The Journal of Infectious Diseases

BRIEF REPORT

Proper Assignation of Reactivation in a COVID-19 Recurrence Initially Interpreted as a Reinfection

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A 77-year-old man (case R) with previous diagnosis of a mild COVID-19 episode was hospitalized 35 days later. On day 23 postadmission, he developed a second COVID-19 episode, now severe, and finally died. Initially, case R's COVID-19 recurrence was interpreted as a reinfection due to the exposure to a SARS-CoV-2 RT-PCR–positive roommate. However, whole-genome sequencing indicated that case R's recurrence corresponded to a reactivation of the strain involved in his first episode. Case R's reactivation had major consequences, leading to a more severe episode, and causing subsequent transmission to another 2 hospitalized patients, 1 of them with fatal outcome.

Keywords. COVID-19; nosocomial transmission; reactivation; SARS-CoV-2; WGS.

Whole-genome sequencing (WGS) has been essential to clarify a key aspect in the coronavirus disease 2019 (COVID-19) pandemic, namely, the analysis of recurrences, allowing identification of those due to reinfections [1, 2]. Genomic research has demonstrated the prolonged persistence of viable severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in severely immunosuppressed patients [3, 4], but it has not equally been used to support reactivations and the scarce reports focus primarily on clinical descriptions [5]. Furthermore, the potential relationship between SARS-CoV-2 reactivation and associated nosocomial outbreaks has not been described to date. In this study we present a SARS-CoV-2 reactivation and its consequences in the nosocomial setting.

METHODS

Clinical Data

Baseline characteristics and clinical and laboratory parameters at COVID-19 diagnosis and their outcome were obtained from the electronic medical records. The study was approved (reference, MICRO.HGUGM.2020–042) by the ethical research committee of Gregorio Marañón Hospital.

Diagnostic Tests

SARS-CoV-2 RT-PCR

Viral RNA was extracted and purified from 300 µL of nasopharyngeal exudates with the aid of a KingFisher (Thermo Fisher Scientific) instrument. Next, reverse transcription polymerase chain reaction (RT-PCR) was performed using a TaqPath COVID-19 CE-IVD RT-PCR kit (Thermo Fisher Scientific).

SARS-CoV-2 Serology

Determinations of antibodies in sera were performed by specific qualitative detection of anti-SARS-CoV2 immunoglobulin G (IgG) anti-N using a chemiluminescent immunoassay of microparticles in the ARCHITECT system (Abbott).

Whole-Genome Sequencing

Eleven µL of RNA was used as template for reverse transcription using Invitrogen SuperScript IV reverse transcriptase (ThermoFisher Scientific) and random hexamers (ThermoFisher Scientific). Whole-genome amplification of the coronavirus was done with an Artic_nCov-2019_V3 panel of primers (Integrated DNA Technologies; artic.network/ncov-2019) and Q5 Hot Start DNA polymerase (New England Biolabs). Libraries were prepared using the Nextera Flex DNA Library Preparation Kit (Illumina) following manufacturer´s instructions.

Libraries were quantified with a Quantus Fluorometer (Promega), before being pooled at equimolar concentrations (4 nM). Next, they were sequenced in pools of up to 17 libraries on the Miseq system (Illumina) and the MiSeq Reagent Micro kit v2 (2 × 151 pb) or in pools of up to 96 libraries with the MiSeq Reagent (2 × 201 pb).

FastQ files above the GISAID thresholds were deposited at GISAID EPI_ISL_654287, EPI_ISL_654203, EPI_ISL_654284, EPI_ISL_654176, and EPI_ISL_1173765. An in-house analysis pipeline was applied to analyze the sequencing reads. The

Received 8 April 2021; editorial decision 28 May 2021; accepted 7 June 2021; published online June 9, 2021.

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The Journal of Infectious Diseases® 2021;XX:1–5

DOI: 10.1093/infdis/jiab302

pipeline can be accessed at https://github.com/pedroscampoy/covid_multianalysis. Briefly, the pipeline went through the following steps: (1) removal of human reads with Kraken [6]; (2) preprocessing and quality assessment of fastq files using fastp [7] v0.20.1 (arguments: --cut tail, --cut-window-size, --cut-mean-quality , -max_len1 ,-max_len2) and fastQC v0.11.9 [8]; (3) mapping with bwa version 0.7.17 [9] and variant calling using IVAR version 1.2.3 [10] using Wuhan-1 sequence (NC_045512.2) as reference; and (4) recalibration of punctual low-coverage positions using joint variant calling. When necessary, informative noncovered positions were analyzed by standard Sanger sequencing with the corresponding flanking primers from the ARTIC set.

RESULTS

Our case (case R; Figure 1) was a 77-year-old man with hypertension and dyslipidemia, a diagnosis of cutaneous B-cell lymphoma in remission, a previous stroke, and chronic obstructive pulmonary disease associated with mild interstitial lung disease without exacerbation or need of supplemental oxygen. His first positive SARS-CoV-2 RT-PCR was on 28 July 2020 when he had a mild infection with fever without developing pneumonia or other complications. Hospital admission was not required. SARS-CoV-2 serology was not performed at that time.

On 1 September (35 days after his first positive RT-PCR; Figure 1) he was admitted to the hospital due to an acute obstructive cholangitis secondary to choledocholithiasis that was removed by endoscopy. Chest X-ray on admission showed chronic alterations compatible with idiopathic pulmonary fibrosis. The images were no different from the previous episode. The patient received piperacillin-tazobactam. After the endoscopic procedure, he developed mild acute pancreatitis, hemobilia, and acute kidney injury related to acute tubular necrosis. In addition, he developed catheter-related *Enterococcus faecium* bacteremia successfully treated with vancomycin. During this time, he obtained 2 negative SARS-CoV-2 RT-PCR tests (1 and 14 September; Figure 1).

On day 23 following admission (57 days after his first positive RT-PCR from his previous COVID-19 episode), extensive bilateral lung opacities were identified in a control abdominal computed tomography (CT). After these unexpected radiological findings, SARS-CoV-2 RT-PCRs were performed for 2 consecutive days, both positive (cycle threshold [Ct] 19 and 21). SARS CoV-2 IgG serology was negative (Figure 1).

Case R developed mild dyspnea and hypoxemia (oxygen saturation of 92% at room air). He received remdesivir for 5 days and dexamethasone 20 mg once daily for 4 days. After a slight improvement, on day 29, he developed fever and respiratory worsening. On day 31, high-flow oxygen therapy and a single 400-mg dose of tocilizumab (interleukin 6 level, 226 pg/mL) were administered. The patient was transferred to the intensive care unit where he received full ventilatory support and continuous changing between prone and supine positions. However, the patient rapidly developed multiorgan failure with hemodynamic instability, mixed metabolic and respiratory acidosis, and renal impairment requiring continuous renal replacement therapy. Body CT scan revealed nonspecific colitis and worsening of the bilateral pulmonary opacities with pleural effusion. A colonoscopy ruled out ischemic colitis. Despite

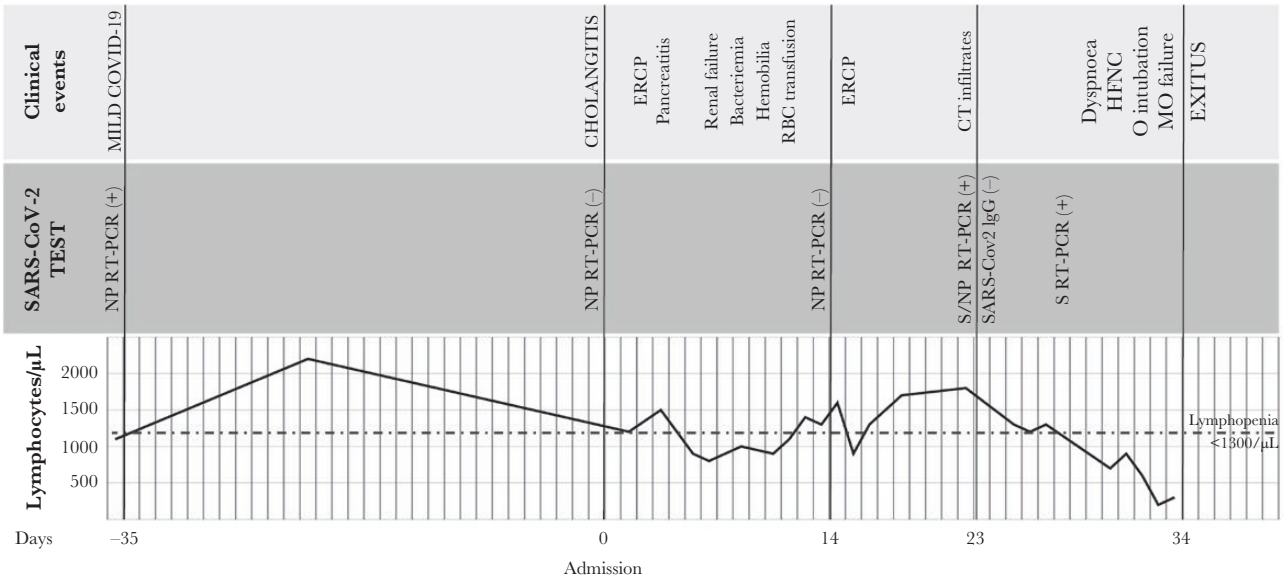

Figure 1. Clinical timeline for case R. Abbreviations: COVID-19, coronavirus disease 2019; CT, computerized axial tomography scan; ERCP, endoscopic retrograde cholangiopancreatography; HFNC, high-flow nasal cannula; MO failure, multiorgan failure; NP, nasopharyngeal sample; O intubation, orotracheal intubation; RBC, red blood cells transfusion; RT-PCR, reverse-transcription polymerase chain reaction; S, serum sample; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

all therapeutic interventions, the patient developed refractory multisystem organ failure and finally died on day 34. Retrospectively, we recovered 3 sera specimens (from days 23 [the day the nasopharyngeal RT-PCR result was positive], 27, and 30) and all were positive for SARS-CoV-2 by RT-PCR (Ct value in all 3 was 37). Clinical outcomes are shown in Figure 1.

Whole-Genome Sequencing Analysis

Prior to having the WGS data, several findings, that is the chronology of SARS-CoV-2 infections, dates of symptom onset, positive SARS-CoV-2 RT-PCRs, and room coincidences, led clinicians to assume that case R recurrence was a reinfection due to exposure to a patient with whom he had shared a hospital room (case A) and who had been admitted 11 days before due to an intestinal obstruction, had a bilateral pneumonia, and subsequent positive SARS-CoV-2 RT-PCR. However, WGS data (obtained in a larger study analyzing a wide nosocomial outbreak in the gastroenterology ward, under evaluation) indicated that fully different strains were identified in case A and case R (Figure 2A). In addition, case R was part of a cluster that also included cases S and T, infected by an identical strain (0 SNPs; Figure 2A). Cases S and T had shared a room, but case R at the time of his positive RT-PCR was in a different one. However, tracking back his previous movements revealed that case R had shared room with case S 7 days before, confirming a link between them; SARS-CoV-2 infection in case S had a fatal outcome.

WGS data ruled out our initial hypothesis of reinfection after nosocomial exposure and led us to consider, alternatively, case R as a reactivation, causing a subsequent nosocomial transmission. The sequences of the positive specimens collected from case R's first and second episodes (July and September 2020) belonged to the same lineage (B.1.177) and showed nearly identical sequences; they shared 16 SNPs and differed in 2 (Figure 2A and 2C and Supplementary Table). The marked diversity of circulating SARS-CoV-2 in the second COVID-19 wave (Figure 2B), the differences between the strains circulating in July and September, and the high similarity between the case R's sequences and those from the 2 related nosocomial cases, together, strongly support that case R recurrence most likely represented a reactivation causing subsequently a nosocomial transmission.

DISCUSSION

This study shows the importance of WGS-based analysis to correctly understand COVID-19 recurrences and, additionally,

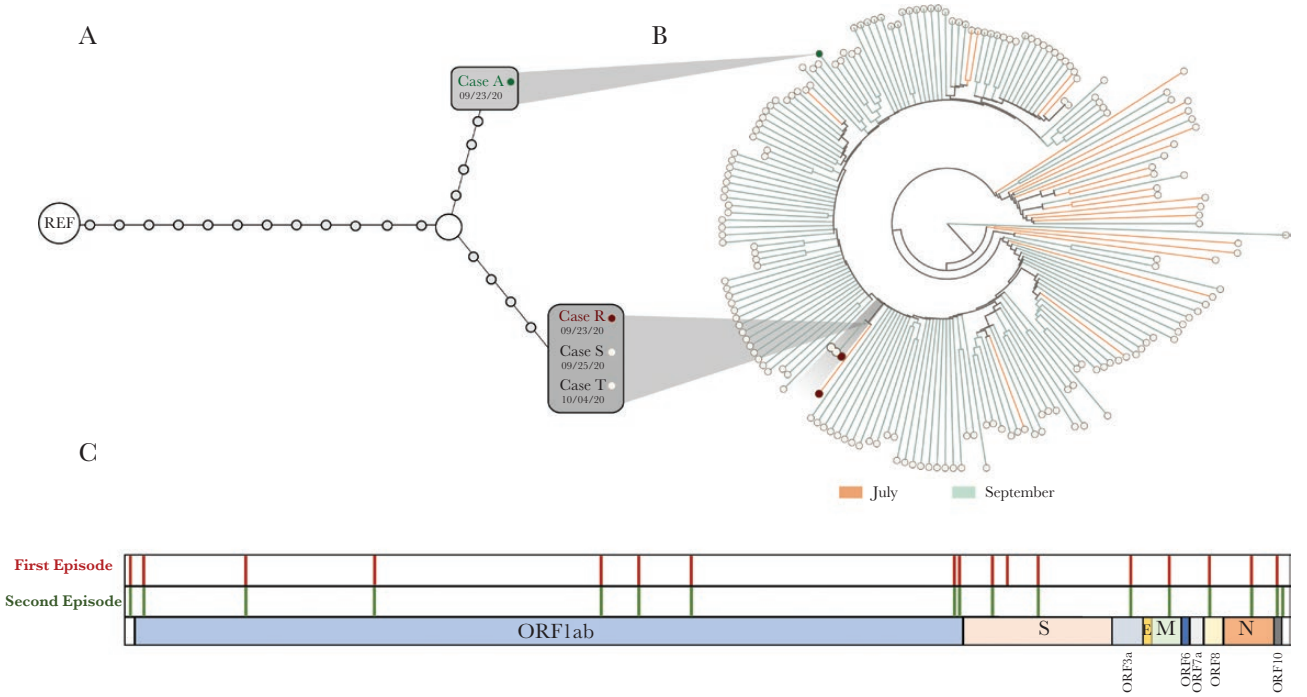

Figure 2. *A,* Network of relationships obtained from whole-genome sequencing analysis for the outbreak strains. Each dot corresponds to a single nucleotide polymorphism. When 2 or more cases share identical genome (zero single nucleotide polymorphisms between them) they are included in the same box. The median vector, the recent common ancestor for the 2 branches, was not sampled. *B,* Phylogenetic tree including 183 representative sequences from SARS-CoV-2 circulating in July 2020 (case R's first episode) and September 2020 (case R's second episode). The 2 sequences from case R are indicated and also those from the 2 other cases involved in the nosocomial outbreak. *C,* Distribution along the SARS-CoV-2 chromosome of the single nucleotide polymorphisms identified in the 2 sequential episodes of case R. Each vertical bar corresponds to a single nucleotide polymorphism. Abbreviations: ORF, open reading frame; REF, Wuhan-1 reference strain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

the true links within nosocomial transmission events. This technique provided key data to describe a COVID-19 reactivation, which was subsequently responsible for another 2 nosocomial cases.

The similarities between the strains infecting case R in the July and September episodes may be explained by either a persistently active infection or a reactivation after a clinical resolution. The persistently active infection hypothesis was less likely because the patient fully recovered from mild clinical symptoms experienced during his first episode. Furthermore, X-rays at admission did not show abnormal SARS-CoV-2–related findings and 2 sequential negative PCR results just before being diagnosed again in September (at admission and 14 days later) were obtained. Finally, during the 23 days of hospital stay before reactivation, the patient had close contact with 4 roommates, none of which had a COVID-19 diagnosis.

All the previous findings make more likely the alternative explanation, namely reactivation, for the high sequence similarities between the specimens collected during the 2 episodes experienced by case R. The subtle differences (2 different SNPs and 16 identical SNPs) found for this case are similar to those described in a reactivation reported elsewhere [11]. The reactivation hypothesis implies that SARS-CoV-2 had remained undetected (or unsampled) in a reservoir between the 2 sequential episodes. The presence of SARS-CoV-2 in extrapulmonary tissues (eyes, gastrointestinal tract, liver, and brain) has been reported [12], due to the ubiquity of the ACE2 receptors. However, reservoirs for SARS-CoV-2 after the resolution of a COVID-19 episode have not been defined yet and the presence of SARS-CoV-2 in nonrespiratory tissues from asymptomatic cases [13] suggests that further studies are needed to identify other viral reservoirs [14].

If the reservoir hypothesis were correct, we would expect reactivations to be mainly associated with immunosuppression, which would trigger the replication of the latent strain. Few studies have proposed reactivation as the explanation for COVID-19 recurrence [5, 15], some involving immunosuppression. However, only 2 were supported with viral genome analyses [11] (D.A. Molina, unpublished). Several factors suggest the presence of immunosuppression in case R. Firstly, he had stayed hospitalized 23 days with a severe conditions before his first positive RT-PCR. Acute care settings are a risk factor for malnutrition. Before the diagnosis of COVID-19, case R had lymphopenia for 12 days; this may impair immunity, a factor associated with increased morbidity and mortality [16, 17]. Secondly, the patient had severe gastrointestinal conditions (acute cholangitis, post-ERCP [endoscopic retrograde cholangiopancreatography] acute pancreatitis, and gastrointestinal bleeding requiring blood transfusion) that could have worsened his immune system. Finally, he presented 2 infections (cholangitis and a catheter-related infection) and acute kidney injury that might have further worsened his already weakened immune system. SARS-CoV-2 IgG determination was negative at the time of the second episode diagnosis, which might be consistent with immunosuppression, although we should also consider that the detection of specific responses months after acute infection sometimes may be not optimal.

A relevant retrospective finding in case R is the positive SARS-CoV-2 RT-PCR in 3 serum specimens taken the same day he had his first diagnostic SARS-CoV-2 RT-PCR, and 4 and 6 days later. SARS-CoV-2 may be detected in plasma samples from patients with respiratory disease and this may have value to predict the severity of the disease [18]. However, SARS-CoV-2 RNAemia has not been found close to diagnosis, even in cases with pneumonia [19]. Therefore, the presence of SARS-CoV-2 in plasma just at the same time as diagnosis of the second episode experienced by case R, would suggest that this was not a new infection but a likely longer-term disease, which may support the reactivation scenario.

In summary, we report genomic viral analysis that allowed identification of a reactivation case with major consequences, leading to a more severe second episode with fatal resolution and subsequent nosocomial transmission of the same strain with an additional COVID-19–related death.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Tejerina, Maricela Valerio, Mª Cristina Veintimilla, Lara Vesperinas, Teresa Vicente, Sofía de la Villa.

Acknowledgments. We are grateful to Dainora Jaloveckas (cienciatraducida.com) for editing and proofreading assistance.

Financial support. This work was supported by Instituto de Salud Carlos III (grant number COV20/00140 SeqCOVID-Consorcio para la Epidemiología Genómica de SARS-CoV-2 en España); and Consejo Superior de Investigaciones Científicas (CSIC) (PTI Salud Global). L. P. L. holds a Miguel Servet contract (grant number CP15/00075).

Potential conflict of interests. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Data availability. The data that support the findings of this study (FastQ files) are openly available on GISAID at https://www.gisaid.org/, reference numbers EPI_ISL_654287, EPI_ISL_654203, EPI_ISL_654284, EPI_ISL_654176, and EPI_ISL_1173765.

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