Longitudinal study of a SARS-CoV-2 infection in an immunocompromised patient with X-linked agammaglobulinemia

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Abstract

We describe the case of a 23-year-old immunocompromised male patient with clinically diagnosed X-linked agammaglobulinemia who was admitted to the hospital on the 14th April 2020 due to coronavirus disease of 2019 (COVID-19). Despite COVID-19 test negativizations, the patient was hospitalized most of the time and finally admitted to the intensive care unit where he died from multiorgan failure and shock. Over 149 days, 26 respiratory samples were collected, subjected to viral genome sequencing, and all assigned to the same lineage, supporting a single viral infection event. The accumulation of mutations throughout the course of the infection was accelerated and suggested the presence of compartmentalized viral subpopulations that evolved independently in the upper and lower respiratory airways. These results support that long-term viral shedding in immunocompromised patients is one possible mechanism for the emergence of variants of concern and provide evidence towards the infection control guidelines in these patients.

Main Text

A 23-year-old immunocompromised male patient with clinically diagnosed X-linked agammaglobulinemia (XLA) (**Supplementary Material**), who was treated monthly with intravenous immunoglobulin, was admitted to the hospital on the 14th April 2020 with left bilobar pneumonia likely due to coronavirus disease 2019 (COVID-19). At the time of admission, the patient reported cough and chronic diarrhoea, fever over the last four days, and a nasopharyngeal (NP) swab sample testing positive for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by RT-qPCR (**Figure 1A**). The patient was immediately treated with antibiotics (azithromycin, ceftazidime) and compassionate use of hydroxychloroquine (12-day course). On day 16, four days after the end of the hydroxychloroquine treatment, the RT-qPCR assay on NP swab was negative. On day 18, the assay was positive again turning back negative three days later. From day 21 to 44, RT-qPCR assays of five NP swab samples were negative for SARS-CoV-2. On day 24, the patient developed respiratory symptoms, including cough, and, following computed tomography (CT), he was diagnosed with viral pneumonia with bacterial superinfection. Despite clinical findings, no microbial pathogens were isolated from the bronchoalveolar lavage (BAL) samples. He was given another course of antibiotics (meropenem, linezolid and co-trimoxazole (a combination of trimethoprim and sulphamethoxazole)), antifungal treatment (amphotericin B), and intravenous glucocorticoids (methylprednisolone). On day 31, following the detection of SARS-CoV-2 in a BAL sample (day 29), the patient received the antiviral drug remdesivir for compassionate use (10-day course).

On day 45, since he was asymptomatic and had a negative RT-qPCR from a NP swab sample suggestive of a resolved infection, he was discharged from the hospital. However, the following day, he returned to the hospital reporting fever and bleeding from his right ear. He was again admitted to the hospital and tested for SARS-CoV-2 due to concern for a possible COVID-19 recurrence. The RT-qPCR of a NP swab sample was positive on day 47. From this moment onwards, the patient was persistently positive for SARS-CoV-2 infection. Infectious SARS-CoV-2 was also successfully cultured from a BAL sample on day 50, showing that the virus was actively replicating in the lower respiratory airways of the patient

(Supplementary Material). The patient was given another course of antibiotics (cefepime, meropenem, linezolid, piperacillin/tazobactam, and prophylactic co-trimoxazole), antifungal treatments (amphotericin B, voriconazole), intravenous (methylprednisolone, dexamethasone) and oral (prednisone, dexamethasone) corticosteroids, and a second course of remdesivir (8-day course from day 103). Since no clinical improvement was observed one week after the remdesivir antiviral treatment, he was given a cocktail of the HIV (human immunodeficiency virus) protease inhibitors lopinavir/ritonavir in addition to ceftazidime, a third-generation cephalosporin beta-lactam antibiotic. No other pathogens were detected from respiratory samples up to this moment **(Supplementary Material)**. On day 128, given continuous respiratory decline characterised by dyspnea and tachypnea, the patient was admitted to the intensive care unit (ICU) **(Supplementary Figure 1)**. On day 131, he was administered hyperimmune serum from a COVID-19 convalescent donor. On day 133, after many desaturation events despite high-flow nasal cannula therapy, the patient was provisioned with non-invasive mechanical ventilation. On the same day, *Pseudomonas aeruginosa* was isolated in a throat swab from multidrug-resistant surveillance cultures and the patient received treatment with ceftolozane/tazobactam. Three days later, he underwent endotracheal intubation because of respiratory failure. From this moment onwards, the patient was sedated and mechanically ventilated. On day 147, since blood culturing showed the growth of a Gram-negative pathogen, which was later identified as being multidrug-resistant *P. aeruginosa*, he was given colistin (polymyxin E) and meropenem antibiotics, as a last-resort treatment. On day 149 (10th September 2020), he died from multiorgan failure and shock.

Throughout the period described, 26 respiratory samples were collected from the patient (22 NP swabs and 4 BAL samples) **(Figure 1A)**. Whole SARS-CoV-2 viral genomes of a subset of 13 NP and 3 BAL samples were sequenced using either Illumina or Oxford Nanopore Technologies sequencing **(Supplementary Material)**. A urine, faeces, and peripheral blood sample (on day 44), and an additional peripheral blood sample (on day 87) were collected, but viral genome was not detected in any of their RNA extractions. All genome sequences were assigned to the PANGO lineage A.2 (Clade 19B), which was predominant in Spain during the early months of the pandemic [1]. Assignment of the sequences to the same lineage suggests that the patient had a single viral infection event. Synonymous and non-synonymous mutations accumulated throughout the course of the infection in NP swab and BAL samples (Spearman correlation, $r=0.77$, $p=0.00072$) **(Figure 1B)**. Different constellations of mutations were observed in the sequences isolated from NP swab and BAL samples, suggesting the presence of compartmentalized viral subpopulations that evolved independently, as was previously suggested [2]. The median mutation rate, calculated as the accumulated mutations per day since diagnosis, was 0.09 mutations/day, higher than the one originally estimated for SARS-CoV-2 (0.06 mutations/day [3]) (One-sample Wilcoxon test, $p=0.005$), indicating accelerated mutation rate during the infection. There was no significant difference in the mutation rate calculated for NP swab and BAL samples in the infection period (Mann-Whitney test, $p=0.18$).

On day 0, viral genome sequences harboured the characteristic mutational pattern of lineage A.2 (ORF1a:F3701Y, ORF3a:G196V, ORF8:L84S, N:S197L) in addition to two other substitutions and four

synonymous mutations (**Figure 1B**). In particular, the spike (S) gene sequence was characterized by the I197V substitution and one synonymous mutation. These were the only two mutations observed in the S gene throughout the course of this five-month period infection in the NP swab samples. However, we observed a different evolutionary pattern in the BAL samples. On day 50, the A653V amino acid substitution was observed. This has been associated as part of the mutational pattern of two variants spreading in France [4] and Germany [5] at the beginning of 2021. On day 87, the P384L in the receptor-binding domain (RBD) emerged but disappeared together with the A653V on day 136, five days after treatment with the hyperimmune serum from a convalescent patient, when R158S and N501T substitutions emerged. Strikingly, the N501T mutation is associated with an increased binding affinity of the S protein to the human angiotensin-converting enzyme 2 (ACE2) receptor and has been recently identified as an escape mutation against several anti-SARS-CoV-2 neutralizing antibodies (NABs) (e.g., B38, CC12.1, and S309 S2H12 S304, among others) [6]. Interestingly, the position R158 of the S protein is part of the N-terminal domain (NTD) antigenic supersite, a region being recognized by all known NABs directed to the NTD [7], and the R158S substitution has been included among the escape mutations of anti-SARS-CoV-2 monoclonal NABs targeting the NTD of the viral S protein [8]. Besides the S gene, we highlight the emergence, on day 50, of the G204R in the nucleocapsid (N) gene, a mutation characteristic of the P.2 lineage, and, on day 136, of the K1795Q in the ORF1a and the P67S in the N gene, which are distinctive signatures of P.1 and B.1.617.3 lineages, respectively. P67S was also reported in the 20C-US emerging variant within the B.1.2 lineage [9].

This study describes the case of an XLA-immunocompromised patient with prolonged SARS-CoV-2 infection, supporting evidence that these patients undergo viral shedding for long periods of time, which can last up to several months [9–12]. The patient presented RT-qPCR negative NP swab samples in different time intervals throughout the course of infection that either matched to a positive BAL sample or were followed by a positive RT-qPCR sample. This indicates that a negative RT-qPCR result in NP swab samples, which are the specimens usually adopted for diagnosis and surveillance, may not imply remission from infection, as was previously observed [10–12]. Viral genome sequencing of NP and BAL samples revealed an accelerated intra-host viral evolution, as previously described in immunocompromised patients [10]. Different mutations were accumulated in samples collected from NP swabs and BAL throughout the course of infection, which may point to differential viral adaptation to the upper and the lower respiratory airways. Several host factors could account for this phenomenon, such as temperature and immune response disparities and/or tissue differences in the ACE2 expression. In fact, the upper respiratory tract shows lower temperature and type-I IFN-mediated immune responses compared to those observed in the lower respiratory tract [13], together with a higher density of the ACE2 [14]. These factors could favour viral infection and replication in the upper respiratory tract, contributing to the intra-host viral diversity in patients. Furthermore, it is important to note that the mutations emerging in the viral population present in the lower respiratory tract are not detected by sequencing NP swab samples, highlighting that the emergence of potentially worrying viral variants may be underestimated by sequencing standard diagnostics NP samples. We observe the remarkable appearance of amino acid substitutions linked to immune evasion in the BAL sample collected three days after treatment with

hyperimmune serum. Of note, the appearance of the same or other mutations of interest in the NP swab samples days after hyperimmune serum treatment could not be ruled out. In fact, we were able to sequence only one NP swab sample 24 h after treatment, which may not be enough time to observe a possible viral population shifting in these samples. One limitation of our study is that we have no data on the Abs composition and SARS-CoV-2 neutralizing activity of the hyperimmune serum used. Lastly, the emergence of mutations distinctive of currently circulating SARS-CoV-2 variants of concern (VOCs) support the hypothesis for long-term viral shedding in immunocompromised patients as one possible mechanism for the emergence of VOCs.

Declarations

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical approval

The University Hospital Nuestra Señora de Candelaria (Santa Cruz de Tenerife, Spain) review board approved the study (ethics approval number: CHUNSC_2020_24).

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Figures

Figure 1

Five-month longitudinal study of SARS-CoV-2 positive samples collected from an XLA-immunocompromised patient. A) Chronological visualization of samples collected throughout the course of infection until patient death (day 149). RT-qPCR cycle threshold (Ct) are shown for collected nasopharyngeal swab (NP, circle) and bronchoalveolar (BAL, triangle) samples, with sequenced samples highlighted in blue. Vertical bars represent the accumulated number of mutations in the sequenced

genome compared to the consensus viral sequence obtained from the first NP sample (day 9). † At day 50, a BAL sample was shown to have actively replicating SARS-CoV-2 viruses. B) Graphical representation of SARS-CoV-2 whole-genome consensus sequences with synonymous (blue asterisks) and non-synonymous mutations (orange asterisks) identified as compared to the Wuhan-Hu-1 reference sequence (NC_045512.2). Only non-synonymous mutations are identified with the amino acid changes in the figure. On day 50, in the N gene, the amino acid substitution S197L is replaced by S197T.

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