patient 3

A 35-year-old Tunisian patient with rheumatoid arthritis under Rituximab (B lymphopenia with zero CD19+ cells/mm³) presented on April 28 with fever, cough, and mild dyspnea. He had a positive NP sample for SARS-CoV-2 (21 Ct) with COVID-19 CT scan lesions (25% lung involvement). He did not require oxygen therapy and was discharged at day 3. On day 49, he was readmitted for persistent cough, exertional dyspnea, and intermittent fever. CT scan presented different topography of COVID-19 lesions. SARS-CoV-2 PCR was negative on a NP swab collected on day 51 but positive on a BAL performed on day 56 was (25 Ct). In the light of imaging findings, negative bacterial investigations and the lack of antibiotics response, diagnosis of post-COVID-organized pneumonia was established. A one-week corticosteroid

treatment was initiated on day 66 with significant clinical improvements. On day 73, the patient had recurrence of fever, cough, and increased inflammatory markers (CRP at 125mg/L) that gradually improved within 4 weeks. A NP swab collected on day 84 was positive on RT-PCR (19 Ct) and viral culture. SARS-CoV-2 PCR was negative at day 104. The patient's serology remained negative up to day 121.

SARS-CoV-2 whole-genome was obtained from a NP swab at day 2, a lower respiratory tract sample at day 73, and from a NP swab after symptom's relapse at day 84. Sequences from days 2 and 73 were similar, aside from a C5147T mutation detected in at 68% frequency at day 2 (figure 1). On day 84, we detected the appearance of seven mutations at frequencies close to 70%. Two pairs of close mutations were present in the same PCR tiles. The mutations in these pairs were strongly linked, i.e., about 99% of tiles amplicons contained either none or both mutations (supp. Table 1), suggesting a co-infection with a second viral strain presenting seven additional mutations, linked with a symptom relapse at day 73.

DISCUSSION

This work reports three severely immunocompromised COVID-19 patients shedding infectious viruses up to 4 months post-symptom onset, illustrating different situations leading to long-term infectiousness. This highlights the need for caution and virological controls in deeply immunocompromised populations.

The first patient presented a single continuous infection with high viral load and regular positive culture during 123 days. Long-term replication, lack of respiratory symptoms, and absence of any mutation-selection were permitted by the patient's complete immunosuppression. The second patient presented a positive RT-PCR for 121 days and a positive viral culture on day 103 (27 days after his readmission). Unfortunately, we could not sequence the viral genome during the first hospitalization to rule out a potential reinfection. The last patient presented a quickly resolved COVID-19 episode followed by a post-COVID organized pneumonia with still active viral replication in the lower respiratory tract. At day 84, 32 days after his second hospitalization, he presented a probable superinfection with symptom relapse, high viral loads, positive viral culture and seven new mutations (see also the phylogenetic reconstruction in supp. Figure 1). Those mutations seem unlikely to have arisen during the 11-day period from the previous sequence, especially as SARS-CoV-2

present an evolution speed estimated to 1.10^{-3} mutations per nucleotide per year (i.e. two to three mutations per month) and as highlighted by the extremely low number of mutations selected in our two other immunocompromised patients over large time periods. Those seven new mutations were never present at a 100% frequency. Moreover, we observed a strong linkage for two pairs of these mutations. This, along with the symptom relapse, reinforces the hypothesis of a superinfection with a probable cohabitation of two viral strains. The patient homeless condition also allowed multiple re-exposure until day 52, however, the symptom relapse on day 73 suggest a nosocomial infection. No sequence data from the other patients or healthcare workers of the ward could be explored to explore the infection source. Isolation precaution were maintained and observed during the whole hospitalization.

Two cases of prolonged viral shedding for more than 100 days were previously described in two patients presenting B-cell immunodeficiency [4,10]. Interestingly, all our patients also presented deep CD19 depletion. Convalescent plasma to reduce viral shedding in such population could be evaluated. The T-cell immunity, which could not be explored in our patients, may also play a role in prolonged viral shedding. Several observational studies identified patients with positive SARS-CoV-2 RT-PCR 100 days after their initial detection [11,12]. However, differentiation between re-infections and prolonged viral shedding was not established. Moreover, these studies did not follow patient's infectiousness using viral culture, as we did here. Although several case reports published in different countries showed SARS-CoV-2 reinfection [13–15] a superinfection with a second SARS-CoV-2 strain has not been described to date.

In conclusion, immunodeficiency plays a major role in prolonged viral shedding that can be observed in immunocompromised patients without any respiratory symptom (patient 1), late symptom relapse (patient 2), or with SARS-CoV-2 superinfection (patient 3). Further studies are needed to better understand the frequency and dynamic of long-term viral infectiousness. In the meantime, guidelines should recommend virological assessment of infectiousness, using viral culture and/or Ct value measure (low CT value), prior to stopping isolation in immunocompromised patients.

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FIGURE LEGEND

Figure 1. Virological follow up of the three patients. For each patient, the viral load in respiratory samples are indicated by the observed Ct (Cycle threshold) values. The nature of sample is indicated by the point shape and viral culture status is indicated by the color. The viral strains successfully sequenced are indicated by an asterisk and the sequence differences with the reference strain are indicated in the table along each graphic.

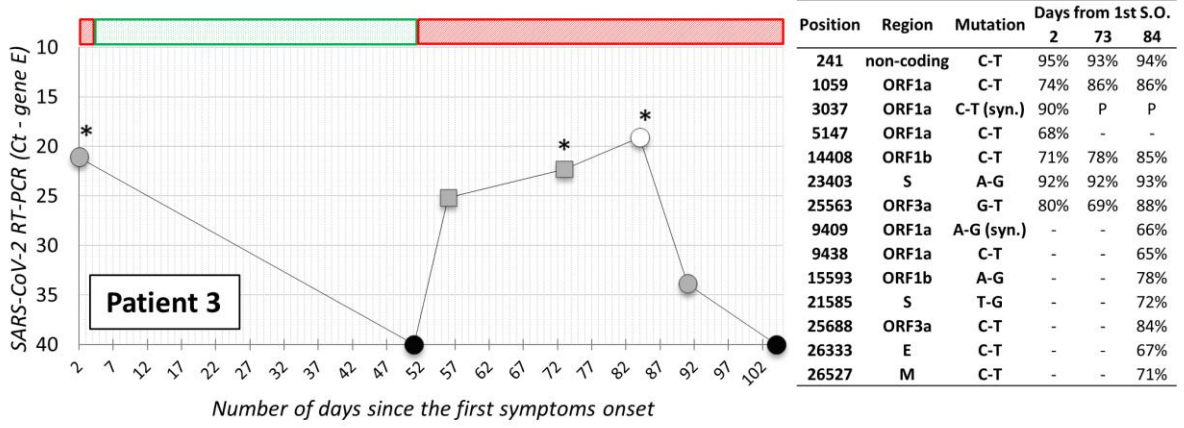
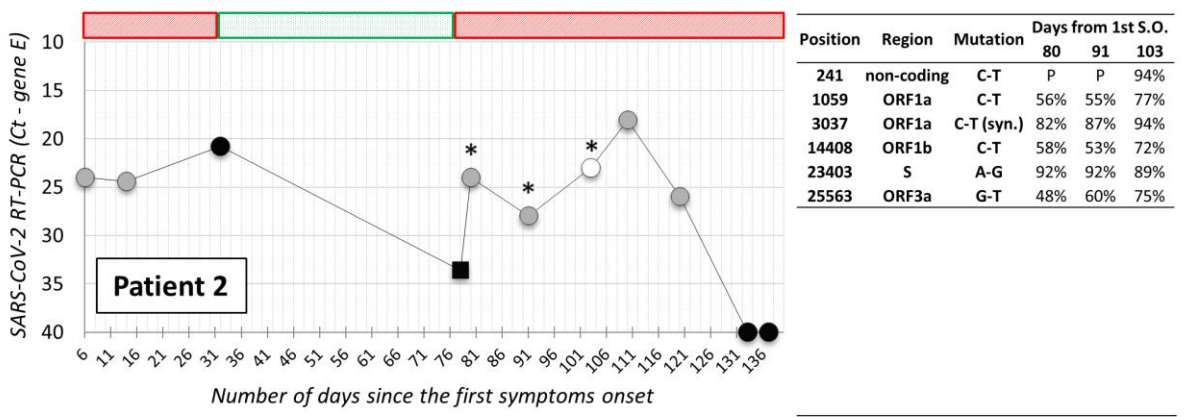
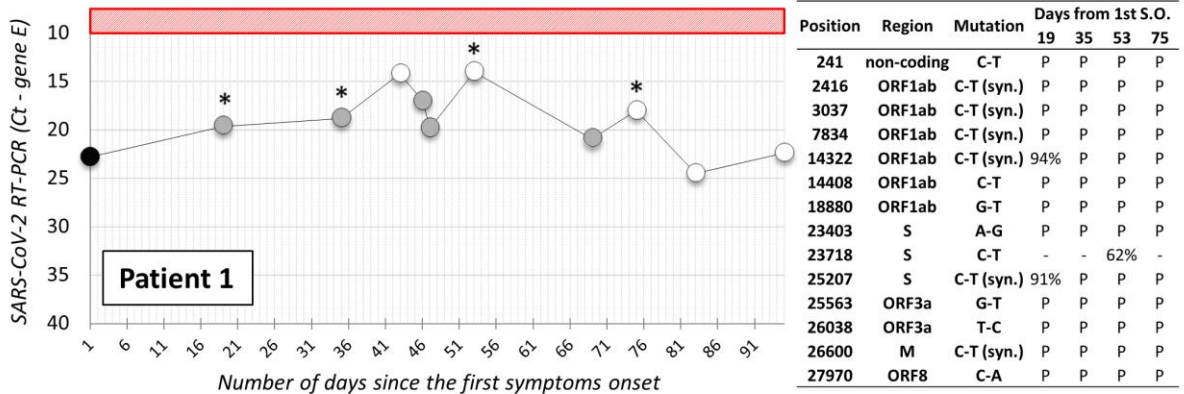
Table 1. Main characteristics of the three patients presenting prolonged infectious viral shedding.

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Table 1. Main characteristics of the three patients presenting prolonged infectious viral shedding.

	Patient 1	Patient 2	Patient 3
Age (years)	66	71	35
Sex	Male	Male	Male
Immunocompromised condition	HIV	Cardiac transplantation	Rheumatoid arthritis
Comorbidities	None	Obstructive sleep apnea, gout disease, osteoarthritis, chronic kidney disease, arterial hypertension, diabetes	None
Characteristics at initial admission			
Symptoms	Confusion	Asthenia, dry cough, myalgia, low-grade fever	Fever, cough, mild dyspnea
White-blood cells (/mm ³)	2570	4070	4560
Neutrophils (/mm ³)	1600	2400	2580
Lymphocytes (/mm ³)	490	840	1150
Eosinophils (/mm ³)	30	10	260
Hemoglobin (g/dL)	11.2	10.9	13.5
Platelets (/mm ³)	290 000	209 000	250 000
CRP (mg/L)	48	26	25
Creatinine (μmol/L)	64	207	60
SGOT (UI/L)	59	38	18
SGPT (UI/L)	21	34	28
Bilirubin (mg/L)	8	9	5
LDH (U/L)	453	234	199
CD4 (/mm ³)	10	110	1150
CD8 (/mm ³)	270	650	810
CD19 (/mm ³)	60	20	0
Co-infections			
Respiratory	<i>Mycobacterium gordonae</i>	None	None
CSF	JC virus	None	None
Blood	CMV, EBV		
Other	Oral candidiasis	None	None

Figure 1



Patient location:	Nasopharyngeal sample:	B.A.L. or tracheal aspirate:	Sequences:
● Hospital	● Not tested by culture	● Not tested by culture	* Strain sequenced
● Home	● Culture negative	● Culture negative	P Presence (tables)
	○ Culture positive	○ Culture positive	- Not detected (tables)
			syn. Synonymous mutation (tables)