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Letters to the Editor

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

Dear Editor,

In this journal, Walsh and colleagues[1] recently reviewed the evidence supporting that immunocompromised patients may remain positive for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection for long periods of time, up to 20 days. Here, we report the case of an immunocompromised patient with clinically diagnosed X-linked agammaglobulinemia (XLA) (*Supplementary Material*) who was persistently infected with SARS-CoV-2 for almost five months. He was admitted to the hospital on the 14th April 2020 with left bilobar pneumonia, reporting cough, chronic diarrhoea, and fever over the previous four days, and a nasopharyngeal (NP) sample tested positive for SARS-CoV-2 by RT-qPCR (Fig. 1A). In the hospital, he was treated with hydroxychloroquine, two courses of remdesivir, lopinavir/ritonavir, antibiotics, antifungal treatments, and glucocorticoids. Infectious SARS-CoV-2 was successfully cultured from a bronchoalveolar lavage (BAL) sample on day 50, showing that the virus was actively replicating in the lower respiratory airways (*Supplementary Material*). On day 133, he was treated with hyperimmune serum from a convalescent patient. Despite treatments and two coronavirus disease 2019 test negativizations, the patient stayed in the hospital most of the time and died in the intensive care unit from multiorgan failure and shock on day 149 (10th September 2020) (Fig. 1A). See the *Supplementary Material* for further details.

Throughout the period described, 26 respiratory samples were collected from the patient (22 NP swabs and 4 BAL samples) (Fig. 1A). A urine, faeces, and peripheral blood sample (on day 44), and another peripheral blood sample (on day 87) were collected, but viral genome was not detected in any of their RNA extractions. SARS-CoV-2 viral genomes of a subset of 13 NP and 3 BAL samples were sequenced using alternative methodologies (Supplementary Material). All genomes were assigned to the PANGO lineage A.2 (Clade 19B), which was predominant in Spain during the early months of the pandemic.[2] Assignation 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 and BAL samples (Spearman correlation, $r = 0.77$, $p = 0.00072$) (Fig. 1B). Different constellations of mutations were observed in the sequences isolated from NP and BAL samples, suggesting compartmentalization of viral subpopulations evolving independently. The median mutation rate -accumulated mutations per day since diagnosis- was 0.09 mutations/day, higher than the originally estimated for SARS-CoV-2 (0.06 mutations/day[3]) (One-sample Wilcoxon test, $p = 0.005$), indicating accelerated mutation rate during infection. There was no significant difference in the mutation rate calculated for NP and BAL samples (Mann-Whitney *U* 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 (Fig. 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 infection period in NP samples. However, we observed a different evolutionary pattern in BALs. On day 50, the A653V substitution was observed. This was found 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 emerged but disappeared together with the A653V at day 136, five days after treatment with hyperimmune serum, when R158S and N501T emerged. Strikingly, the N501T is associated with an increased binding affinity of the S protein to the human angiotensin-converting enzyme 2 (ACE2) receptor and has been identified as an escape mutation against anti-SARS-CoV-2 neutralizing antibodies (NAbs).[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 has been included among the escape mutations of anti-SARS-CoV-2 monoclonal NAbs targeting the NTD of the S protein.[8] Besides, 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.

This study describes an XLA-immunocompromised patient with prolonged SARS-CoV-2 infection, supporting evidence that these patients undergo viral shedding for long periods of time.[9,10] The patient presented RT-qPCR negative NP 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 samples may not imply remission from infection.[9] Viral genome sequencing revealed an accelerated intra-host viral evolution. Different mutations were accumulated in samples collected from NPs and BALs throughout the course of infection, which may point to viral adaptation to the upper and lower respiratory airways. Several host factors may account for this phenomenon, such as temperature and immune response disparities and/or differences in the ACE2 expression. Furthermore, it is worth noting that the mutations emerging in the lower respiratory tract were not detected by sequencing NP samples. Thus, the emergence of potentially worrying viral variants may be underestimated by sequencing standards focusing on NP samples. The emergence of substitutions linked to

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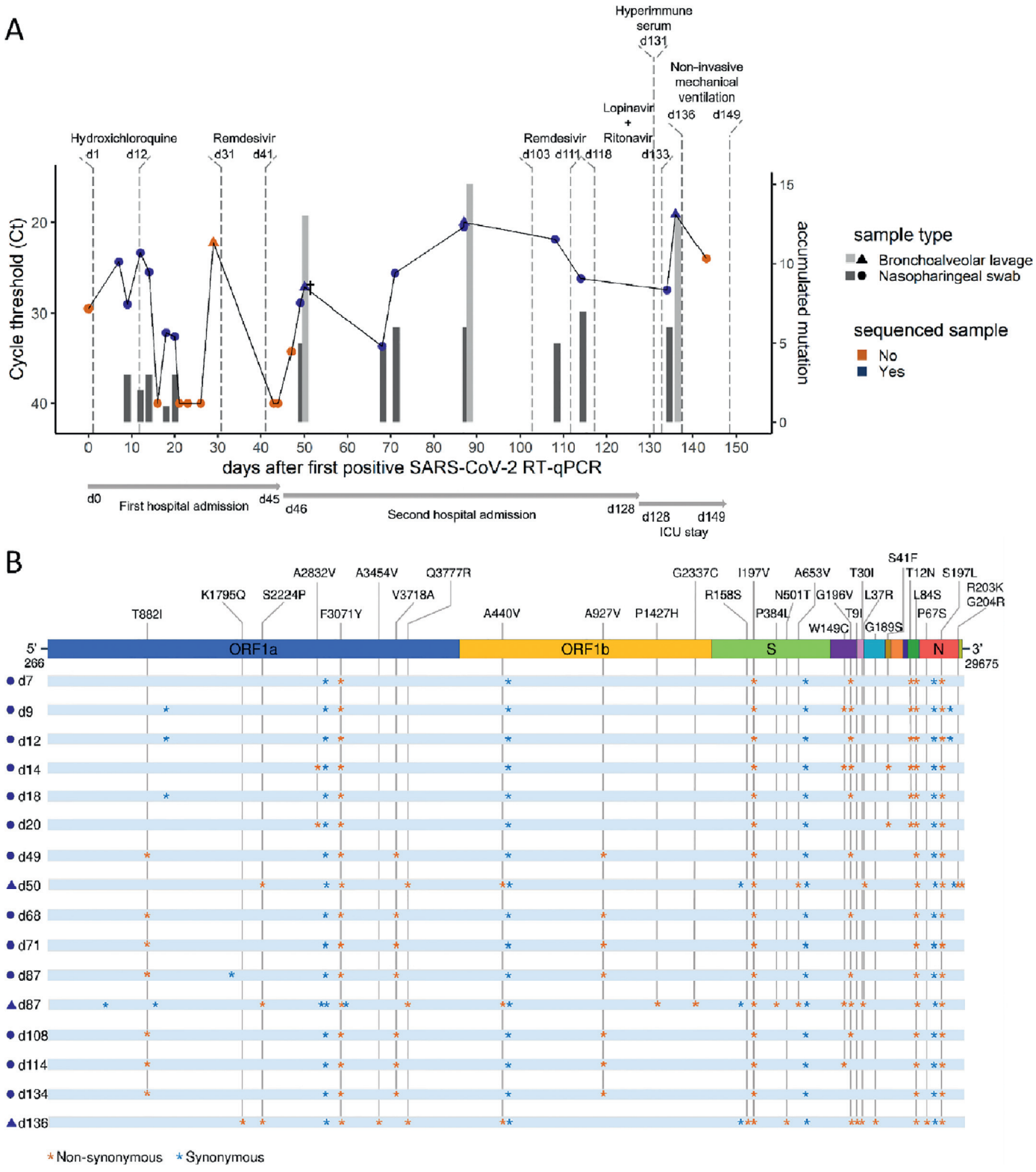

Fig. 1. *Five-month longitudinal study of SARS-CoV-2 positive samples collected from a XLA-immunocompromised patient.* **(A)** Chronological visualization of samples collected throughout the course of infection until patient death (day 149). RT-qPCR cycle threshold (Ct) are shown for collected nasopharyngeal 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 nonsynonymous 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article)

immune evasion in the BAL sample collected three days after treatment with hyperimmune serum is remarkable. Of note, the presence of the same or other mutations of interest in the NP samples days after hyperimmune serum treatment could not be ruled out. In fact, we were able to sequence only one NP sample 24 h after treatment, which may not be enough time to observe a possible viral population shifting in these samples. One limitation 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.

Declaration of Competing 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.

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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).

Authors Contribution

JAF and CF conceived the idea, the experimental design and supervised the project. LC, JMLS, HRP, HGC, AIC, RGM, and DGMA conducted the sequencing experiments. SRA and MEAA conducted the viral culture experiments. MHP, JAF, HGC, ODG, and DGMA collected patient data. LC, JMLS, AVF, and CF performed the analysis and interpreted the results. AVF and CFA obtained the funding. LC drafted the first version of the manuscript and prepared the figures. All authors contributed to manuscript revision and read and approved the submitted version.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.07.028.

References

1. Walsh K.A., Spillane S., Comber L., Cardwell K., Harrington P., Connell J., et al. The duration of infectiousness of individuals infected with SARS-CoV-2. *J Infect [Internet].* 2020 Dec 1;**81**(6):847–56. Available from https://doi.org/10.1016/j.jinf.2020.10.009.
2. Latif AA, Mullen JL, Alkuzweny M, Tsueng G, Cano M, Haag E, et al. A.2 Lineage report [Internet]. outbreak.info. 2021 [cited 2021 Jun 21]. Available from: https://outbreak.info/situation-reports?pango=A.2.
3. Harvey W.T., Carabelli A.M., Jackson B., Gupta R.K., Thomson E.C., Harrison E.M., et al. SARS-CoV-2 variants, spike mutations and immune escape. *Nat Rev Microbiol [Internet].* 2021;**19**(7):409–24. Available from https://doi.org/10.1038/s41579-021-00573-0.
4. Colson P., Levasseur A., Delerce J., Pinault L., Dudouet P., Devaux C., et al. Spreading of a new SARS-CoV-2 N501Y spike variant in a new lineage. *Clin Microbiol Infect [Internet].* 2021 Jun 9. Available from https://doi.org/10.1016/j.cmi.2021.05.006.
5. Mallm J.P., Bundschuh C., Kim H., Weidner N., Steiger S., Lander I., et al. Local emergence and decline of a SARS-CoV-2 variant with mutations L452R and N501Y in the spike protein. *medRxiv [Internet].* 2021. Available from: https://www.medrxiv.org/content/early/2021/04/29/2021.04.27.21254849.
6. Wang R., Chen J., Gao K., Wei G.W.. Vaccine-escape and fast-growing mutations in the United Kingdom, the United States, Singapore, Spain, India, and other COVID-19-devastated countries. *Genomics [Internet].* 2021;**113**(4):2158–70. Available from https://www.sciencedirect.com/science/article/pii/S0888754321001798.
7. McCallum M., De Marco A., Lempp F.A., Tortorici M.A., Pinto D., Walls A.C., et al. N-terminal domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2. *Cell [Internet].* 2021 Apr 29;184(9):2332–2347.e16. Available from: https://doi.org/10.1016/j.cell.2021.03.028
8. Suryadevara N., Shrihari S., Gilchuk P., VanBlargan L.A., Binshtein E., Zost S.J., et al. Neutralizing and protective human monoclonal antibodies recognizing the N-terminal domain of the SARS-CoV-2 spike protein. *Cell [Internet].* 2021 Apr 29;**184**(9). 2316–2331.e15. Available from https://doi.org/10.1016/j.cell.2021.03.029.
9. Choi B., Choudhary M.C., Regan J., Sparks J.A., Padera R.F., Qiu X., et al. Persistence and evolution of SARS-CoV-2 in an immunocompromised host. *N Engl J Med [Internet].* 2020 Dec 3 [cited 2021 Feb 17];**383**(23):2291–3. Available from: http://www.nejm.org/doi/10.1056/NEJMc2031364.
10. Baang J.H., Smith C., Mirabelli C., Valesano A.L., Manthei D.M., Bachman M.A., et al. Prolonged severe acute respiratory syndrome coronavirus 2 replication in an immunocompromised patient. *J Infect Dis [Internet].* 2021 Jan 1;**223**(1):23–7. Available from https://doi.org/10.1093/infdis/jiaa666.

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Avian influenza H10 subtype viruses continuously pose threat to public health in China

Dear editor,

Wang and colleges recently reported in this journal the first case of human infection with H10N3 virus in China.[1] In China, H10 subtype (H10N2, H10N3, H10N6, H10N7, H10N8, and et. al.) avian influenza virus (AIV) had distributed in poultry and wild bird populations in China.[2] Because poultry showed no clinical symptoms when infected with H10 subtype viruses, their eradication had not been a priority for the control of zoonosis diseases in China. However, H10 subtype viruses had continuously contributed to some zoonotic spillover events. In 2010, a number of cases of human infected with H10N7 were reported in Australia[2], and subsequently, China reported that the first human case of H10N8 infection that resulted in a human death in 2013 and the recently emerged human-infecting H10N3 virus in 2021.[1,3] Therefore, the H10 subtype AIV poses continuous public health concerns. To that purpose, we systematically analyzes the evolutionary dynamics and dissemination pathways of H10 subtype AIV in China.

In the present study, to elucidate the evolutionary process of H10 subtype influenza viruses, we firstly examined HA genes of global H10 subtype virus by performing multiple sequence alignment and phylogenetic analysis.[3,4] H10 subtype viruses had divided into two lineages—North American-lineage and Eurasian-lineage (Fig. 1A). We observed that the H10 subtype viruses in Eurasian-lineage were more complex embodying different neuraminidases, while the H10N7 virus was concentrated on North American-lineage (Fig. 1A). All of the H10 subtype viruses isolated from China were derived from Eurasian-lineage. It is interesting to note that 78.5% of the H10 subtype viruses (N2-N9) were isolated from Jiangxi province (Fig. 1B–D), indicating that Jiangxi province of China is the epicenter of the H10 subtype viruses. In addition, we also found that the number of H10 subtype viruses increased during 2000–2015, and subsequently decreased after 2016 (Fig. 1B). The H10N3 and H10N8 influenza viruses exhibited the highest number of H10 subtype virus in China. H10N8 virus had become the dominant subtype in poultry during 2011–2015; however, the number of H10N3 virus had increased during 2016–2021 (Fig. 1B), indicative of dominant H10 subtype switch in China. Previous study showed that genetic diversity of H10N8 viruses were much higher prior to human spillover event.[2] In this study, we found that the genetic diversity of human-origin H10N3 viruses were lower than that of H10N8 viruses (Supplementary Fig. 1), and we also found that the genotype of human-infecting H10N3 virus was same as the A/chicken/Jiangsu/0110/2019(H10N3) and A/chicken/Jiangsu/0104/2019(H10N3). These findings supported the finding that the avian-to-human transmission of H10N3 virus might have occurred recently without further reassortment.

To estimate the population dynamics of H10 subtype influenza virus in China, we inferred the HA genes of H10 subtype virus demographic history using Bayesian Skyride plots (Supplementary Fig. 2). Our finding suggested that from 2012 to 2014, the decreasing effective population size indicated that the diversity of H10 subtype viruses declined, while genetic diversity increased dramatically during 2014 to 2015 and then stably maintained (Fig. 2A and B). Subsequently, we analyzed the dissemination pathways of H10 subtype viruses in China in different sampling locations based on the HA phylogenetic tree. Among the result, we found that Jiangxi province was regarded as epicenter for the viral spread (Fig. 2C). Specifically, Jiangxi was linked with three locations—Hunan, Hubei, and Hebei. In addition, we found that Zhejiang was linked with closer province including Jiangsu(Fig. 2C). However, there are some limitations that sampling bias might have affected the results. Among our findings, the transmission routes of H10 subtype viruses were primarily concentrated in Jiangxi province of China. Previous study showed that the role of wild birds in the dispersal of AIVs during the seasonal migration.[5,6,7] In the case of Jiangxi province, the Poyang Lake, with its excellent ecology and vast wetlands, has become a world-famous migratory bird wintering hub, which increase the close contact between wild birds carrying H10 subtype viruses and poultry, accelerating the reassortment and mammalian adaptive mutations.

The emerged H10N3 and H10N8 influenza viruses had caused human infection in China, posing public health threat. In previous study, we found that the key substitutions in PB2 protein of H10N8 viruses including I292V, A588V, and T598M were associated with mammalian adaption.[8,9]. Thus, a key concern is whether the H10 subtype viruses in poultry had acquired the mammalian adaption in recent years. In the present study, we found that the proportion of I292V, A588V, and T598M substitutions in the PB2 protein of H10 subtype viruses in poultry increased sharply during 2013 to 2021 (Fig. 2D), and the human-infecting H10N3 and H10N8 viruses all harbored these amino acids substitutions, indicating that H10 subtype viruses in China pose an increasing threat to hu-