COVID-19

Potential SARS-CoV-2 kidney infection and paths to injury

Luise Hassler and Daniel Batlle

Although direct kidney infection by SARS-CoV-2 remains controversial, a study based largely on autopsies shows increased tubulointerstitial fibrosis in patients with COVID-19 and suggests direct kidney infection. Moreover, in human kidney organoids, SARS-CoV-2 infection upregulates several pro-fibrotic and pro-inflammatory pathways.

Refers to Jansen, J. et al. SARS-CoV-2 infects the human kidney and drives fibrosis in kidney organoids. *Cell Stem Cell* **29**, 217–231.e8 (2022).

In hospitalized patients with COVID-19, the presence of acute kidney injury (AKI) is associated with poor survival[1,2], but the pathophysiology of AKI in these patients is complex and not fully understood[3]. Although the importance of AKI as a major complication of COVID-19 is now recognized, controversy remains as to whether SARS-CoV-2 infects the kidneys directly. Moreover, if direct kidney infection is present, it remains to be proven whether it initiates and/or contributes to the development of AKI in patients with COVID-19. To shed light on these questions, Jansen and colleagues[4], on behalf of the COVID Moonshot consortium, examined the presence of SARS-CoV-2 in the kidneys of patients with COVID-19 and report that the virus infects kidney cells.

Jansen and colleagues[4] used 62 kidney samples from patients with COVID-19 (61 autopsy specimens and 1 biopsy sample). Although it was not stated in the original article, the researchers have clarified to *Nature Reviews Nephrology* that they detected SARS-CoV-2 nucleocapsid protein staining by immunofluorescence in 6 out of 6 samples tested and that all 62 kidney specimens tested positive for SARS-CoV-2 by PCR (R. Kramann, personal communication). Staining for SARS-CoV-2 was mainly detected in proximal tubular cells, which express the receptor for viral entry into cells — angiotensin-converting enzyme 2 (ACE2)[5]. These data might suggest that everyone with severe COVID-19 and associated kidney disease would have positive staining for the nucleocapsid protein of SARS-CoV-2 by immunofluorescence and that this technique is a very sensitive method for detection of SARS-CoV-2 in the kidney. In our recent survey of the literature, we noted that SARS-CoV-2 was detected in kidneys of only 102 of 235 patients with COVID-19, using different methods[6]. Of note, immunofluorescence staining was positive in 10 of 13 (77% of) kidney samples, which is a much higher percentage than that observed for RT–qPCR, immunohistochemistry or in situ hybridization[6].

> Kidney organoids infected with SARS-CoV-2 upregulated pro-fibrotic signalling pathways

Importantly, evidence that implicates direct kidney infection in the pathogenesis of acute tubular injury or collapsing glomerulopathy, which are the two best described forms of kidney disease in patients with COVID-19, is still lacking. Clinical data related to kidney function, such as blood urea nitrogen, creatinine or proteinuria, were not provided in the study by Jansen and colleagues[4]. However, the researchers report evidence of proximal tubule injury assessed by staining for kidney injury molecule 1 (KIM1), which is a marker of this pathology; the number of samples stained was not specified. They also found increased tubulointerstitial fibrosis in the COVID-19 cohort compared with a COVID-19-negative control cohort (n = 57) matched for age, sex and comorbidities[4]. A subset of this control cohort is of particular interest because it comprised patients who received treatment for acute respiratory distress syndrome in an intensive care unit (n = 14); 71% of those patients had AKI. Single-nucleus RNA sequencing (RNA-seq) of kidney autopsy tissue from one patient with COVID-19 detected SARS-CoV-2 RNA expression in almost all of the 13 identified cell clusters, including proximal tubule cells and podocytes. Moreover, the data revealed upregulation of fibrosis-driving pathways compared with a control adult human kidney.

It should be noted, for comparison, that the largest series of kidney biopsy samples from patients with COVID-19 to date (n = 284) reported positive staining for nucleoprotein of SARS-CoV-2 assessed by immunohistochemistry in only 3.7% of cases; moreover, these positive cases were all negative by in situ hybridization[7]. To avoid selection bias, all kidney biopsy samples with temporal association to a positive nasopharyngeal SARS-CoV-2 RT–PCR test up to 3 months before biopsy were included. This relative long interval between the nasopharyngeal positivity and biopsy might be crucial in explaining the negative findings in the kidney. However, 43.3% of the biopsies were performed within 1 week of confirmed COVID-19 infection, and therefore such samples would likely show the presence of virus if direct kidney infection was present[7]. Nonetheless, the time point in the course of the disease at which kidney infection is most likely to be detected is unknown and might depend on the stage and/or severity of disease. Of note, the cohort analysed by Jansen and colleagues might be representative of extreme COVID-19 severity since all samples except for one kidney biopsy specimen were obtained from autopsies. Recently, Caceres and colleagues[8], detected the nucleocapsid protein of SARS-CoV-2 by immunofluorescence in kidney biopsy samples from two patients with COVID-19, as in the findings in the single biopsy sample analysed by Jansen et al.[4]. Of interest, these researchers also detected the presence of SARS-CoV-2 in urine sediment cells that co-expressed ACE2 and found that higher SARS-CoV-2 viral load in urine sediments correlated with increased incidence of AKI and mortality[8].

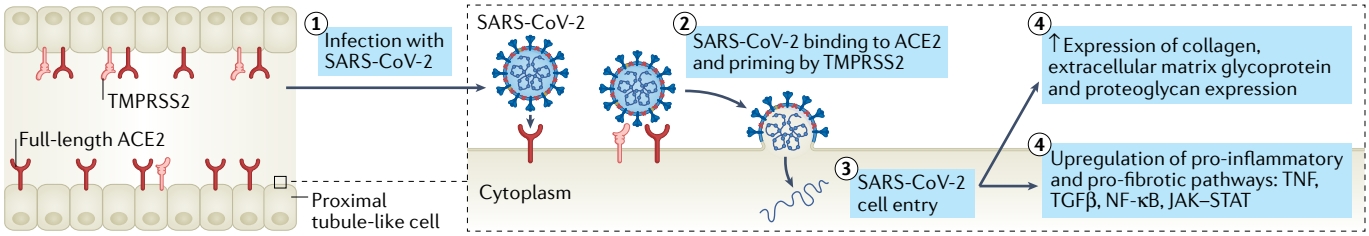

Fig. 1 | **Direct SARS-CoV-2 infection in human kidney organoids.** Human kidney organoids form proximal tubule-like structures and express angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2), both of which enable the entry of SARS-CoV-2 into the cells. The virus binds to ACE2 via its spike protein, which is cleaved by TMPRSS2; this cleavage enables viral–cell membrane fusion and release of viral RNA into the cytosol. SARS-CoV-2 infection of these organoid cells results in the activation of pro-fibrotic and pro-inflammatory pathways. JAK, Janus kinase; NF-κB, nuclear factor-κB; STAT, signal transducer and activator of transcription; TGFβ, transforming growth factor-β; TNF, tumour necrosis factor.

Perhaps the most interesting part of the study by Jansen and colleagues[4] are the data obtained from human kidney organoids. This model has been used previously to assess responses to experimental COVID-19 therapies because human kidney organoids express ACE2 and transmembrane protease serine 2 (TMPRSS2) in proximal tubule-like structures[9]. TMPRSS2 is necessary for priming of the spike protein of SARS-CoV-2 and subsequent viral entry into cells[10] (FIG. 1). Kidney organoids are readily infectable with SARS-CoV-2 and provide a model independent of haemodynamic or systemic factors to examine the impact of direct viral invasion on fibrosis. Kidney organoids infected with SARS-CoV-2 upregulated pro-fibrotic signalling pathways compared with uninfected organoids (FIG. 1). Single-cell RNA-seq (scRNA-seq) of the infected organoids revealed 15 distinct cell clusters; SARS-CoV-2 gene expression was detected in 4–25% of proximal tubule cells and 1.4–18% of podocytes. The scRNA-seq data also revealed potential pathways activated by SARS-CoV-2 infection that might lead to cellular injury, dedifferentiation and pro-fibrotic signalling. The authors speculate that activation of these pathways might explain why AKI is so common in patients with severe COVID-19 and might contribute to the possible development of chronic kidney disease. Of note, the protease inhibitor MAT-POS-b3e365b9-1 inhibited SARS-CoV-2 infection in kidney organoids in a dose-dependent manner and decreased both SARS-CoV-2 RNA (by RT-qPCR) and viral titres (by plaque assay)[4].

> “Whether SARS-CoV-2 infects the kidney directly …, even in severe cases of AKI, remains unclear”

In our opinion, whether SARS-CoV-2 infects the kidney directly and how often this might happen, even in severe cases of AKI, remains unclear, and more work is needed to clarify these questions. The study by Jansen and colleagues[4] lends support to direct kidney infectivity having a role in the complex pathophysiology of kidney complications associated with COVID-19 but lacks information on clinical counterparts such as AKI and collapsing glomerulopathy. By showing enhanced kidney fibrosis after SARS-CoV-2-infection using autopsy samples and human kidney organoids, however, the study does provide insight into this possible connection. Further studies to demonstrate direct kidney invasion and its timing should include kidney biopsies coupled with urine viral studies, as well as staining of tubule cells for SARS-CoV-2 nucleocapsid or spike protein.

Luise Hassler and Daniel Batlle ✉

Division of Nephrology/Hypertension, Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA.

✉*e-mail: d-batlle@northwestern.edu*

https://doi.org/10.1038/s41581-022-00551-6

1. Hirsch, J. S. et al. Acute kidney injury in patients hospitalized with COVID-19. *Kidney Int.* **98**, 209–218 (2020).
2. Cheng, Y. et al. Kidney disease is associated with in-hospital death of patients with COVID-19. *Kidney Int.* **97**, 829–838 (2020).
3. Batlle, D. et al. Acute kidney injury in COVID-19: emerging evidence of a distinct pathophysiology. *J. Am. Soc. Nephrol.* **31**, 1380–1383 (2020).
4. Jansen, J. et al. SARS-CoV-2 infects the human kidney and drives fibrosis in kidney organoids. *Cell Stem Cell* **29**, 217–231.e8 (2022).
5. Ye, M. et al. Glomerular localization and expression of angiotensin-converting enzyme 2 and angiotensin-converting enzyme: implications for albuminuria in diabetes. *J. Am. Soc. Nephrol.* **17**, 3067–3075 (2006).
6. Hassler, L., Reyes, F., Sparks, M. A., Welling, P. & Batlle, D. Evidence for and against direct kidney infection by SARS-CoV-2 in patients with COVID-19. Clin. *J. Am. Soc. Nephrol.* **16**, 1755–1765 (2021).
7. May, R. M. et al. A multi-center retrospective cohort study defines the spectrum of kidney pathology in coronavirus 2019 disease (COVID-19). *Kidney Int.* **100**, 1303–1315 (2021).
8. Caceres, P. S. et al. High SARS-CoV-2 viral load in urine sediment correlates with acute kidney injury and poor COVID-19 outcome. *J. Am. Soc. Nephrol.* **32**, 2517–2528 (2021).
9. Wysocki, J. et al. A novel soluble ACE2 variant with prolonged duration of action neutralizes SARS-CoV-2 infection in human kidney organoids. *J. Am. Soc. Nephrol.* **32**, 795–803 (2021).
10. Davidson, A. M., Wysocki, J. & Batlle, D. Interaction of SARS-CoV-2 and other coronavirus with ACE (angiotensin-converting enzyme)-2 as their main receptor: therapeutic implications. *Hypertension* **76**, 1339–1349 (2020).

Acknowledgements
We acknowledge Jan Wysocki and Juan Carlos Velez for the careful reading of the manuscript and their suggestions before submission. We acknowledge the support of a gift from the Joseph and Bessie Feinberg Foundation, NIH grant 1R21 AI166940-01 (D.B.) and a stipend from the BMEP to L.H.

Competing interests
D.B. is co-inventor of patents entitled “Active Low Molecular Weight Variants of Angiotensin Converting Enzyme 2 (ACE2)”, “Active low molecular weight variants of Angiotensin Converting Enzyme 2 (ACE2) for the treatment of diseases and conditions of the eye” and “Soluble ACE2 Variants and Uses therefor”; is founder of Angiotensin Therapeutics Inc.; has received consulting fees from AstraZeneca, Relypsa and Tricida (all unrelated to this work); and has received unrelated support from National Institute of Diabetes and Digestive and Kidney Diseases grant R01DK104785, as well as from a grant from AstraZeneca. L.H. declares no competing interests.