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Letter to the Editor

Molecular evolution of the SARS-CoV-2 omicron BA.2 variant in kidney transplant recipients with prolonged viral shedding

Dear editor,

Response to: Yi Zhang et al. *Intra-host SARS-CoV-2 single-nucleotide variants emerged during the early stage of COVID-19 pandemic forecast population fixing mutations*, J Infect 2022;84:722–746.

We read with interest the article by Zhang et al.[1] who reported the emergence of numerous SARS-CoV-2 variants during the early stages of the COVID-19 pandemic. The authors forecast these variants' potential ability to evade immune responses. Research investigating the occurrence of prolonged SARS-CoV-2 shedding from the upper respiratory tract has detected that this phenomenon is common[2] and associated with the emergence of *de novo* mutations in the viral genome. Here, we describe the emergence of SARS-CoV-2 mutations in a cohort of kidney transplant recipients (KTRs) who experienced prolonged viral shedding despite being treated with anti-SARS-CoV-2 monoclonal antibodies.

Several of the 197 KTRs who were diagnosed with COVID-19 at our center between December 20, 2021 and April 15, 2022 (i.e., throughout the omicron wave in France) experienced a late clinical deterioration. We identified 15 KTRs (8%) who had detectable viral loads in nasopharyngeal swabs for more than 21 days. Of them, eight harbored the BA.2 variant and had at least two specimens collected during the course of infection (i.e., the first at diagnosis and the second after day 21 [D21] or at the time of clinical deterioration). Quantification of both viral loads and anti-RBD IgG as well as Spike gene sequencing was carried out in the available specimens according to a previously described methodology.[3] All of the eight patients received between two and four mRNA vaccine doses in the year preceding the diagnosis of COVID-19. Prophylactic injections of casirivimab–imdevimab were given to three participants. In addition, four received pre-exposure prophylaxis with tixagevimab–cilgavimab (300 mg) between 36 and 76 days prior to the onset of COVID-19. Symptoms at presentation were mild in all cases (Table 1). Tixagevimab–cilgavimab (600 mg) was administered with curative intent to five participants, and four received sotrovimab infusions (500 mg). Consequently, all patients received tixagevimab–cilgavimab in either a prophylactic or curative setting.

A total of 36 nasopharyngeal swabs were collected between D0 and D72 post infection. All tested specimens remained positive by real-time PCR for a median of 36 days (interquartile range [IQR]: 28–46 days; Fig. 1A). The SARS-CoV-2 Spike gene was analyzed in 29 samples and 22 were exploitable.

One deletion and 11 non-synonymous nucleotide substitutions were identified between D11 and D72 post-infection (Fig. 1A). Early mutations—which included K147E (n = 1), E340D (n = 2), E340K (n = 1), R346T (n = 3), K356T (n = 2), and K444R (n = 5)—emerged after a median of 28 days from symptom onset (IQR: 24–30 days). Conversely, we identified the following late mutations between D70 and D72 post infection (Fig. 1B): K147N (n = 1), H245N (n = 1), Y449S (n = 1), L452R (n = 1), T547K (n = 1), and KN187–188del (n = 1).

Five patients (P1–P5) showed an unusual symptom worsening as of 21 days after the diagnosis of COVID-19. Hospitalization was required in four cases and one was admitted to an intensive care unit (ICU). Table 1 summarizes patient characteristics both at baseline and at the time of clinical deterioration. It is worth noting that the mutation burden observed in SARS-CoV-2 isolates at the beginning of the infection—when symptoms were mild—was markedly lower. Conversely, the number of mutations tended to increase during the course of follow-up, being evident between 11 and 72 days from symptom onset.

The most potently neutralizing antibodies to SARS-CoV-2—which are directed against the RBD of the viral Spike protein—act by blocking the RBD–ACE2 interactions. Interestingly, six (E340, R346, K356, K444, Y449, and L452) of the eleven mutations identified during the course of follow-up are known to affect the RBD region[4] and can act as escape mutations to neutralizing antibodies.[5,6] Previous studies have shown that SARS-CoV-2 can evolve to respond to immune pressures conferred by specific mAbs. For example, the E340K substitution confers reduced susceptibility to sotrovimab (> 297-fold change in EC50 value), whereas mutations at R346 and K444 not only confer resistance to cilgavimab but may also facilitate transmission primarily by enhancing binding to ACE2.[7] The R346K and K444R mutations have been previously reported in nine immunocompromised patients who had been treated with tixagevimab–cilgavimab; however, differently from our study, no significant clinical deterioration was observed at follow-up.[8] The question as to whether the prophylactic or therapeutic use of tixagevimab–cilgavimab could have increased viral mutation rates remains unanswered. While all patients in our study had received tixagevimab–cilgavimab, this association does not prove a causative link between the use of mAbs and the emergence of mutations. Notably, prolonged viral shedding did not occur in any of the 120 KTRs with COVID-19 who were followed at our center and had not been treated with tixagevimab–cilgavimab. There are at least two potential reasons to link the use of this mAbs combination with the onset of mutations. First, the ability of tixagevimab–cilgavimab to neutralize any omicron sublineage is limited—with cilgavimab being the only ingredient of the cocktail with some effectiveness.[9] Second, the use of low-dose cilgavimab–tixagevimab (150 mg of each antibody)—as generally performed in France at the beginning of the omicron wave—does not confer an adequate protection in a high

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Table 1
General characteristics of the eight kidney transplant recipients (P1–P8) with COVID-19 who showed prolonged SARS-CoV-2 shedding.

Variable	P1	P2	P3	P4	P5	P6	P7	P8
Age (years)	54	57	73	80	71	46	59	73
Sex	M	M	F	M	M	F	M	M
Interval from TX (years)	27	0.2	2	6	1	5	0.1	0.1
Number of mRNA vaccine doses	3	4	4	3	3	2	3	3
Prophylactic casirivimab–imdevimab	No	No	Yes	Yes	Yes	No	No	No
Prophylactic tixagevimab–cilgavimab	No	Yes	Yes	Yes	Yes	Yes	No	No
Administration delay until diagnosis (days)	-	55	36	44	76	-	-	-
Anti-Spike IgG titers at diagnosis (BAU/mL)	219	2224	5685	3405	3567	179	232	97
Symptoms at presentation	Rhinitis	Cough, rhinitis	Cough, rhinitis	Cough, anosmia, ageusia, diarrhea, asthenia	Rhinitis	None	Cough, rhinitis	None
Curative tixagevimab–cilgavimab	Yes	No	No	No	Yes	No	Yes	Yes
Curative sotrovimab	No	Yes	Yes	Yes	No	No	No	Yes
Duration of viral shedding (days)	30	49	53	26	42	72	28	70
Clinical worsening	Yes	Yes	Yes	Yes	Yes	No	No	No
Interval from diagnosis to worsening (days)	30	44	35	14	27	-	-	-
Symptoms at the time of worsening	Severe asthenia, cough	Dyspnea, lung involvement (10–25%)	Asthenia, fever, myalgia, cough, pneumopathy (10–25%)	Dyspnea, fever, lung involvement (10–25%)	ARDS	-	-	-
Hospitalization	No	Yes	Yes	Yes	Yes (ICU)	-	-	-
Treatment	None	High steroid doses	High steroid doses	High steroid doses	Non-invasive ventilation	-	-	-

Abbreviations: M, male, F, female; TX, transplantation; ARDS, acute respiratory distress syndrome; ICU, intensive care unit.

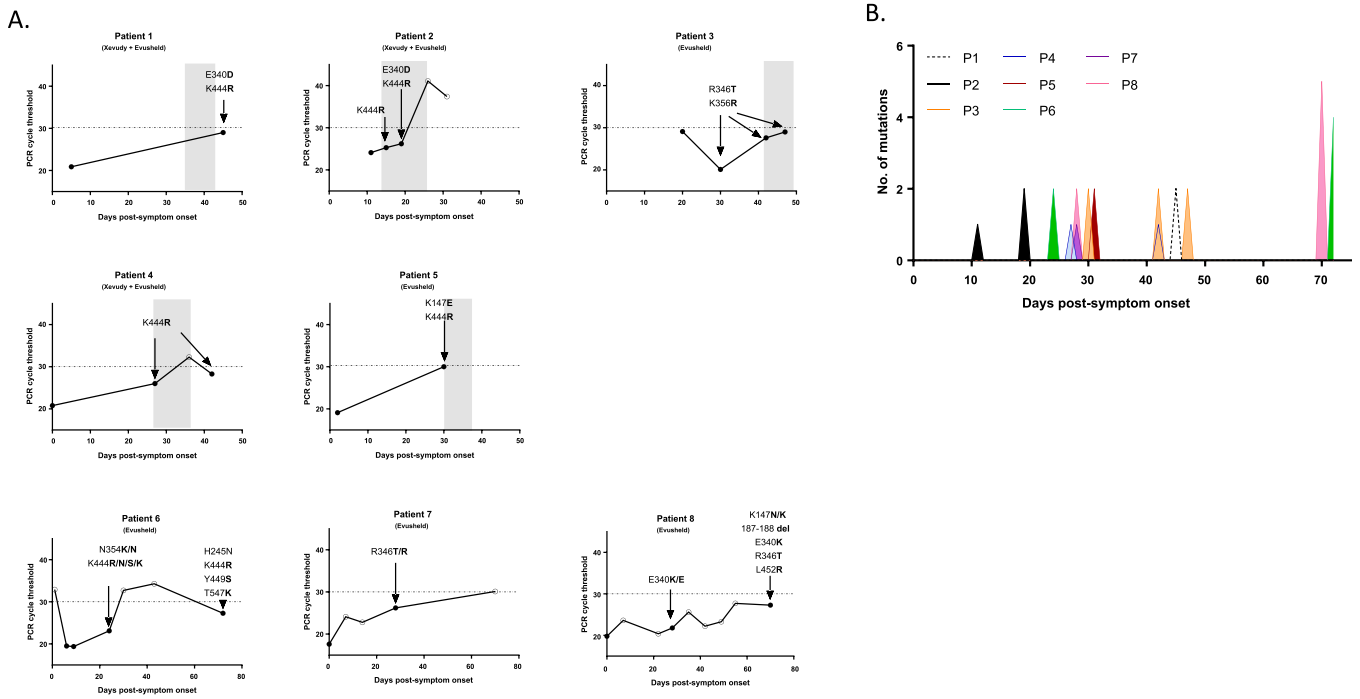

Fig. 1. Temporal evolution of SARS-CoV-2 mutation burden and viral loads. Panel A: patient-based analysis of the temporal evolution of SARS-CoV-2 viral loads plotted against the identification of emerging Spike gene mutations. Each mutant amino acid residue is reported. Empty circles denote the day of sample collection, whereas full circles the day of gene sequencing. Arrows indicate the days on which mutations were detected. Shaded areas denote the period characterized by clinical deterioration, whereas the treatment approach is reported in parentheses. Panel B: temporal course of the detected mutations with respect to the day of symptom onset. Abbreviation: P, patient.

proportion of KTRs.[10] This may ultimately lead to the possible emergence of escape mutations to evade immune responses.

Despite its rarity, prolonged SARS-CoV-2 shedding in KTRs with COVID-19 may result in adverse molecular (i.e., onset of escape mutations) and clinical (i.e., symptom deterioration) consequences—even in patients with an initially mild presentation. Further research is needed to clarify the potential causative role of neutralizing mAbs given in either a prophylactic or curative setting. It can be anticipated that their potential detrimental effects may be more pronounced when given at suboptimal doses and/or to patients infected with resistant variants of concern.

Declaration of Competing Interest

Sophie Caillard received honoraria from Astra Zeneca (board expert). Other authors have no conflict of interest.

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