SARS-CoV-2 re-infection in a cancer patient with a defective neutralizing humoral response

Cinzia Borgogna[1], Marco De Andrea[2,3], Gloria Griffante[1], Alessia Lai[4], Annalisa Bergna[4], Massimo Galli[4], Gianguglielmo Zehender[4], Luigi Castello[5], Paolo Ravanini[6], Carlo Cattrini[7], Alessia Mennitto[7], Alessandra Gennari[7], and Marisa Gariglio[1].

[1] Virology Unit, Department of Translational Medicine, University of Piemonte Orientale, Novara, Italy

[2] Viral Pathogenesis Unit, Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy

[3] CAAD-Center for Translational Research on Autoimmune and Allergic Disease, University of Piemonte Orientale, Novara, Italy.

[4] Department of Biomedical and Clinical Sciences L. Sacco, University of Milan, Milan, Italy.

[5] Division of Internal and Emergency Medicine, Department of Translational Medicine, University of Piemonte Orientale and "Maggiore della Carità" Hospital, Novara, Italy.

[6] Unit of Microbiology and Virology, Department of Laboratory Medicine, "Maggiore della Carità" Hospital, Novara, Italy.

[7] Division of Oncology, Department of Translational Medicine, University of Piemonte Orientale and "Maggiore della Carità" Hospital, Novara, Italy.

Running Title: SARS-CoV-2 re-infection in a cancer patient

Keywords: SARS-CoV-2, Re-infection, Neutralization humoral response, Cancer patient.

Corresponding author: Marisa Gariglio, MD, PhD
University of Piemonte Orientale
Dept. of Translational Medicine
Via Solaroli, 17 - 28100 Novara (ITALY)
tel ++39 0321 660649
e-mail marisa.gariglio@med.uniupo.it

Text

To the Editor,

Since August 2020, a series of cases of reinfection by a phylogenetically distinct variant of SARS-CoV-2 have been reported, raising pertinent questions on the heterogeneity of the natural immune response to SARS-CoV-2 infection that may not uniformly confer protective immunity to all individuals.[1-5] Specifically, reinfection seems more likely to occur in individuals whose immune system has been weakened by underlying comorbidities or therapies.[6-8] Here we report a case of a 52-year-old male patient suffering from transitional cell carcinoma of the renal pelvis and ureter who was infected at two separate times with two genetically distant SARS-CoV-2 strains, with reappearance of the first strain four months after the first infection. The patient's past medical history and treatments are summarized in Supplementary Figure 1. On June 23, 2020 (day 0), he had cough and fever and was diagnosed with COVID-19 by SARS-CoV-2 reverse transcriptase-polymerase chain reaction (RT-PCR) assay of a nasopharyngeal swab specimen (cycle threshold, Ct, values for SARS-CoV-2 E, RdRp, and N genes ranged from 25 to 26) (Figure 1A). Chest X-ray did not reveal any abnormality, and his clinical conditions improved with resolution of cough and fever within two weeks. On day 35 and 36, two consecutive nasopharyngeal swabs resulted negative for SARS-CoV-2 infection. In the next few months, the patient did not show any respiratory symptoms. However, the deterioration of his cancer condition leading to urinary tract infection and sepsis required further hospitalization. On day 110, the patient had fever caused by an ongoing *Escherichia coli*-induced sepsis. RT-PCR assay of a nasopharyngeal swab resulted positive again, causing concern for a recurrence of COVID-19 (Ct values of 34 and 36 for E and N genes, and over 40 for the RdRp gene). An abdominal CT scan performed on day 113 showed thrombosis of the inferior vena cava, of the right iliac vein, and of both femoral veins. On day 115, the patient died from septic shock and respiratory failure.

Quantitative SARS-CoV-2 viral loads by droplet digital PCR detected 546, 1, and 53 copies/μl on day 0, 110, and 115 nasopharyngeal swabs, respectively (Figure 1A). Whole genome sequencing

and phylogenetic analysis of RNA from the first two specimens showed that the viral genome found at day 0 could be grouped in the Nextstrain clade 20B and Pangolin lineage B.1.1, while the strain isolated on day 110 belonged to the Nextstrain clade 20A and Pangolin lineage B.1 (Figure 1B). However, when we sequenced the RNA from the third sample harvested on day 115, we detected again the Nextstrain clade 20B, suggesting that the first infection strain had never been cleared completely. With regard to amino acid changes, by analyzing minority variants in the day 115 specimen, the mutations R203K and G204R, which distinguish B.1 and B.1.1 lineages, were the predominant ones until 65% coverage, but below this cut-off we were also able to detect significant levels of the wild-type virus (Figure 1C). Furthermore, the D614G variant was always present in specimens isolated on day 110 and 115, whereas it was absent, even as a minority variant, in the specimen harvested on day 0. No evidence of recombination events was observed. Phylogenetic analysis was congruent with both persistent infection with B.1.1 strains (specimens from day 0 and 115) and re-infection with B.1 strain on day 110.

The patient's blood was only available on day 110 and resulted negative for the presence of SARS-CoV-2 genome by droplet digital PCR analysis. Rapid immunochromatographic test on blood resulted positive for IgG anti-SARS-CoV-2 N protein. Very low levels of IgG anti-SARS-CoV-2 spike protein were found in this sample (1200 AU/ml with the low threshold < 2.544 AU/ml). In addition, anti-receptor binding domain (RBD) antibodies were determined by a different ELISA, which confirmed the presence of a very low reactivity. Consistently, the neutralizing activity performed using the replication-competent chimeric VSV expressing the SARS-CoV-2 spike protein (rVSV-SARS-CoV-2-SΔ21) was very low when compared to convalescent positive control. The inhibitory concentration (IC_{50}) of the positive control was 0.0007 while the patient' serum was a thousand times less potent (IC_{50}: 0.1) and barely reached 50% of neutralization at the lowest serum dilution of 1:10 (Figures 1D and S2).[9] The fact that VSV harbors the D614 form of the spike protein—the same found in the strain isolated on day 0—and that the G614 form is reportedly unable to interfere with the

neutralizing titer[10] rules out any detection bias of our approach, indicating that the patient did fail to mount an appropriate neutralizing humoral response.

Overall, this case highlights the concerning risk of re-infection in cancer patients who fail to mount an efficient neutralizing humoral response along with the underlying existence of persistent asymptomatic/undetectable infection.

Acknowledgements: We thank Marcello Arsura for critically reviewing the manuscript.

Financial support: This study was funded by the AGING Project – Department of Excellence – DIMET, Università del Piemonte Orientale.

Potential conflict of interest: The authors declare no conflict of interest.

Author contributions: Methodology: Gloria Griffante; Resources: Paolo Ravanini, Luigi Castello, Carlo Cattrini, Alessia Mennitto; Investigation: Gloria Griffante, Alessia Lai, Annalisa Bergna, Paolo Ravanini; Data curation: Cinzia Borgogna, Marco De Andrea; Formal analysis: Cinzia Borgogna, Gloria Griffante, Alessia Lai, Annalisa Bergna; Visualisation: Cinzia Borgogna, Marco De Andrea; Conceptualisation: Marisa Gariglio, Alessandra Gennari; Writing– original draft: Cinzia Borgogna, Marisa Gariglio; Writing – review & editing: Marco De Andrea, Alessia Lai, Massimo Galli, Gianguglielmo Zehender, Luigi Castello, Carlo Cattrini, Alessia Mennitto, Alessandra Gennari; Supervision: Marisa Gariglio, Massimo Galli, Gianguglielmo Zehender.

Figure legend

Figure 1. A) Timeline of clinical presentations and SARS-CoV-2 testing, including viral loads (copies/μl) and the strains found in the study patient. Timing of relevant clinical events, such as outcome of diagnostic tests, is shown; **B)** Phylogenomic analyses of described SARS-CoV-2 strains in the study patient. The tree was constructed by the maximum likelihood method. Clade information as inferred by Nextstrain and Pangolin nomenclatures is shown; **C)** Viral genome classification and amino

acid mutations identified according to Nextclade and Pangolin among the three specimens harvested on day 0, 110, and 115; **D)** Serum neutralizing assay against rVSV-SARS-CoV-2-SΔ21 with a sample harvested at day 110. Data are representative of two independent experiments performed in duplicate. Error bars represent the standard deviation. Patient (blue dot), normal human serum (Neg) (black triangle), positive serum = COVID-19 convalescent serum (Pos) (red square).

References

1. Tillett RL, Sevinsky JR, Hartley PD, Kerwin H, Crawford N, Gorzalski A, et al. Genomic evidence for reinfection with SARS-CoV-2: a case study. Lancet Infect Dis. 2020;21:52-58.
2. Prado-Vivar B, Becerra-Wong M, Guadalupe JJ, Márquez S, Gutierrez B, Rojas-Silva P, et al. A case of SARS-CoV-2 reinfection in Ecuador. Lancet Infect Dis. 2020;23:S1473-3099(20)30910-5.
3. Gupta V, Bhoyar RC, Jain A, Srivastava S, Upadhayay R, Imran M, et al. Asymptomatic reinfection in two healthcare workers from India with genetically distinct SARS-CoV-2.Clin Infect Dis. 2020:ciaa1451. doi: 10.1093/cid/ciaa1451.
4. To KK, Hung IF, Ip JD, Chu AW, Chan WM, Tam AR, et al. COVID-19 re-infection by a phylogenetically distinct SARS-coronavirus-2 strain confirmed by whole genome sequencing. Clin Infect Dis. 2020;ciaa1275. doi: 10.1093/cid/ciaa1275.
5. Van Elslande J, Vermeersch P, Vandervoort K, Wawina-Bokalanga T, Vanmechelen B, Wollants E, et al. Symptomatic SARS-CoV-2 reinfection by a phylogenetically distinct strain Clin Infect Dis. 2020; ciaa1330. doi: 10.1093/cid/ciaa1330.
6. Choi B, Choudhary MC, Regan J, Sparks JA, Padera RF, Qiu X, et al. Persistence and evolution of SARS-CoV-2 in an immunocompromised host. N Engl J Med. 2020;383:2291-2293.
7. Avanzato VA, Matson JM, Seifert SN, Pryce R, Williamson BN, Anzick SL, et al. Case study: Prolonged infectious SARS-CoV-2 shedding from an asymptomatic immunocompromised individual with cancer. Cell. 2020;183:1901-1912.e9.

8. Babiker A, Marvil C, Waggoner JJ, Collins MH, Piantadosi A. The importance and challenges of identifying SARS-CoV-2 reinfections. J Clin Microbiol. 2020;59(4):e02769-20.
9. Case JB, Rothlauf PW, Chen RE, Liu Z, Zhao H, Kim AS, et al. Neutralizing antibody and soluble ACE2 inhibition of a replication-competent VSV-SARS-CoV-2 and a clinical isolate of SARS-CoV-2. Cell Host Microbe. 2020;28:475-485.e5.
10. Zou J, Xie X, Fontes-Garfias CR, Swanson KA, Kanevsky I, Tompkins K, et al. The effect of SARS-CoV-2 D614G mutation on BNT162b2 vaccine-elicited neutralization. NPJ Vaccines. 2021;6:44.