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 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. 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.[1] Perez *et al*[2] 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.[3] Currently, case definition of COVID-19 reinfection is lacking, but ECDC guidelines,[3] 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*,[4] 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 subjetcs 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 (PaO_2 59 mm Hg, $PaCO_2$ 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 $SatO_2$ was 96% while he was breathing $O_2$90%, by non-invasive mechanical ventilation (facial mask, PEEP 10 cmH_20; PS 10 cmH_2O).

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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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

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,[5] 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 08, 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[7] 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 COVIDEX[12] 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

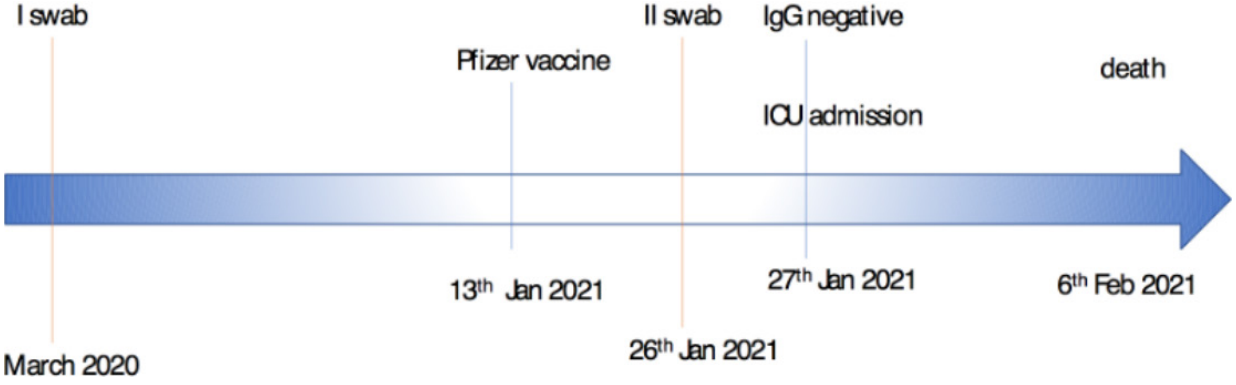

Figure 1 Timeline of symptom onset, molecular diagnosis and ICU admission.

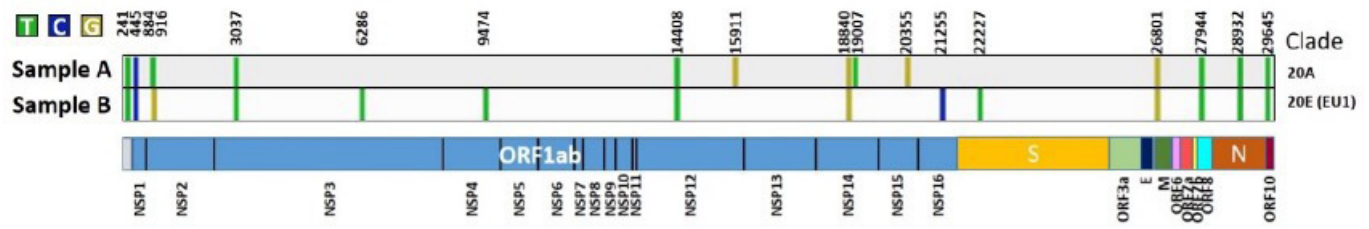

Figure 2 Genome annotation of variants in sample A and B compared with the reference genome Wuhan_Hu_1 (GeneBank MN908947). Variants identified are shown as coloured vertical bars (green, nucleotide change to T; blue, nucleotide change to C; yellow, nucleotide change to G), genomic position with respect to the reference genome are indicated at the top. The lower bar indicates the genomic organisation of SARS-Cov-2 and its encoded proteins.

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[15] 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

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*	*5'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*
	14408	*C*	*T*	*NSP12b*	*P314L*	*SNP*	*RNA-dependent RNA polymerase, post-ribosomal frameshift*	*NSP12b:P314L*
	15911	A	G	NSP12b	D815G	SNP	RNA-dependent RNA polymerase, post-ribosomal frameshift	NSP12b:D815G
	18840	*A*	*G*	*NSP14*	*A267A*	*SNP_silent*	*3'-to-5' exonuclease*	*NSP14:A267A*
	19007	C	T	NSP14	A323V	SNP	3'-to-5' exonuclease	NSP14:A323V
	20355	A	G	NSP15	L245L	SNP_silent	endoRNAse	NSP15:L245L
	26801	*C*	*G*	*M*	*L93L*	*SNP_silent*	*Membrane*	*M:L93L*
	27944	*C*	*T*	*ORF8*	*H17H*	*SNP_silent*	*ORF8 protein*	*ORF8:H17H*
	28932	*C*	*T*	*N*	*A220V*	*SNP*	*Nucleocapsid protein*	*N:A220V*
	29645	*G*	*T*	*ORF10*	*V30L*	*SNP*	*ORF10 protein*	*ORF10:V30L*
Sample B	*241*	*C*	*T*	*5'UTR*	*241*	*extragenic*	*NA*	*5'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	C	T	NSP4	A307V	SNP	Transmembrane protein	NSP4:A307V
	14408	*C*	*T*	*NSP12b*	*P314L*	*SNP*	*RNA-dependent RNA polymerase, post-ribosomal frameshift*	*NSP12b:P314L*
	18840	*A*	*G*	*NSP14*	*A267A*	*SNP_silent*	*3'-to-5' exonuclease*	*NSP14:A267A*
	21255	G	C	NSP16	A199A	SNP_silent	2'-O-ribose methyltransferase	NSP16:A199A
	22227	C	T	S	A222V	SNP	Spike	S:A222V
	26801	*C*	*G*	*M*	*L93L*	*SNP_silent*	*Membrane*	*M:L93L*
	27944	*C*	*T*	*ORF8*	*H17H*	*SNP_silent*	*ORF8 protein*	*ORF8:H17H*
	28932	*C*	*T*	*N*	*A220V*	*SNP*	*Nucleocapsid protein*	*N:A220V*
	29645	*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)

	Values
WCC (4,5-10 × 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.

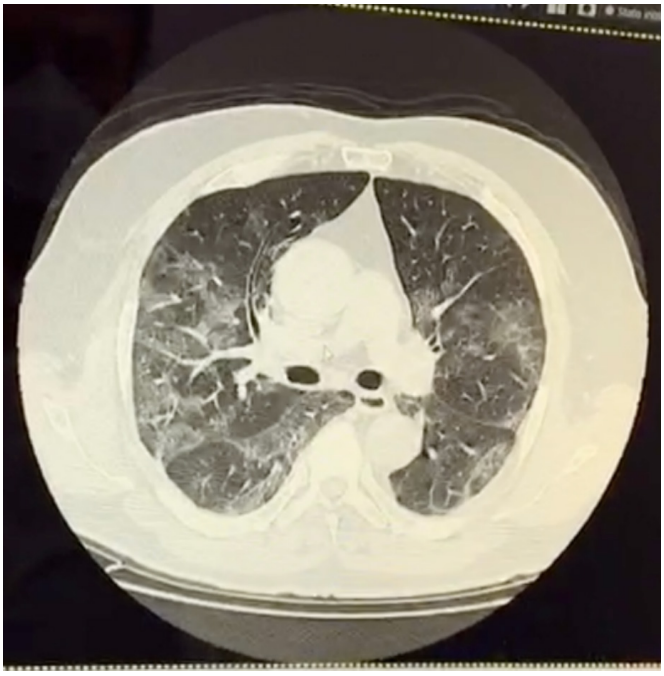

Figure 3 Chest CT scan at ICU admission, the patient was on spontaneous non-invasive ventilation (facial mask).

effective (the Moderna mRNA-1273 vaccine and the one received by our patient- the first dose out of two of the mRNA vaccine developed by Pfizer/BioNTek). When the patient was admitted to our ICU, 14 days after his vaccine shot, had a very low level of antibodies IgG directed against the spike protein. It is not known what level of neutralisation is required for the efficacy of Pfizer/BioNTech mRNA vaccine, even if it demonstrated substantial efficacy prior to the second (final) dose.

Reporting the unfortunate case of this patient, the Authors signal that we, as citizens exposed to the risk of infection and physicians in care of COVID-19 19 patients, deeply need the work of dedicated genomic labs that provide useful systems for genomic situation reporting globally, in order to understand regional outbreaks and variants, that later may become dominant because of some selective advantage. SARS-CoV-2 variants may hypothetically render vaccines less effective (vaccine escape), or being associated with differences in symptoms and disease course. May the difference between the two variants reported in our patient (clade 20A (Clade GISAID O) and clade 20E (EU1) (Clade GISAID GV, the SARS-CoV-2 variant with the A222V substitution in the spike glycoprotein) mediate an altered immune response, which could explain such different clinical scenarios in the same subject? Unfortunately, we have 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]

Learning points

- Consider a suspected COVID-19 reinfection case when a positive PCR follows a previous positive PCR after more than 60 days.
- Even if most of the documented SARS-CoV-2 reinfections were milder than first encounters with the virus, they can be letal.
- It is not known what level of antispike IgG is required for the efficacy of Pfizer/BioNTech mRNA vaccine.

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REFERENCES

1 Iwasaki A. What reinfections mean for COVID-19. *Lancet Infect Dis* 2021;21:3–5.
2 Perez G, Banon T, Gazit S. 1 to 1000 SARS-CoV-2 reinfection proportion in members of a large healthcare provider in Israel: a preliminary report. *MedRxiv* 2021.
3 European Centre for Disease Prevention and Control. *Reinfection with SARS-CoV: considerations for public health response*. ECDC, 2020.
4 Kapoor R, Nair RK, Nayan N. Reinfection or reactivation of Coronavirus-19 in patients with hematologic malignancies: case report series. *SN Compr Clin Med* 2021:1–5.
5 Hodcroft EB, Zuber M, Nadeau S. Emergence and spread of a SARS-CoV-2 variant through Europe in the summer of 2020. *medRxiv* 2020.
6 Farfour E, Lesprit P, Visseaux B, *et al*. The Allplex 2019-nCoV (Seegene) assay: which performances are for SARS-CoV-2 infection diagnosis? *Eur J Clin Microbiol Infect Dis* 2020;39:1997–2000.
7 Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet* 2011.
8 Li H, Durbin R, Richard D. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 2009;25:1754–60.
9 Garrison E, Marth G. Haplotype-based variant detection from short-read sequencing. *arXiv* 2012.
10 Seemann T. Snippy: fast bacterial variant calling from NGS reads. Available: https://github.com/tseemann/snippy
11 Li H. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics* 2011;27:2987–93.
12 Mercatelli D, Triboli L, Fornasari E, *et al*. Coronapp: a web application to annotate and monitor SARS-CoV-2 mutations. *J Med Virol* 2021;93:3238–45.
13 Cacciabue M, Aguilera P, Gismondi MI. Covidex: an ultrafast and accurate tool for virus subtyping. *BioRXiv* 2020.

14 Hadfield J, Megill C, Bell SM, *et al*. Nextstrain: real-time tracking of pathogen evolution. *Bioinformatics* 2018;34:4121–3.

15 Hodcroft EB HJ, Neher RA, Bedford T. *Year-letter genetic clade naming for SARS-CoV-2 on Nextstain.org*, 2020.