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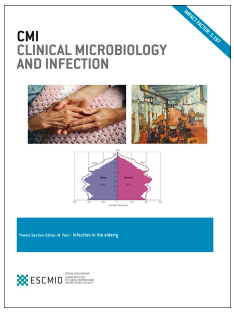

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LETTER

Persistent SARS-CoV-2 infection with repeated clinical recurrence in a patient with common variable immunodeficiency

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To the Editor,

A 22-year-old man with common variable immunodeficiency (CVID) complicated with granulomatous–lymphocytic interstitial lung disease (GLILD) previously treated with azathioprine and a 4-weekly course of rituximab 3 years before who was receiving subcutaneous (SC) immunoglobulin replacement therapy (IRT) was diagnosed of COVID-19 by SARS-CoV-2 reverse-transcriptase-polymerase-chain-reaction (RT-PCR) of a nasopharyngeal swab specimen after a 4-day history of fever. He quarantined at home but was later admitted to the hospital with COVID-19 bilateral pneumonia and hypoxemia on day 20. He was treated with ceftriaxone, dexamethasone, and an extra dose of SC-IRT, and was discharged on day 27 with positive RT-PCR assay of a nasopharyngeal swab (Ct value 30.1, Fig. 1B and 2B). The patient did not develop serologic response at any time.

On day 38, he was readmitted for relapsing fever and dyspnoea. As SARS-CoV-2 RT-PCR assay negativized, he was diagnosed of nosocomial pneumonia and received treatment with piperacillin-tazobactam for 7 days. However, on day 55, hypoxemia and new bilateral pulmonary infiltrates developed, requiring hospital admission and supplemental oxygen. A bronchoalveolar-lavage specimen on day 57 revealed an RT-PCR Ct value of 27.2 (Fig. 1B). A transbronchial biopsy was performed, detecting tissue SARS-CoV-2 RNA. After treatment with piperacillin-tazobactam, azithromycin, and dexamethasone, he was discharged without need for supplemental oxygen and infiltrate resolution.

The last admission for fever, radiologic progression, and mild hypoxemia was on day 72, showing again positive RT-PCR assay of a sputum specimen on day 77. The patient received a 10-day course of remdesivir, convalescent plasma therapy, dexamethasone, and meropenem, with complete clinical and radiographic resolution. Subsequent RT-PCR assays were negative in both upper and lower respiratory samples. No anti-SARS-CoV-2 monoclonal antibodies were available.

Samples on days 4, 26, and 57 were sequenced given the suspected relapse or reinfection. Phylogenetic analysis confirmed persistent infection after a single infection event with clinical relapse. All three isolates belonged to lineage B.1.416.1 and formed a common clade. They showed seven mutations with respect to the reference sequence, including one non-coding mutation, one synonymous SNPs, and five non-synonymous SNPs in nsp6, nsp12, and spike (Fig. 1B, Table S4). Intra-host dynamics were revealed by

nineteen single-nucleotide-polymorphisms (SNPs) that varied in frequency between sampling times where all but one SNPs showed intermediate frequencies in at least one of the samples (Fig. 1B). The temporal dynamics observed were not compatible with increased diversity corresponding to higher viral load, as previously described in secondary humoral immunocompromised patients,[1] because sampling points 2 and 3 showed a higher number of polymorphic sites and higher Ct values (Fig. 1B and 2A). Two of the SNPs in the spike gene arising in the second sampling time have been also described in the lineage B.1.1.7 (Alpha) but they did not become fixed in the population. The sequence on day 57 owned the largest number of fixed changes. Plasma immunoglobulin levels were normal (Fig. S1C), but viral-induced low B and T-cell counts (Table S3) were revealed by flow cytometry analysis.

Interestingly, accelerated viral evolution was observed in this case as previously described in secondary humoral immunocompromised patients[1-4], due to mutations without recombination evidence seen in other coronaviruses such as HCoV-OC43. This fact may advocate for immunocompromised patients as important contributors in generating viral genetic diversity and emergence of new variants, especially after confirming its selective active persistence in the lower respiratory tract with negative nasopharyngeal swab samples, as recently suggested in a report of a patient with X-linked agammaglobulinemia[5]. Furthermore, the clinical relapses of this patient without detectable SARS-CoV-2 in the upper airways may imply that standard nasopharyngeal swab samples are not adequate in patients with primary immunodeficiencies (PID) and, perhaps, also in secondarily immunocompromised patients. Despite plasma Rt-PCR is not routinely performed, it should be considered in B-cell depleted patients, especially after Hueso et al[5] effectively monitored RNAemia and correlated it with clinical improvement after early convalescent plasma therapy. Additionally, treatment with the combination of remdesivir and hyperimmune plasma was able to finally eradicate this reservoir as also reported by Palomba and colleagues[6]. However, the selection pressure after plasma therapy in patients with PID with reduced inherent clearance capability could potentially give raise to escape mutations with reduced susceptibility to polyclonal neutralizing antibodies[2]. However, early plasma therapy should be considered in patients unable to elicit a specific humoral response against SARS-CoV-2 as previously demonstrated in B-cell depleted patients with protracted COVID-19[5]. Further information is needed on viral intra-host dynamics

in immunocompromised patients with prolonged viral shedding for clinical management, public health purposes, as well as understanding the arising of new variants of concern (VOC).

This report highlights the potential capability of SARS-CoV-2 of causing prolonged relapsing infections in patients with treated PID and its associated accelerated within-host genomic evolution, as well as the role of the combination of remdesivir and convalescent plasma therapy in eliminating its reservoir in the lower respiratory tract. [3,4, 5]

Figure legends

Figure 1. Whole-genome viral sequencing of subsequent SARS-CoV-2 patient's isolates during hospital admissions

(A). Maximum-likelihood phylogeny of 191 SARS-CoV-2 genomes rooted with reference sequence from Wuhan. Bootstrap values ranging from 70 to 100 are represented in nodes as circles whose size is proportional to the value. Branches are colored by identified PANGO lineages. Branches leading to the three sequences of interest are colored with red and marked as a red star. The scale bar indicates the number of nucleotide substitutions per site. (B). Intra-host variation along three sampling times and its corresponding Cycle thresholds (Ct) values detected for N gene. Nonsynonymous changes are plotted in panels on the left and synonymous changes on the right. Upper panel shows non-fixed SNPs in the first sample, middle panel shows non-fixed SNPs in the second sample, and lower panel shows non-fixed and fixed SNPs in the third sample.

Figure 2. Aminoacidic replacements in the sequenced samples and timeline of the SARS-CoV-2 infection course in our patient

(A). Amino acid replacements found in at least one of three analyzed samples indicated on a graphical representation of the SARS-CoV-2 genome. Amino acid replacements in black indicate those fixed in at least one of the samples, in gray those that only appeared transitorily but did not get fixed. Underlined amino acid replacements are the ones shared by all three sampling points. (B). Timeline of patient's clinical evolution, hospital admissions, virological studies, and treatments. BAL; bronchoalveolar lavage.

Conflict of interest

Authors report no conflicts of interest for the present work.

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Author contributions

MDCN, VGB and PMM conceived the idea, searched the bibliographic materials, reviewed the existing literature, and wrote the article. PRR, IC and MC developed the figures, aided in the search of the bibliographic materials, and contributed to the writing of the article. LMP sequenced the samples. JTP reviewed the literature and contributed to the writing of the article. VGB and PMM were responsible for the care of the patient during hospital admissions. PMM supervised the work. All authors provided critical feedback and helped shape the final version of the manuscript.

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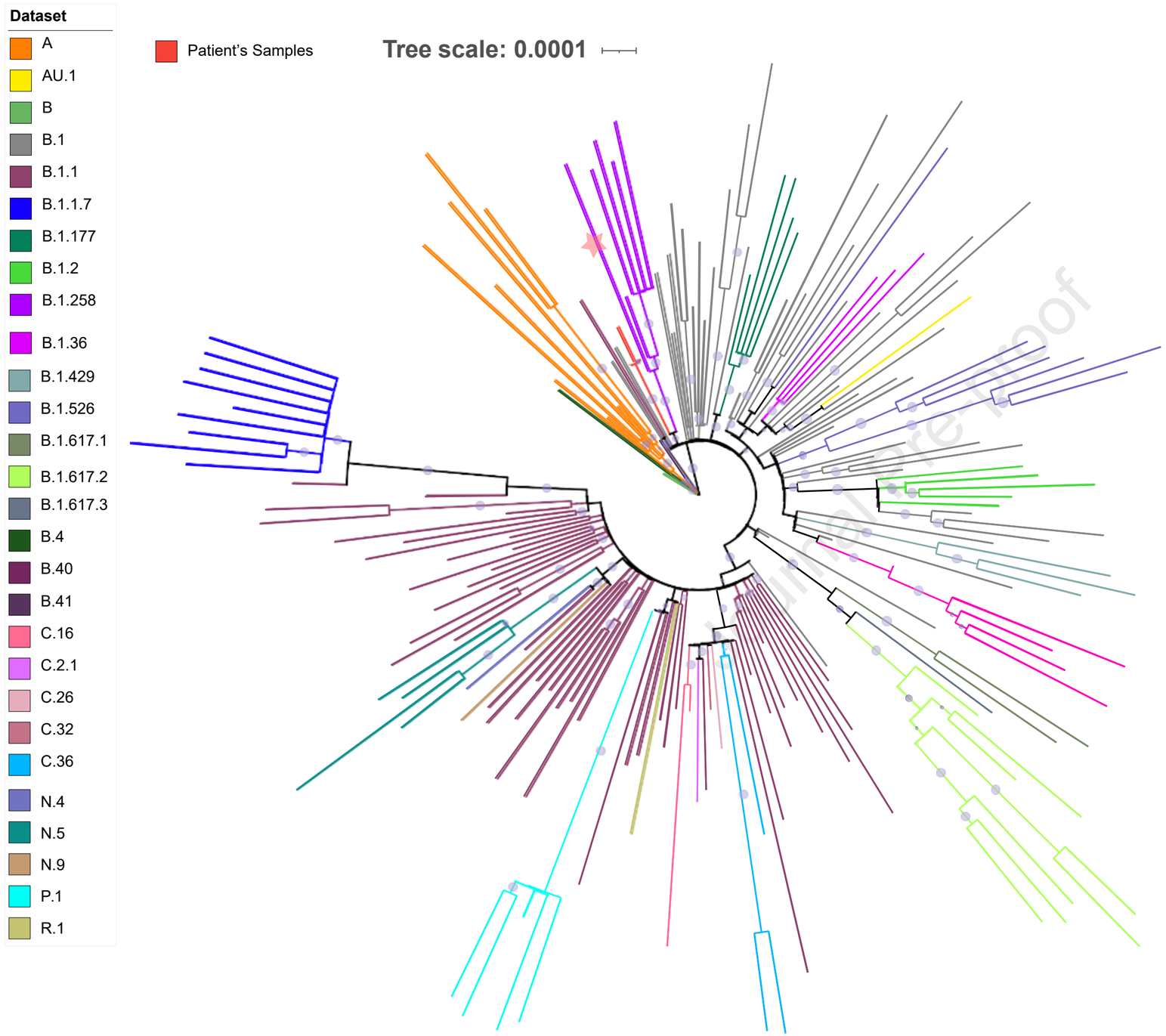

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