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BRIEF COMMUNICATION



# Long-term shedding of viable SARS-CoV-2 in kidney transplant recipients with COVID-19

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The exact duration of viable SARS-CoV-2 shedding in kidney transplant recipients (KTRs) remains unclear. Here, we retrospectively investigated this issue using cell cultures of SARS-CoV-2 RT-PCR-positive nasopharyngeal samples ($n$ = 40) obtained from 16 KTRs with symptomatic COVID-19 up to 39 days from symptom onset. A length of viable SARS-CoV-2 shedding >3 weeks from the onset of symptoms was identified in four KTRs (25%). These results suggest that a significant proportion of KTRs can shed viable SARS-CoV-2 for at least 3 weeks, which may favor the emergence of new variants. Based on these data, we recommend prolonging the isolation of KTRs with COVID-19 until negative SARS-CoV-2 RT-PCR testing.

**KEYWORDS**
clinical research/practice, complication: infectious, infection and infectious agents - viral, infectious disease, kidney transplantation/nephrology, translational research/science

## 1 | INTRODUCTION

Immunocompetent individuals with non-severe coronavirus disease 2019 (COVID-19) may shed SARS-CoV-2 for up to 10 days.[1,2] Understanding whether immunocompromised kidney transplant recipients (KTRs) with COVID-19 may experience prolonged viral shedding has important public health implications, especially with respect to patient isolation. A previous study demonstrated that SARS-CoV-2 ribonucleic acid (RNA) was detectable for a longer period in nasopharyngeal swabs collected from KTRs than in those from immunocompetent individuals.[3] However, the presence of SARS-CoV-2 RNA does not necessarily indicate that the virus is vital and infectious. As a result, limited data are available on the shedding dynamics of viable SARS-CoV-2 in KTRs. Here, we retrospectively investigated this issue using cell cultures of SARS-CoV-2 RT-PCR-positive nasopharyngeal samples ($n$ = 40) obtained from 16 KTRs with symptomatic COVID-19 up to 39 days from symptom onset.

## 2 | MATERIALS AND METHODS

### 2.1 | Study population

The study participants ($n$ = 16) were retrospectively recruited from among adult KTRs who were hospitalized with symptomatic COVID-19 at our transplant center between March 4 and April 15, 2020. All KTRs had an objectively confirmed diagnosis of COVID-19 based on positive testing of nasopharyngeal swabs by reverse transcription-polymerase chain reaction (RT-PCR). KTRs who had at least two positive nasopharyngeal swabs (of which one collected at least 7 days after symptom onset) during the follow-up period were deemed eligible. Patient data were extracted from medical records on admission and during follow-up. Patients requiring high concentrations of oxygen (>6 L/min) or admitted to an intensive care unit were considered to have severe COVID-19. The study protocol complied with the tenets of the Helsinki Declaration and was granted

**Abbreviations:** CNI, calcineurin inhibitors; COVID-19, coronavirus disease 2019; Ct, cycle threshold; IQR, interquartile range; KTR, kidney transplant recipients; RDRP, RNA-dependent RNA polymerase; RNA, ribonucleic acid; RT-PCR, reverse transcription-polymerase chain reaction.

ethical approval by the local Institutional Review Board (approval number: DC-2013-1990).

### 2.2 | Reverse transcription-polymerase chain reaction

Quantitative RT-PCR testing for SARS-CoV-2 was carried out according to the COVID-HUS study protocol (ClinicalTrials.gov identifier: NCT04405726) using nasopharyngeal swabs obtained at admission and, subsequently, on a weekly basis until discharge. Thereafter, RT-PCR testing was performed at 30 and 45 days (±7 days) from the onset of symptoms. We used an in-house RT-PCR-based assay targeting two different regions of the RNA-dependent RNA polymerase (*RdRp*) gene in accordance with the WHO technical guidance. Viral loads were quantified using the cycle threshold (Ct) values of the *RdRp* gene.

### 2.3 | Cell infection and assessment of viral cytopathic effect

Viral cytopathic effect assays were performed using the Buffalo green monkey kidney (BGMK) cell line (ATCC; PTA-4594). In brief, filtered SARS-CoV-2 RT-PCR-positive nasopharyngeal samples (50 µL) were added in duplicate to BGMK cells seeded in a 96-well plate and cultured at 37°C for 72 h in a 5% $CO_2$ atmosphere. Cultures were observed daily for cytopathic effects. On the third day, cells were fixed and probed with a recombinant rabbit anti-SARS-CoV-2 (2019-nCoV) Spike S2 antibody (Interchim). Foci were counted on an AID ELIPSOT reader using the ELISPOT 8.0 software (AID Autoimmun Diagnostika GmbH) as previously described.[4] All experiments were carried out in a biosafety level-3 laboratory.

## 3 | RESULTS

The study sample consisted of 16 KTR (88% men; median age: 63.3 years; interquartile range [IQR]: 54–68.7 years). The median body mass index was 24 kg/m$^2$ (IQR: 23–30.8 kg/m$^2$), whereas the median time elapsed from the date of transplantation to the diagnosis of COVID-19 was 3.8 years (IQR: 1.6–7.2 years). With respect of immunosuppressive drugs, 13 (81.3%) and 14 (87.5%) patients received calcineurin inhibitors (CNI) and mycophenolate mofetil/mycophenolic acid, respectively. Inhibitors of the mammalian target of rapamycin and steroids were given to 2 (12.5%) and 10 (62.5%) patients, respectively. Only two patients presented with severe COVID-19. Clinical and demographic characteristics are summarized in Table 1.

From symptom onset until 38 days thereafter, we collected a median of two nasopharyngeal swabs per patient (IQR: 2–3.3). Viable SARS-CoV-2 shedding was identified in a total of 17 (42.5%) swabs and 9 (56.2%) KTRs showed at least one positive virus isolation. Most (9/16, 56.2%) samples collected within 10 days of symptom onset had positive viral cultures of SARS-CoV-2. Notably, a viable virus was detected in 8 of the 24 (33.3%) samples collected after at least 10 days from symptom onset. In addition, a length of viable SARS-CoV-2 shedding >3 weeks from the onset of symptoms was identified in four KTRs (25%; Figure 1, panels A and B). While none of these four patients had severe COVID-19, high viral loads were noted in their nasopharyngeal swabs on admission (24, 21, 16, and 17 Ct), respectively; median in the remaining 12 patients: 26 Ct, IQR: 23–30 Ct. Moreover, they showed lower C-reactive protein peak levels (36, 17, 5, 33 mg/L, respectively; median in the remaining 12 patients: 62 mg/L, IQR: 44–96 mg/L,) and had a trend toward lower lymphocyte counts before COVID-19 (0.66, 0.85, 0.84, and 0.88 × 10$^9$/L, respectively; median in the remaining 12 patients: 1.16 × 10$^9$/L, IQR: 0.85–1.94 × 10$^9$/L,). Other patient characteristics in relation to viable SARS-CoV-2 shedding duration are summarized in Table 1.

## 4 | DISCUSSION

Using *in vitro* cell cultures, we demonstrate that a significant proportion of KTRs with COVID-19 can have a prolonged SARS-CoV-2 shedding period. A positive cytopathic effect in cell cultures can be considered as a surrogate for the presence of a viable virus with infectious potential. Previous studies have shown that viral shedding in immunocompetent subjects is generally limited to less than 10 days from symptom onset,[1,2] although one report found a duration of up to 20 days.[5] Another investigation suggested that the length of viral shedding in immunocompetent patients may be dependent on the severity of COVID-19—being as high as 32 days in those with severe clinical manifestations.[6,7] Herein, we identified four KTRs with a shedding of viable SARS-CoV-2 longer than 3 weeks from symptom onset (up to 38 days). While none of these patients had severe COVID-19, they showed higher viral loads in their nasopharyngeal swabs. Moreover, they had less severe inflammation—possibly as a result of a reduced immune response to SARS-CoV-2. In a patient with mild COVID-19 who had recently undergone heart transplantation, Decker et al.[8] identified viral shedding after 21 days from symptom onset. Another study conducted in 20 patients under immunosuppressive therapy for cancer reported prolonged viral shedding up to 61 days from the onset of symptoms.[9] Currently, the European Center for Disease Prevention and Control guidelines for immunocompromised patients with COVID-19 recommend discontinuing isolation in the following conditions: (1) clinical resolution of symptoms; (2) isolation for at least 20 days from the onset of symptoms; or (3) two consecutive negative SARS-CoV-2 RT-PCR tests of respiratory specimens collected ≥24 h apart. The Centers for Disease Control and Prevention guidance regarding discontinuing transmission-based precautions in this population maintains that isolation should end (1) 20 days after the symptoms first appeared, (2) at least 24 h have passed since last fever, and (3) when symptoms have improved.[10,11] Based on the current data, we believe that an extension of isolation precautions

**TABLE 1** Clinical and demographic characteristics, management of immunosuppression, and antiviral and immunomodulatory therapies of kidney transplant recipients (*n* = 16)

| | Entire cohort (*n* = 16) | Viable SARS-CoV–2 shedding <21 days (*n* = 12) | Viable SARS-CoV–2 shedding >21 days (*n* = 4) |
|---|---|---|---|
| Men | 14 (87.5%) | 11 (91.7%) | 3 (75%) |
| Age (years) | 63.3 (54–68.8) | 63.3 (57.3–68.7) | 58.4 (44.3–68.8) |
| Comorbidities | | | |
| BMI (kg/m²) | 24 (23–30.8) | 26.5 (23–33) | 24 (22.5–24.3) |
| Cardiovascular disease | 6 (37.5%) | 5 (41.7%) | 1 (25%) |
| Respiratory disease | 6 (37.5%) | 5 (41.7%) | 1 (25%) |
| Diabetes | 6 (37.5%) | 5 (41.7%) | 1 (25%) |
| Hypertension | 13 (81.3%) | 11 (91.7%) | 2 (50%) |
| Interval from kidney transplantation (years) | 3.8 (1.6–7.2) | 4.9 (1.4–12.8) | 2.9 (1.8–3.8) |
| Antithymocyte globulin | 8 (50%) | 5 (41.7%) | 3 (75%) |
| Anti-CD25 | 8 (50%) | 7 (58.3%) | 1 (25%) |
| Tacrolimus | 10 (62.5%) | 8 (66.7%) | 2 (50%) |
| Ciclosporin | 3 (18.8%) | 1 (8.3%) | 2 (50%) |
| MMF/MPA | 14 (87.5%) | 11 (91.7%) | 3 (75%) |
| mTOR inhibitors | 2 (12.5%) | 1 (8.3%) | 1 (25%) |
| Steroids | 10 (62.5%) | 6 (50%) | 4 (50%) |
| Belatacept | 2 (12.5%) | 2 (16.7%) | 0 |
| Dyspnea | 9 (56.3%) | 8 (66.7%) | 1 (25%) |
| Cough | 12 (75%) | 9 (75%) | 3 (75%) |
| Fever | 15 (93.8%) | 11 (91.7%) | 4 (100%) |
| Myalgia | 10 (62.5%) | 7 (58.3%) | 3 (75%) |
| Headache | 6 (37.5%) | 4 (33.3%) | 2 (50%) |
| Diarrhea | 14 (87.5%) | 10 (83.3%) | 4 (100%) |
| SARS-CoV–2 Ct[a] | 25 (22–29) | 26 (23–30) | 20 (16–24) |
| CRP peak, mg/L | 46 (28.9–76.8) | 61.5 (43.7–95.5) | 24.8 (13.7–33.9) |
| Severe disease | 2 (12.5%) | 2 (16.7%) | 0 |
| Lopinavir/ ritonavir | 1 (6.3%) | 1 (8.3%) | 0 |
| Hydroxychloroquine | 8 (50%) | 5 (41.7%) | 3 (75%) |
| Tocilizumab | 2 (12.5%) | 2 (16.7%) | 0 |
| CNI withdrawal | 4/13 (30.1%) | 3 (33.3%) | 1 (25%) |
| MMF/MPA withdrawal | 14/14 (100%) | 11 (100%) | 3 (100%) |
| mTORi withdrawal | 2/2 (100%) | 1 (100%) | 1 (100%) |

Continuous variables are presented as medians (interquartile ranges), whereas categorical variables are presented as counts (percentages).

Abbreviations: BMI, body mass index; CNI, calcineurin inhibitors; CRP, C-reactive protein; MMF, mycophenolate mofetil; MPA, mycophenolic acid; mTORi, inhibitors of the mammalian target of rapamycin.

[a]SARS-CoV-2 cycle threshold measured in the nasopharyngeal swab obtained on admission.

should be considered for KTRs. It is also possible that a prolonged shedding of viable SARS-CoV-2 in immunocompromised patients with a weak immunological pressure can lead to the emergence of viral variants.[12–14] Surprisingly, we identified viable SARS-CoV-2 shedding in three cases characterized by low viral loads (Ct ≥ 35). While the presence of a viable virus generally shows an inverse relationship with Ct, this is not invariably the case. For example, a previous study demonstrated a successful viral propagation in 5 of 60 samples with Ct > 35,[15] a finding which has been independently confirmed by another report (for 2 of the 74 specimens with Ct > 35).[16] The presence of positive cultures in certain patients who had a low viral load in nasopharyngeal swabs is surprising. A potential explanation may lie in the presence of a low viral load in the upper respiratory tract coupled with a higher load in the lower tract. Alternatively, a sampling issue can be hypothesized.

In conclusion, our results indicate that a significant proportion of KTRs can shed viable SARS-CoV-2 for at least 3 weeks. Based on these findings, we also suggest prolonging the isolation of KTRs with

**(A)**

| Patient ID | Days from symptos onset | SARS-CoV-2 culture |
|---|---|---|
| KTR 1 | 5 | - |
| | 14 | - |
| KTR 2 | 21 | - |
| | 29 | - |
| KTR 3 | 25 | - |
| | 39 | - |
| KTR 4 | 2 | + |
| | 9 | - |
| | 16 | - |
| | 35 | - |
| KTR 5 | 1 | + |
| | 8 | + |
| | 23 | - |
| | 30 | - |
| KTR 6 | 6 | + |
| | 9 | + |
| | 23 | + |
| | 29 | + |
| KTR 7 | 7 | + |
| | 38 | + |
| KTR 8 | 1 | - |
| | 8 | + |
| | 28 | + |
| KTR 9 | 11 | + |
| | 30 | - |
| KTR 10 | 2 | - |
| | 8 | - |
| KTR 11 | 11 | + |
| | 27 | + |
| KTR 12 | 17 | - |
| | 27 | - |
| KTR 13 | 2 | + |
| | 9 | + |
| | 31 | - |
| KTR 14 | 3 | - |
| | 19 | - |
| KTR 15 | 4 | - |
| | 17 | - |
| KTR 16 | 20 | + |
| | 30 | - |

**(B)**

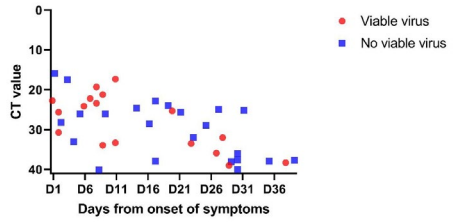


**FIGURE 1** Duration of viral shedding in kidney transplant recipients ($n$ = 16). (A) Results of viral culture in relation to the days from symptom onset for each kidney transplant recipient. A total of 40 nasopharyngeal specimens from 16 recipients were analyzed. Each sample was tested with (1) an RT-PCR-based assay targeting two different regions of the RNA-dependent RNA polymerase (*RdRp*) gene in accordance with the WHO technical guidance and (2) SARS-CoV-2 viral cell culture. Four patients (KTR6, KTR7, KTR8, and KTR11) were found to shed viable SARS-CoV-2 for at least 3 weeks from the onset of symptoms. (B) Presence (red dots) or absence (blue dots) of viable SARS-CoV-2 shedding (based on the results of viral culture) in relation to cycle threshold (CT) values and days after symptoms onset (DSO). KTR, kidney transplant recipient; RT-PCR, reverse transcription-polymerase chain reaction; SARS-CoV-2, severe acute respiratory distress syndrome coronavirus 2; WHO, World Health Organization [Color figure can be viewed at wileyonlinelibrary.com]

COVID-19 in the following conditions: (1) until clinical resolution of symptoms and negative SARS-CoV-2 RT-PCR testing of respiratory specimens or (2) after at least 30 days from symptom onset when RT-PCR testing is not available. The main caveats of the current study include the limited sample size and the lack of data concerning viral variants; therefore, our findings require confirmation in independent investigations.

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## DISCLOSURE

The authors of this manuscript have conflicts of interest to disclose as described by the *American Journal of Transplantation*. Dr Caillard reports personal fees and non-financial support from Novartis, nonfinancial support from Sanofi, and nonfinancial support from Astellas, unrelated to the current study. The other authors have no conflicts of interest to disclose.

## DATA AVAILABILITY STATEMENT

Data supporting the findings from this study are available from the corresponding author upon reasonable request.

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