SARS-CoV-2 superinfection and reinfection with three different strains

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Abstract

We report a COVID-19 case with unprecedented viral complexity. In the first severe episode, two different SARS-CoV-2 strains (superinfection) were identified within a week. Three months after discharge, patient was readmitted and was infected in a nosocomial outbreak with a different strain, suffering a second milder COVID-19 episode.

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Running title : Three sequential SARS-CoV-2 infections

Abstract

We report a COVID-19 case with unprecedented viral complexity. In the first severe episode, two different SARS-CoV-2 strains (superinfection) were identified within a week. Three months after discharge, patient

was readmitted and was infected in a nosocomial outbreak with a different strain, suffering a second milder COVID-19 episode.

Keywords: COVID-19, SARS-CoV-2, superinfection, reinfection, genomics.

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First COVID-19 episode (E1)

Sixty-five-year old male with past medical history of hypertension admitted in the emergency department on February 6, 2020 (Day 0 – E1). Diagnosis of hypertensive cerebellar haemorrhage with intraventricular and subarachnoid haemorrhage that required decompressive craniectomy (Day 1). Patient was moved post-operatively to the neurosurgery ward (Day 4). Condition of the patient deteriorated on Day 50: diminished oxygen saturation (93%), no consolidation on chest X-ray, with lymphopenia of 800, IL-6 of 4.6, CRP of 1.1, and D-dimer of 800 ng/ml. On the same Day 50, patient´s SARS-COV2 RT-PCR (Specimen-1) result was positive; he was moved to a COVID-19 ward in a different building and given lopinavir/ritonavir and hydroxychloroquine for 10 days. On Day 69, a second SARS-CoV-2 RT-PCR assay was done with positive result (Ct 19; Specimen-2). Patient was returned to the neurosurgery ward, which had been turned into a COVID-19 area. On day 74 radiological worsening was noted in the left lung, coinciding with an inflammatory process: lymphocytopenia (0.7 10E3/µ), low platelet count (125 10E3/µL), increased DD (989 ng/mL), CRP of 4.4 mg/dL, ferritin of 778 µg/L, and IL6 of 13.6 pg/mL. Treatment with methylprednisolone was initiated and a SARS-CoV-2 RT-PCR (Specimen-3) performed on the same day (Day 74) with positive result (Ct 25). SARS-CoV-2 RT-PCR assay performed on Day 87 was negative, as well as subsequent tests on Days 108, 117, 147, 195, and 206. Patient was discharged on Day 207 to a long-term facility due to persistent generalised muscle weakness.

Second COVID-19 episode (E2)

On December 6 (three months after the previous discharge) (Day 304), patient was admitted due to a fortuitous fall from a seated position. Diagnosis of head trauma and subdural hematoma over left frontoparietal lobe. At admission, severe malnutrition and muscular hypotrophy were found. On Day 350, he developed fever and SARS-COV2 RT-PCR result was positive (Ct 16; Specimen-4); no SARS-COV2 IgG antibodies were detected. No signs of consolidations on chest X-rays; patient presented mild hypoxemia and completed a five-day course of dexamethasone (6 mg/day). By Day 364 respiratory syndrome was controlled and positive serology for SARS-COV-2 determined (IgG of $>$ 40000 UA/mL).

Genomic analysis

Two positive SARS-CoV-2 RT-PCR from E1 (Specimens 2 and 3) and one positive specimen (Specimen-4) from E2 were available for whole genome sequencing (WGS).

A B.1.258 lineage strain (Strain 1, Figure) was identified in E1 from the Specimen 2. When analysing the data from the Specimen-3, many heterozygous calls were obtained (Figure), suggesting the presence of two SARS-CoV-2 strains. Cross-contamination with a positive specimen from another patient was ruled out by confirming identical human genetic content (short tandem repeat human DNA profile analysis, Supplementary Figure) in specimens 2 and 3. Heterozygous calls indicated superinfection with a different strain (Strain 2; (lineage B.1)) differing in 13 single nucleotide polymorphisms (SNPs) from Strain 1. Frequency determination of alternative alleles in the heterozygous calls allowed us to determine that superinfecting Strain 2 was overrepresented (59-79% frequency, Supplementary Table). Strain 1 and 2 sequences were analysed along with 2,249 SARS-CoV-2 sequences from specimens collected from COVID-19 cases among the Madrid population throughout the pandemic. A tree with the results was created and Strains 1 and 2 are presented on the branch corresponding to the strains circulating in the first COVID-19 wave (before July 2020, Figure).

A new strain (Strain 3; lineage B.1.177) was identified in E2 from Specimen-4, with 16 SNPs not shared with Strains 1 or 2, and without the 14 SNPs identified in Strains 1 and 2 (Figure). This indicated that COVID-19

E2 was a reinfection. Strain 3 sequence was positioned in the phylogenetic tree among the sequences from strains circulating after July 2020, ruling out its circulation during our case´s first episode. Short tandem repeat human DNA analysis confirmed that specimens from the two sequential COVID-19 episodes were from the same individual (Supplementary Figure).

E1 SARS-CoV-2 superinfection occurred during the first COVID-19 wave, when prevalence of SARS-COV-2 among our population was very high (1,182 cases/100,000 inhabitants) and most hospitalized cases were COVID-19 patients. Moreover, our patient was highly dependent due to neurological damage and remained hospitalized in two different buildings. Thus, the patient was probably exposed to different nosocomial circulating strains that might have caused E1 superinfection. To the best of our knowledge, only another likely SARS-CoV-2 superinfection has been reported (Tarhini, 2021), in an immunosuppressed patient, for whom two SARS-CoV-2 strains were identified on Day 56. There is higher risk of prolonged viral shedding in immunosuppressed SARS-CoV-2 positive cases (Choi et al., 2020), which may explain the superinfection. Our patient was not immunosuppressed and superinfection was detected only one week after the identification of a single-strain infection in a previous specimen. Two coexisting SARS-CoV-2 strains have been also reported in a COVID-19 case who was reinfected 26 days after the first infection (Lee JS, 2020).

E1 was severe and identification of two coinfecting strains coincided with patient´s clinical deterioration, suggesting some clinical impact of superinfection on the severity of E1. On the other hand, E2, associated to a third different strain, was milder, similar to other reinfection reports (Van Elslande et al., 2020), with no consolidation on X-ray and mild hypoxemia. E2 infection resulted from a nosocomial exposure, as Strain 3 was responsible for a hospital outbreak involving at least 11 cases (0 SNPs among them) from three wards. An epidemiological survey confirmed that the health care worker who attended the patient who had shared a room with our case was also infected with the same strain (0 SNPs), suggesting a potential role in the reinfection.

Summarizing, we describe a COVID-19 case with unprecedented viral complexity in SARS-CoV-2 infection. Initially, the patient was superinfected by two different strains within a short period, followed months later by a COVID-19 reinfection by a third distinct strain.

Figure

Upper panel: maximum likelihood unrooted tree with genetic distances of the three identified strains (highlighted as coloured circles within the phylogenetic tree; Strain 1 in orange, Strains 1 + 2 in ochre, and Strain 3 in purple) based on sequence alignment of 2,249 positive SARS-CoV-2 samples from cases among the Madrid population throughout the pandemic. Coloured branches are used for SARS-CoV-2 sequences from specimens collected during the first COVID-19 wave (black) and after July 2020 (grey). The B.1.1.7 variant is showed purple.

Lower panel: location of single nucleotide polymorphisms along the SARS-CoV-2 reference genome for the three identified strains. Relative allele frequency of each strain (Strains 1 and 2; See also Supplementary table) in the superinfection event is indicated for the positions with heterozygous calls. Fasta files were deposited in GISAID (accession numbers strain 1 - EPI_ISL_1547368 strain 2 - EPI_ISL_1547369 and strain 3 - EPI_ISL_1547363).

Supplementary Figure

Short tandem repeat-PCR results on the specimens used for the SARS-CoV-2 RT-PCR (also sequenced). Twelve non-coding STR loci and the gender-specific locus amelogenin were examined.

Supplementary Table

Single nucleotide polymorphisms and allele frequencies for the three SARS-CoV-2 studied strains.

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Conflict of interest . The authors declare no conflicts of interest.

Ethical aspects

This study was approved by the ethical research committee of Gregorio Marañón Hospital (REF: MICRO.HGUGM.2020-042). Informed consent was obtained from the patient for the publication of this case report.

Data availability

The data that support the findings of this study (FastA files) are openly available

in GISAID at https://www.gisaid.org/ . Reference numbers (strain 1 - EPI_ISL_1547368 strain 2 - EPI_ISL_-1547369 and strain 3 - EPI_ISL_1547363).

Contribution statement

Laura Pérez Lago: Conception, Design, Analysis, Manuscript revision

Martha Kestler: data compilation, clinical data, epidemiological research.

Pedro J. Sola-Campoy: Bioinformatic analysis,

Cristina Rodriguez-Grande : methodology, investigation, data analysis

Rubén Francisco Flores-García : epidemiological reasearch, data analysis

Sergio Buenestado-Serrano Bioinformatic analysis,

Marta Herranz, Analysis, investigation

Luis Alcala: Data compilation, data analysis

Carolina Martínez-Laperche: data analysis , investigation

Julia Suárez-González : Data analysis, sequencing.

Pilar Catalán : data analysis, methodology, investigation

Patricia Muñoz : resources, manuscript revision

Darío García de Viedma: Conception, Design, Analysis, Manuscript writing

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