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Case Report

SARS-CoV-2 dual infection with Delta and Omicron variants in an immunocompetent host: a case report

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ABSTRACT

Despite the high number of SARS-CoV-2 infections, only a few cases of dual infection have been reported. Here, we describe a case of COVID-19 caused simultaneously by Delta and Omicron variants in an immunocompetent individual during the early emergence of Omicron variant. A 73-year-old man was hospitalized with suspected acute coronary syndrome and a positive test result for SARS-CoV-2 RNA was received during routine testing at the hospital. He experienced mild symptoms of COVID-19 and was discharged on the ninth day. We sequenced the SARS-CoV-2 whole genome from the sample obtained on admission. The viral sequence was classified as PANGO lineage B.1.1.10 by the Galaxy pipeline; however, on detailed manual analysis, we identified the presence of both Delta and Omicron variants. After excluding the possibilities of a recombinant virus or contamination in the sample, we confirmed the presence of dual infection in this patient. We highlight that dual infections with SARS-CoV-2 may be more common than expected but are difficult to detect during the waves of one dominant variant.

Dual infections (those caused by two different viral variants) have been described with RNA viruses but are rare among respiratory viral infections (Calistri *et al.*, 2011; Myers *et al.*, 2011). For SARS-CoV-2, few cases of dual infection have been reported, despite an extremely high number of persons being infected worldwide (Francisco *et al.*, 2021; Pedro *et al.*, 2021; Roychoudhury *et al.*, 2022; Samoilov *et al.*, 2021; Vankeerberghen *et al.*, 2021). However, pinning down these cases is complicated; they can be identified with higher probability during the transition from dominance of one SARS-CoV-2 variant of concern to another in the population. We describe a confirmed case of dual infection with Delta and Omicron variants in an immunocompetent patient during the early emergence of Omicron variant in Estonia.

A 73-year-old otherwise-healthy man was admitted to Tartu University Hospital on December 31, 2021 with suspected acute coronary syndrome after a syncope incident. The patient had a head wound (as a result of the fall) and a complaint of thoracic pain. He had mild symptoms suggestive of COVID-19. The initial measured oxygen saturation (SpO_2) was 88% in ambient air; SpO_2 increased to approximately 98% with 3 l/min oxygen supplementation. The initial pO_2 was 139 mmHg; pO_2/FiO2% was 3.48 mmHg/%.

On December 30, the patient's sample tested positive for SARS-CoV-2 RNA by quantitative polymerase chain reaction (TaqPath™ COVID-19 CE-IVD RT-PCR Kit, ThermoFisher Scientific) performed by SYNLAB Eesti Ltd. Cycle thresholds were 20.9, 20.6, and 18.3 for ORF1, S, and N genes, respectively.

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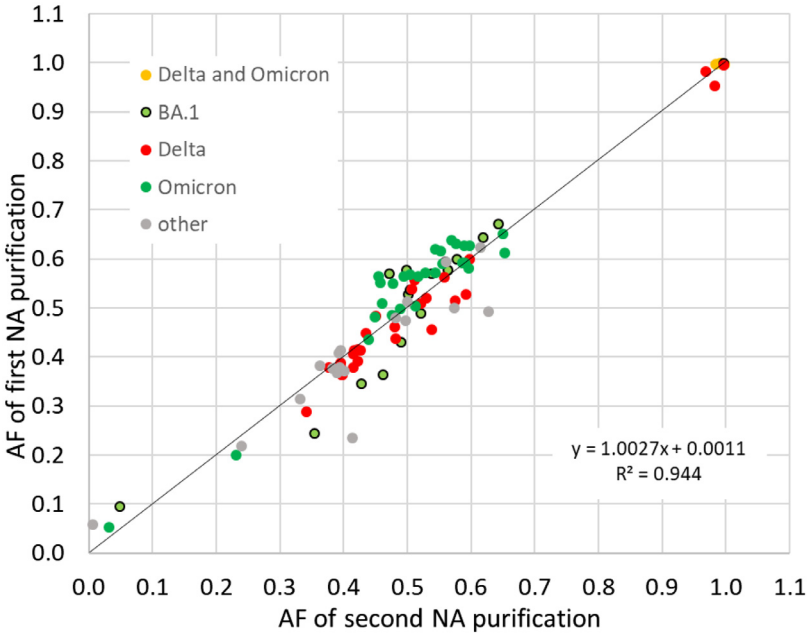

Figure 1. Correlation of variant AF between two NA purifications and NGS runs, indicating that there was no contamination during NA purification and NGS. A single circle represents one mutation. Mutations are color-coded according to specificity (presence in different variants).
AF, allele frequencies; NA, nucleic acid; NGS, next-generation sequencing

A second nasal swab was obtained on December 31, 2021, and the test results were confirmed positive for SARS-CoV-2 RNA by the hospital laboratory. Throughout the hospital stay, the patient presented no other remarkable symptoms. He required oxygen treatment for only the first two days of hospitalization (to resolve the respiratory symptoms). The patient recovered and was discharged on the ninth day. He had been vaccinated with two doses of the AstraZeneca COVID-19 vaccine (administered on April 9 and June 13, 2021).

The nucleic acid isolated (using QIAsymphony DSP Virus/Pathogen Kit) from the nasal swab obtained on December 31, 2021 was used to sequence the SARS-CoV-2 whole genome. Sequencing was performed with the Illumina COVIDSeq Test with ARTIC v4 primer pool on an Illumina NextSeq 500 System using a paired-end 75-base-pair library. Raw sequences were analyzed; PANGO lineage and Nextstrain clade were determined using workflows devised by the Galaxy Project (Maier *et al.*, 2021).

The sequenced sample was classified as PANGO lineage B.1.1.10, which was unexpected and rare at the time in Estonia (KoroGenoEST consortium, 2022). Through detailed evaluation of the sequence, we identified the presence of both Delta and Omicron variant fingerprints; allele frequencies were remarkably similar (approximately 0.5). This suggested a few possibilities: the presence of a recombinant virus, dual infection, contamination, or any combination of these.

We excluded the possibility of a recombinant strain by the detection of signature mutations of Delta and Omicron throughout the whole viral sequence and by the phasing of raw next-generation sequencing reads into two distinct buckets describing the independent strains and lack of any chimeric reads. We ruled out the possibility of cross-contamination by lack of Delta or Omicron signature-specific mutations in samples processed in the same laboratory for several weeks before and after the sample of interest was sequenced.

To exclude any cross-contamination during nucleic acid purification, the RNA from the nasal swab was repurified (QIAamp Viral RNA Kit) and resequenced (paired-end 150- base-pair reads). Variant calling was repeated side-by-side with the raw reads from the first round of sequencing. The high correlation between the two sequencing runs (Figure 1) reconfirmed the presence of the two viral strains in the initial swab sample.

To verify these results with an independent method, we performed Sanger-based single-genome sequencing of the SARS-CoV-2 S gene (amplicon: NC045512.2 nucleotides 21,358-23,823) (Eden *et al.*, 2020). Through sample dilution we obtained a total of 29 single- genome copies assignable to two distinct lineages (Figure 2), confirming the presence of two viral populations in the sample. Finally, we performed human genotyping (EstChip2-GSAv3-MD) on the samples, confirming the presence of DNA from only a single person in the background.

Thus, we conclude that the patient was simultaneously carrying two different variants of SARS-CoV-2 at the time of admission to the hospital.

The fact that independent teams (Francisco *et al.*, 2021; Pedro *et al.*, 2021; Roychoudhury *et al.*, 2022; Samoilov *et al.*, 2021; Vankeerberghen *et al.*, 2021) have also identified SARS-CoV-2 dual infections suggests that such infections are more prevalent than expected but are simply difficult to detect during waves of pandemic driven by one dominant variant. Most importantly, dual-infected people serve as hubs for recombination and selection of new strains. This is especially the case when immunocompromised individuals are involved, but is true with immunocompetent hosts as well. Recently, recombinants of BA.1 and BA.2 have been observed by us and others. Thus, we underline the emerging need for automated screening tools that help to pinpoint samples with dual infection and facilitate in-depth examination of those samples to disclose the true frequency and clinical relevance of dual infections.

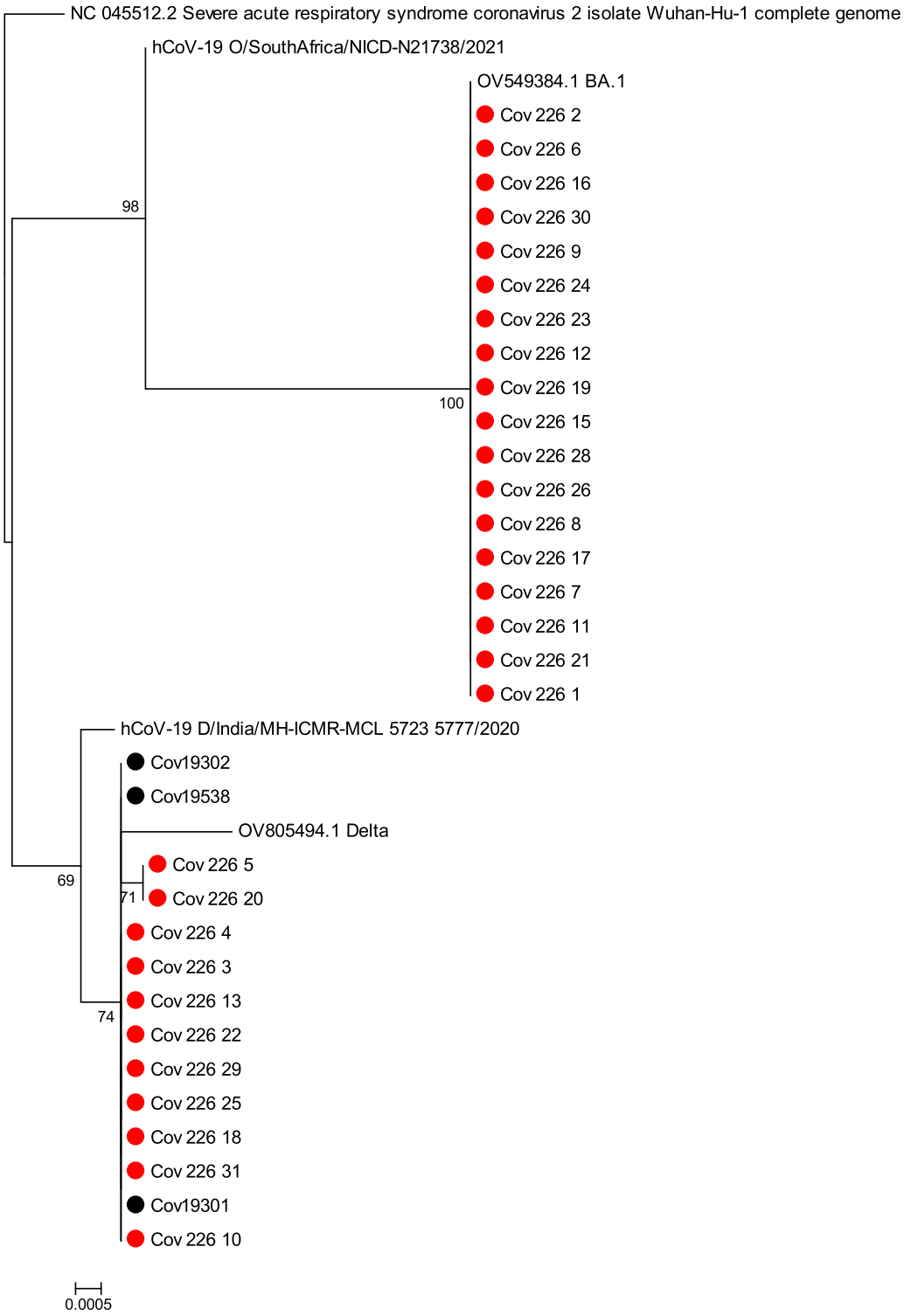

Figure 2. Neighbor-joining tree of 29 SARS-CoV-2 single genomes from the patient's sample (red circles) representing two distinct lineages of single genomes—one clustering with Omicron BA.1 and the other with Delta variant. Single-genome sequencing was carried out by complementary DNA dilution method; the complementary DNA was diluted until approximately 30% of the polymerase chain reactions yielded DNA product. The region was amplified (sequence length 2,310 base pairs; NC045512.2 nucleotides 21,466 to 23,775) using the protocol by Eden *et al.* (2020) and sequenced by Sanger method. The sequences were manually evaluated to exclude all contigs possessing polymorphisms.

Black circles indicate other Estonian Delta variants (closest to predicted Delta in the sample). Reference sequences were retrieved from the National Center for Biotechnology Information sequence database. Delta variants: hCoV-19_D/India/MH-ICMR-MCL_5723_5777/2020 and OV805494.1_Delta. Omicron variants: hCoV-19_O/SouthAfrica/NICD-N21738/2021 and OV549384.1_BA.1. Wuhan genome: NC_045512.2.

Author contributions

M.P., A.S., T.R., M.N. and I.T. performed experiments; A.A., K.H., U.G.T., M.N., and T.P. analyzed data; P.S., I.T. and H.N. provided materials and data; A.A., K.H., U.G.T. and I.L. wrote the paper. All authors read and approved the submitted version.

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Ethical approval

The case belonged to the study approved by the Ethics Committees of University of Tartu.

Declarations of competing interest

The authors have no competing interests to declare.

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