COVID-19: a confirmed case of reinfection in a nurse

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SUMMARY
We describe the case of a 63-year-old man who was reported to have the first confirmed case of COVID-19 reinfection in Campania Region, Italy. We found that the two episodes were caused by virus strains with clearly different genome sequences. The patient, a retired nurse, had a very low level of antibodies IgG directed against the spike protein 14 days after his first Pfizer/BioNTek vaccine shot.

BACKGROUND
Worldwide, several confirmed cases of COVID-19 reinfection have been documented. Perez et al estimated an incidence of COVID-19 reinfections of 1 per 1000 individuals in Israel, considering that, out of 149,735 individuals with a documented positive PCR test between March 2020 and January 2021, 154 had two positive PCR tests at least 100 days apart.

Reporting of reinfection cases can be difficult and their number underestimated since it is necessary to differentiate between a reinfection from a new coronavirus entering the body and a reactivation. This issue of viral reactivation or reinfection with a different strain can be resolved by sequencing of viral genome, but it is possible only if a sample, after the first episode has been obtained, kept and sequenced, and confronted with a second sample from the same patient, which had tested positive for COVID-19. The genomes of the viruses from the two samples need to be different for it to occur as a reinfection. Currently, case definition of COVID-19 reinfection is lacking, but ECDC guidelines suggest considering a suspected COVID-19 reinfection case when a positive PCR or rapid antigen test (RAT) sample follow a previous positive PCR or a previous positive RAT or a previous positive serology (anti-spike IgG Ab), after more than 60 days.

Kapoor et al. in patients with cancer, hypothesise that the oscillating positive/negative PCR reports could be a reactivation of a dormant virus, which is commonly seen in immunosuppressed subjects with viruses like cytomegalovirus, herpes and Ebstein-Barr virus.

To date, most of the documented SARS-CoV-2 reinfections were milder than first encounters with the virus, although some have been more harmful and people have died as a result (table 1). Unfortunately, in patients with malignancies too, the second viral attack (reinfection or reactivation) may be more severe than the previous one, in an unpredictable way. COVID-19 treatment is not changed if a reinfection or a reactivation is known to take place and then distinguishing between reinfection or reactivation is not clinically relevant for the single patient, but the knowledge that reinfection and reactivation both exist, can help in choosing the right public health policy. In fact, even if neutralising antibodies are generated in response to SARS-CoV-2, they do not confer lifelong immunity and this limits the efficacy of strategies based on the so-called ‘herd immunity’.

Here, we describe the case of a 63-year-old man who is reported to have the first confirmed case of COVID-19 reinfection in Campania Region, Italy. We found that the two episodes were caused by virus strains with clearly different genome sequences.

CASE PRESENTATION
A 63-year-old male patient first acquired COVID-19 infection in March 2020, working as a nurse in a surgical ward. At that time, he had no symptoms and proved positive for COVID-19 during an epidemiological testing (14 March 2020). He was not hospitalised but isolated for prevention of onward transmission, until he tested negative twice. He was quite well until 8 months later, even if his past medical history reported chronic obstructive pulmonary disease (COPD), type II diabetes, atrial fibrillation. He got his first shot of Pfizer vaccination (figure 1) on the 13 January 2021, as offered to all the nurses, included those who retired, like our patient did in the meanwhile. On the 26 January 2021, he was admitted in hospital for respiratory failure (PaO₂ 59 mm Hg, PaCO₂ 29 mm Hg, pH 7.44, lactate 1.7 mm/L, respiratory rate (RR) 35). He was afebrile, with a temperature of 36°C. Nasopharyngeal swab on the 26 January 2021 demonstrated the presence of SARS-CoV-2 RNA.

For the rapid worsening of his clinical presentation, the patient was admitted to our Intensive Care Unit (ICU), on his second hospital day. His pulse rate was 101 beats per minute, his blood pressure was 140/70 mmHg and his SatO₂ was 96% while he was breathing O₂ 90%, by non-invasive mechanical ventilation (facial mask, PEEP 10 cmH₂O; PS 10 cmH₂O).

The two specimens positive for SARS-CoV-2 were collected from the Salerno University Hospital Virology Lab and then analysed by whole viral genome sequencing using an amplicon panel. Illumina sequencing yielded 1 048 775 reads for the specimen collected in March 2020 (Sample A), and 3 239 835 reads for the specimen collected in January 2020 (sample B). Sequence data analysis revealed that the virus present in Sample A was a member of clade 20A (Clade GISAID O). Genomic sequence analysis identified
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13 variants, 6 leading to amino acid substitutions (figure 2, table 2). The virus in sample B was a member of clade 20E (EU1) (Clade GISAID GV) and presented 14 variants (figure 2, table 3), 5 leading to amino acid substitutions, including the mutation called A222V on the viral spike protein in the non-terminal domain (NTD), representative of 20E (EU1) clade. The clade 20E (EU1) was first identified in Spain at the end of June and spread successfully through Europe, accounting for the majority of sequences by autumn 2020. Nine SNVs (single-nucleotide variant) were shared between sample A and B (highlighted in table 2).

INVESTIGATIONS
At his ICU arrival, CT scan was positive (figure 3), with bilateral consolidation areas, multiple ground glass opacities, interlobular septa and intralobular lines thickening in both lungs; echocardiography showed left ventricular hypertrophy, with Ejection Fraction (EF) 60%, E/A ratio 0.8, Tricuspid Annular Plane Systolic Excursion (TAPSE) 10 mm, suggestive for cor pulmonale. Lymphocyte count was normal at admission and stayed within acceptable limits for the whole length of our patient’s stay in ICU. C reactive protein level was elevated (17.24 mg/dL) at hospital admission (26 January 2021); total serum IgM were 116 mg/dL on the ICU admission (27 January), while IgG anti spike (COV-2 IgG) were negative (39.2 AU/mL).

SARS-CoV-2 RT-PCR was performed using Allplex assay, following producer instructions and as described by Farfour et al.6 Viral whole genome sequencing and bioinformatics analysis: library preparation was performed using the CleanPlex SARS-CoV-2 FLEX Panel (Paragon Genomics, Hayward, California, USA) for target enrichment according to the manufacturer’s instructions. Briefly, multiplex PCR reactions were performed using 343 pairs of primers separated into two pools covering the entire genome of SARS-CoV-2. Illumina indexes were introduced by PCR. Library quality and concentration was assessed with 4200 TapeStation system using Agilent High Sensitivity (HS) DNA Kit (Agilent Technologies, Santa Clara, California, USA) and Qubit Fluorometer with the double-stranded DNA (dsDNA) HS Assay Kit (Life Technologies, Carlsbad, California, USA). Libraries were pooled in equimolar ratios to reach the recommended final concentration of 4 nM and sequenced on an Illumina MiSeq with 2 × 150 bp using V2 flowcell. The FASTQ files were checked for quality using FASTQC tool (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), and adapter sequences were removed with cutadapt V.3.2 using default parameters. The obtained reads were aligned on SARS-CoV-2 genome (primary assembly MN908947.3) with BWA.7 Mutations were called using Freebayes V.1.0.2,8 requiring a minimum coverage of 10X and low-confidence variants were removed with snippy-vcf_filter,9 setting the following parameters: minqual 100–mincov 10–minfrac 0.1. Consensus sequence were generated using bcftools.10 Annotation of variants were performed using COVID-19 genome annotator,11 while clade assignment was performed using COVIDEX12 and Nextclade.13–15 The two sequence are available in GISAID EpiCoV Database with the following GISAID Accession EPI_ISL_1361596 and EPI_ISL_1361597.

Quantitative determination of IgG antibodies against the spike receptor-binding domain of SARS-CoV-2 in serum was performed by the SARS-CoV-2 IgG II Quant assay (ABBOTT), a chemiluminescent microparticle immunoassay, according to producer instructions.

The patient’s relatives have provided written informed consent for publication.

TREATMENT
Interleukin-6 (IL 6) inhibitor (tocilizumab 8 mg/kg up to a maximum of 800 mg, followed by a second dose after 12 hours) and dexamethasone 6 mg/die intravenous were administered at ICU admission, but without success.

He was ventilated in prone position in spontaneous ventilation (facial mask, NIV) but after 4 days (31 January 2021) he was intubated and sedated, for the clinical worsening and his perceived suffering. He received standard therapy with neuromuscular blockade, prone positioning, and a trial of inhaled nitric oxide. Venovenous extracorporeal membrane oxygenation (ECMO) indications for this patient were collegially discussed, but in absence of local ECMO resources and of a regional ECMO referral networks to expedite patient

Table 1 Characteristics associated with reinfection with SARS-CoV2, modified from Lancet,1 with permission from Elsevier and Copyright clearance Centre

| Sex          | Age (years) | First infection | Second infection | Intervening period (days) |
|--------------|-------------|----------------|-----------------|--------------------------|
| Hong Kong    | Male        | 33             | Mild            | Asymptomatic             | 142                      |
| Nevada, USA  | Male        | 25             | Mild            | Hospitalised             | 48                       |
| Belgium      | Female      | 51             | Mild            | Milder                   | 93                       |
| Ecuador      | Male        | 46             | Mild            | Worse                    | 63                       |

Figure 1 Timeline of symptom onset, molecular diagnosis and ICU admission.
referral and mobile ECMO retrieval, it was desolately dropped.

OUTCOME AND FOLLOW-UP

The patient died after 11 days in ICU.

DISCUSSION

Our patient was an Italian nurse during the first COVID-19 wave and then an unfortunate patient when the second wave hit his/our country again. He was infected twice by two different strains. The second hit was lethal for him. We do not know why the virus in Sample B, first identified in Spain at the end of June and spread through Italy in autumn caused such a different clinical scenario in the same subject, who had no significant problems with the virus in sample A, a member of clade 20A (Clade GISAID O).

Prior to the emergence of the variant A222V, two SARS-CoV-2 vaccines based on ancestral spike proved highly effective.

| Table 2 | Variants identified in sample A and B compared with the reference genome |
|---------|-----------------------------------------------------------------------|
| Sample | Refpos | Ref | Var | Protein | Variant | Varclass | Annotation | Varname |
| Sample A | 241 | C | T | 5'UTR | 241 | extragenic | NA | S'UTR:241 |
|          | 445 | T | C | NSP1 | V60V | SNP_silent | Leader protein | NSP1:V60V |
|          | 884 | C | T | NSP2 | R27C | SNP | Non-Structural protein 2 | NSP2:R27C |
|          | 3037 | C | T | NSP3 | F106F | SNP_silent | Predicted phosphoesterase, papain-like proteinase | NSP3:F106F |
|          | 14 408 | C | T | NSP12b | P314L | SNP | RNA-dependent RNA polymerase, post-ribosomal frameshift | NSP12b:P314L |
|          | 15 911 | A | G | NSP12b | D815G | SNP | RNA-dependent RNA polymerase, post-ribosomal frameshift | NSP12b:D815G |
|          | 18 840 | A | G | NSP14 | A267A | SNP_silent | 3’-to-5’ exonuclease | NSP14:A267A |
|          | 19 007 | C | T | NSP14 | A323V | SNP | 3’-to-5’ exonuclease | NSP14:A323V |
|          | 20 355 | A | G | NSP15 | L245L | SNP_silent | endoRNAse | NSP15:L245L |
|          | 26 801 | C | G | M | L93L | SNP_silent | Membrane | M:L93L |
|          | 27 944 | C | T | ORF8 | H17H | SNP_silent | ORF8 protein | ORF8:H17H |
|          | 28 932 | C | T | N | A220V | SNP | Nucleocapsid protein | N:A220V |
|          | 29 645 | G | T | ORF10 | V30L | SNP | ORF10 protein | ORF10:V30L |
| Sample B | 241 | C | T | 5'UTR | 241 | extragenic | NA | S'UTR:241 |
|          | 445 | T | C | NSP1 | V60V | SNP_silent | Leader protein | NSP1:V60V |
|          | 916 | A | G | NSP2 | E37E | SNP_silent | Non-Structural protein 2 | NSP2:E37E |
|          | 3037 | C | T | NSP3 | F106F | SNP_silent | Predicted phosphoesterase, papain-like proteinase | NSP3:F106F |
|          | 6286 | C | T | NSP3 | T1189T | SNP_silent | Predicted phosphoesterase, papain-like proteinase | NSP3:T1189T |
|          | 9474 | T | C | ORF8 | H17H | SNP_silent | ORF8 protein | ORF8:H17H |
|          | 22 227 | C | T | S | A222V | SNP | Spike | S:A222V |
|          | 26 801 | C | G | M | L93L | SNP_silent | Membrane | M:L93L |
|          | 27 944 | C | T | ORF8 | H17H | SNP_silent | ORF8 protein | ORF8:H17H |
|          | 28 932 | C | T | N | A220V | SNP | Nucleocapsid protein | N:A220V |
|          | 29 645 | G | T | ORF10 | V30L | SNP | ORF10 protein | ORF10:V30L |

*The table shows for every mutation: the mutation position on the reference genome (refpos); the sequence at the mutation site, on the reference genome (ref) and on the sample (var); the protein affected by the mutation (protein); the mutation effect on the amino acid sequence (variant); the class of the mutation (varclass); the extended annotation of the protein region affected by the mutation (annotation); the full name of the variant (varname).

The common variants are highlighted in grey. The mutation patterns suggest that the patient was infected by SARS-CoV-2 on two separate occasions by a genetically distinct virus.

NA, not available.
**Table 3** Blood test results at hospital admission (26 January 2021)

| Parameter                         | Value       |
|----------------------------------|-------------|
| WCC (4.5–10 x 10^9/L)            | 4.83        |
| Neutrophil (40%–75%)             | 85.3        |
| Lymphocyte (20%–50%)             | 10.4        |
| Haemoglobin (130–160 g/L)        | 140.1       |
| Platelet (150–400 cells/L)       | 188         |
| Creatinine (0.6–1.27 mg/dL)      | 1.06        |
| Lactate dehydrogenase (135–500 U/L) | 524       |
| C reactive protein (<5.0 mg/L)   | 17          |

WCC, white cell count.

**Figure 3** Chest CT scan at ICU admission, the patient was on spontaneous non-invasive ventilation (facial mask).

No direct answer to this question, but in case of 20E (EU1), the variant appeared to have similar transmissibility and caused similar clinical presentations in Europe.5

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