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Potential recurrence of COVID-19 in a healthcare professional: SARS-CoV-2 genome sequencing confirms contagiousness after re-positivity

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ABSTRACT

Re-positivity of SARS-CoV-2 tests is widely reported, raising discussion about guidance for patient discharge and ending isolation. The unsuccessful recovery of replication-competent virus and/or absence of secondary cases has suggested that re-positive patients are not contagious. This study reports SARS-CoV-2 re-positivity in a healthcare professional 16 days after three negative tests, with viral genome sequencing supporting contagiousness leading to secondary cases.

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On 27 May 2020, the World Health Organization (WHO) updated criteria for releasing COVID-19 patients from isolation, exempting the need to perform a laboratory RT-PCR test [WHO, 2020a; WHO, 2020b], considering that, even if a positive result is obtained after symptoms resolution, patients are unlikely to be infectious and therefore unable to originate secondary cases (WHO, 2020). Aligned with this, the recovery of replication-competent virus from re-positive patients has been unsuccessful Li et al., 2020; Korea Centers for Disease Control and Prevention, 2020) and no cases of infection among people who had contact with re-positive patients have been reported to date (Azam et al., 2020; Bongiovanni et al., 2020; Dao et al., 2020; Gao et al., 2020; Kang, 2020). This study shows that re-positivity can translate into a contagiousness scenario. This clinical case involved a nurse working at a long-term psychiatric hospital, for which the SARS-CoV-2 infection after re-positivity rendered secondary cases. SARS-CoV-2 genome sequencing revealed similar viral genetic profiles in samples collected from the healthcare professional and her close contacts in both episodes, supporting contagiousness after re-

positivity. A detailed description of the methodological approaches used in this study is provided as Supplementary Material.

Clinical case of SARS-CoV-2 re-positivity in a healthcare professional

In early April 2020, following the death of two patients in a long-term psychiatric hospital located in Braga, Northern Portugal, caused by SARS-CoV-2 (Patient A, female, aged 70 years and Patient B, female, aged 87 years, Figure 1A), healthcare professionals in close contact with the patients and patients from the same ward were screened for SARS-CoV-2 with RT-PCR by nasopharyngeal swabs. Thirty-three of 40 patients and eight of 12 healthcare professionals tested positive for SARS-CoV-2 during this nosocomial outbreak. All infected healthcare workers were sent home for isolation and kept under clinical surveillance. Among these, one nurse (Patient C, female, aged 27 years, Figure 1A), who was asymptomatic during home isolation, was tested again two weeks after the first positive test, on the 27th and the 29th with nasopharyngeal swabs and on the 30th with nasopharyngeal and oropharyngeal swabs. The three RT-PCR tests (performed 13, 15 and 16 days after the first one) were negative, and Patient C was considered cured and returned to work, following the Portuguese General-Directorate of Health guidelines [(Ministério da Saúde 2020)]. Thirteen days after returning to work, the nurse

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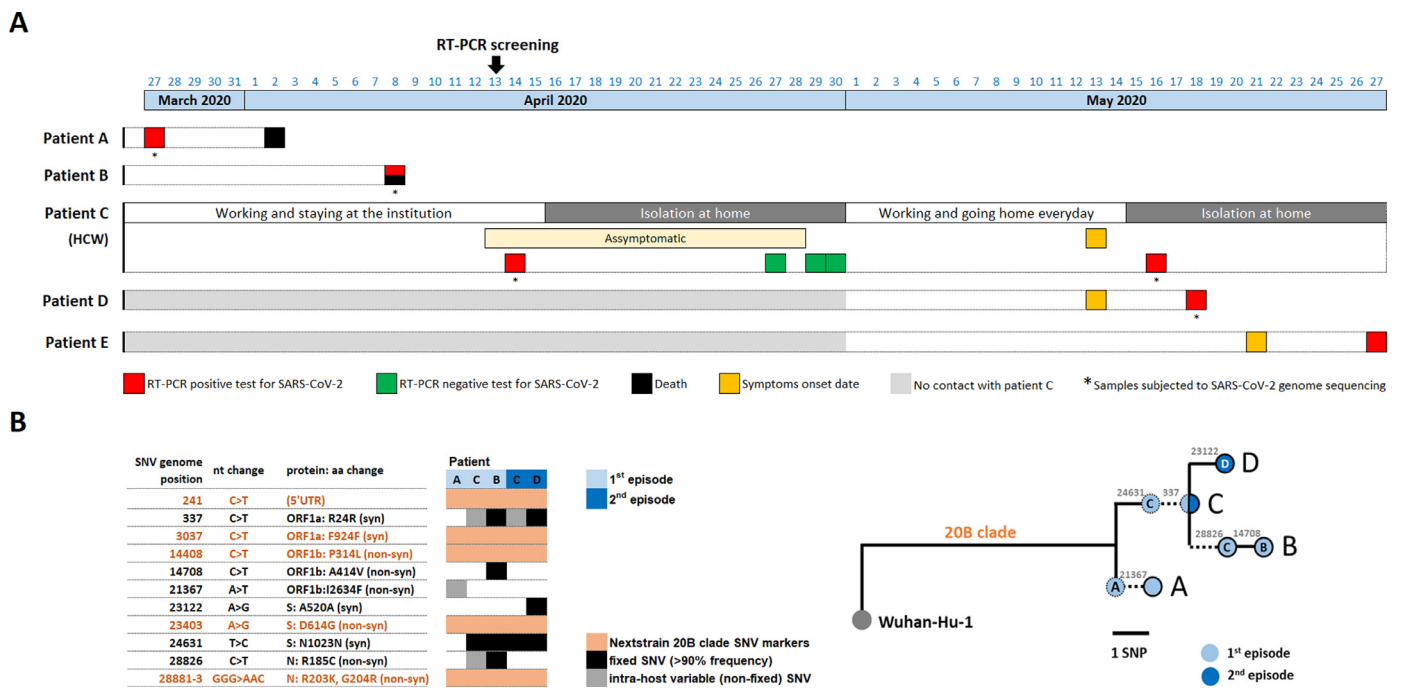


Figure 1. Clinical case study of a recurrent SARS-CoV-2 infection leading to secondary cases. Panel A. Chronogram of events associated with a recurrent SARS-CoV-2 infection in a healthcare worker (HCW) (Patient C) and confirmed contacts. **Panel B.** Viral genome sequencing data. The table indicates the single nucleotide variants (SNV) detected in viral genomes in all samples (details about the SNV frequency and coverage evidence are provided in the Supplementary Material). The phylogenetic tree shows the most plausible transmission trajectory, taking into account the intra-patient frequency of both fixed and non-fixed SNVs. Tree branches with dashed lines denote the detection of intra-patient sub-populations that can place a given sample in different phylogenetic contexts. For instance, the first sample of Patient C is shown in three positions on the tree according to profile of two non-fixed SNVs, namely the sub-population carrying “C337T”, which reached 100% frequency in both secondary cases B (first episode) and D (second episode); and the sub-population carrying “C28826T”, which was transmitted to patient B (becoming fixed), although it was not maintained during the interval elapsed between the two infection episodes of Patient C. The consensus sequences available in GISAID (see Supplementary Material) are indicated in the tree by nodes surrounded by solid lines. Dashed lines indicate the intermediate evolutionary points.

presented anosmia and myalgia, testing positive for SARS-CoV-2 (Figure 1A). She was again removed from work and her cohabitants and other close contacts were quarantined. Two COVID-19 cases were detected among the close contacts: her boyfriend (Patient D, male, aged 29 years, Figure 1A) and his mother (Patient E, female, aged 52 years, Figure 1A); both were in contact with patient C only after 1st May 2020 (i.e., after all of the negative tests (Figure 1A)). Both positive samples from Patient C (first and second episodes) were subjected to short-tandem repeat amplification of 15 human genome targets (AmpFISTR Identifier PCR Amplification kit, Applied Biosystems; see methods for details). Both showed the same genetic profile in all markers (comprising 27 alleles overall), indicating that both samples belonged to the same individual.

SARS-CoV-2 genome sequencing confirms secondary cases after re-positivity

In order to investigate the hypothesis of reinfection, which would have been triggered by the new contacts after recovery, samples collected from Patient C in both episodes were selected for viral genome sequencing at the Portuguese National Institute of Health. Positive samples from close contacts of Patient C that occurred among psychiatric patients (first episode, Patients A and B) and at home (second episode, Patient D) were also sequenced. Viral genome analysis, including inspection of the direction of mutation fixation (i.e., analysis of frequency fluctuation of intra-patient single nucleotide variants - SNV), showed that Patient C was an intermediate case between Patients A and B within the nosocomial transmission chain that took place at the beginning of April 2020 (Figure 1B and Supplementary Material). The direct virus transmission from Patient C to Patient B was further supported by the fixation of two SNPs (C337T and C28826T) in SARS-CoV-2 of Patient B

that were found at ~60% frequency in the sample collected from Patient C (Figure 1B and Supplementary Material). The viral genetic profile detected in Patient C five weeks later was congruent not only with the one that was observed during the first infection episode, but also with the genome sequence collected from Patient D, thus supporting a scenario of prolonged infection instead of reinfection. Of note, the SARS-CoV-2 sub-population carrying the SNP “C28826T”, which was likely transmitted from Patient C to Patient B (where it was fixed), was not maintained during the interval that elapsed between the two episodes of Patient C (Figure 1B and Supplementary Material). Curiously, this mutation leads to the amino acid change in position 185 of nucleocapsid N protein, and is predicted to fall within a B-cell epitope-containing loop, with potential impact on immune response (Corral-Lugo et al., 2020). This study presents a clinical case where SARS-CoV-2 re-positivity (after several negative tests) represented the silencing continuity of the primary infection, leading to secondary cases.

Discussion

The first report of re-positive RT-PCR test in patients who have recovered from COVID-19 after consecutively negative tests was published in February 2020 (Lan et al., 2020). Since then, multiple studies have reported non-negligible rates of RT-PCR re-positivity (Azam et al., 2020; Bongiovanni et al., 2020; Dao et al., 2020; Gao et al., 2020; Kang, 2020; Zou et al., 2020), raising concerns about criteria for releasing COVID-19 patients from isolation, the duration of immune response after recovery, and the eventual impact on vaccination and effectiveness. Several explanations can be considered for re-positive situations, namely: the performance of tests, especially when different laboratories/tests are

rolled, and reinfection and “recurrence” of a prolonged infection (Dao et al., 2020; Gao et al., 2020). In this particular case, even though forensic analysis confirmed that the samples collected in both episodes belonged to the same individual, the possibility of cross-contamination of the first sample during the nosocomial outbreak could not be discarded or investigated. Excluding this hypothesis, the data suggest recurrence, as the possibility of reinfection is very unlikely. Indeed, a short time period elapsed between episodes, and the same viral genetic profile was detected in both samples. The immune protection conferred by the first episode would have been almost nonexistent, allowing Patient C to be re-infected upon contact with positive cases of the still ongoing nosocomial outbreak. Although it would be of interest to assess the immunologic response behind the recurrence of infection in Patient C, no serologic tests were available by that time. The three consecutive negative tests between the two episodes may be explained by a strong virus clearance in the superior respiratory tract, leading to a low virus concentration (below the RT-PCR limit of detection). This observation suggests the existence of potential SARS-CoV-2 body site reservoirs enabling posterior viral load increase in other anatomical sites, namely: the respiratory tract. Supporting this, there are reports of high SARS-CoV-2 RNA levels in non-respiratory sites or signs of COVID-19 extra-pulmonary affection (Choi et al., 2020; Trypsteen et al., 2020), as well as intermittent positivity in the superior respiratory tract. Although the pathobiology behind the present clinical case, involving a presumably healthy individual, is still undisclosed, this study corroborates that the dynamics of SARS-CoV-2 clearance in mild COVID-19 cases is still an under-investigated research line.

This study reports secondary cases after a potential SARS-CoV-2 recurrence of infection, showing that re-positivity after discharge can lead to contagiousness. Considering that negative RT-PCR tests are no longer mandatory for ending isolation of COVID-19 cases (ECDC, 2020b; WHO, 2020b) and that vaccination does not completely prevent infection (Lopez Bernal et al., 2021), these results reinforce that it is advisable to routinely test healthcare workers and other individuals in close contact with high-risk groups (ECDC, 2020a) in order to identify and isolate asymptomatic COVID-19 cases and rapidly prevent transmission chains.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical statement

Verbal and written informed consent were obtained from the patients to allow the use of the clinical and virological data. The SARS-CoV-2 genome sequencing study was approved by the Ethical Committee (“Comissão de Ética para a Saúde”) of the Portuguese National Institute of Health.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.ijid.2021.09.035](https://doi.org/10.1016/j.ijid.2021.09.035).

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