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Kidney Disease and Viral Infection in COVID-19: Why Are Kidney Organoid and Biopsy Studies Not in Agreement?

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Abstract
Context: The clinical course of coronavirus disease-19 (COVID-19) can be complicated by acute kidney injury and proteinuria. Kidney cells express receptors for SARS-CoV-2, the virus responsible for COVID-19. Direct infection of the kidney parenchyma by SARS-CoV-2 has been proposed as the cause of renal dysfunction in COVID-19. ***Subject of Review:*** Kidney organoids derived from human embryonic stem cells or induced pluripotent cells can be reproducibly infected by SARS-CoV-2 in vitro and used to study therapeutics. However, kidney biopsy studies of COVID-19 patients with renal dysfunction have shown no evidence of viral infection. ***Second Opinion:*** Kidney organoids are susceptible to SARS-CoV-2 infection, which is probably facilitated by their limited architectural complexity and maturation compared to the intact organ and by the in vitro culture conditions. Conversely, kidneys in COVID-19 patients appear resistant to infection and may be injured through indirect mechanisms mediated by the host response to the respiratory viral infection, genetic susceptibility to the immune response, physiological disturbances, and therapies. More studies are needed to better understand why kidney organoids are more susceptible than mature kidneys to SARS-CoV-2 infection and further characterize the mechanisms of kidney injury in COVID-19.

Coronavirus disease-19 (COVID-19) patients admitted to the intensive care unit with respiratory failure and pneumonia often develop acute kidney injury (AKI) which complicates the course of the disease [1]. AKI with associated heavy proteinuria can also develop in COVID-19 patients presenting with mild upper respiratory infection in the absence of pneumonia [2]. Some patients may develop proteinuria without concurrent AKI [3]. Understanding the mechanisms of renal dysfunction in COVID-19 is essential to effectively treat patients and prevent progression of the disease.

The main histologic finding in COVID-19 patients with AKI is acute tubular injury/acute tubular necrosis. In some cases, AKI is associated with endothelial injury and thrombotic microangiopathy (TMA). Patients presenting with heavy proteinuria and nephrotic syndrome develop collapsing glomerulopathy in association with acute tubular injury [4]. Two main mechanisms of kidney injury have been proposed: (1) direct infection of the renal parenchyma/vasculature by SARS-CoV-2, the etiologic agent of COVID-19; and (2) indirect effects of infection including exaggerated immune response to the virus, genetic susceptibility to the immune response, physiological disturbances related to lung disease, and therapies to treat the virus-induced alveolar damage [4].

Early studies mostly conducted on autopsy material reported the finding of particles resembling coronavirus not only in the bronchial and lung alveolar epithelium but

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also in peripheral organs including the kidney where viral-like particles were noted by electron microscopy (EM) in tubular epithelial cells, podocytes, and endothelial cells [5–7]. Unfortunately, many studies have misinterpreted as coronavirus cellular organelles such as clathrin-coated vesicles, coatomer-coated vesicles, and multivesicular bodies. In a recently published review by Bullock et al. from the Center for Disease Control and Prevention (CDC, Atlanta, USA), at least 53 papers were found to have incorrectly identified the coronavirus in different organs [8]. Criteria were therefore proposed for the rigorous identification of viruses by EM based on detailed knowledge of the coronavirus morphology and replicative process. Criteria required for the conclusive identification of coronavirus include size (60–140 nm), surface spikes (mostly visible in extracellular particles; intracellular particles rarely have obvious spikes), small electron dense dots in each particle representing the helical nucleocapsid, and the demonstration of viral particles within cytoplasmic vacuoles or attached to the cell surface after their release via exocytosis. Coronavirus particles form by budding through the membranes of the endoplasmic reticulum/Golgi complex into vacuolar structures and are not found free in the cytoplasm. Moreover, the finding of an isolated viral-like particle by EM is not sufficient for diagnosis since coronavirus infection is characterized by large accumulations of virions [8, 9]. For a detailed description of the pitfalls that can be encountered in the ultrastructural diagnosis of coronavirus infection, the reader is referred to previous reports which include EM images [8, 9] and schematics [9] comparing coronavirus particles to their cytoplasmic mimics.

Additional methods that have been used to demonstrate evidence of SARS-CoV-2 infection in the human kidney include immunohistochemistry (IHC), in situ hybridization (ISH), RT-PCR, and single-cell RNA sequencing (scRNAseq) [10, 11]. The finding of viral proteins by IHC or nucleic acids by ISH and/or RT-PCR in the setting of unequivocal demonstration of coronavirus particles by EM provides confirmatory and specific evidence of SARS-CoV-2 infection [8, 9]. However, in the absence of ultrastructural demonstration of productive viral infection and morphologically diagnostic coronavirus particles, positive IHC or ISH results cannot distinguish intact viral particles from viral proteins or nucleic acids that have been produced in excess or released as degradation products and nonspecifically bound to and/or absorbed by cells [11]. Similarly, detection of SARS-CoV-2 in the kidney by RT-PCR may indicate parenchymal infection but can also be a nonspecific finding due to the presence of viral nucleic acid in the circulating blood, glomerular ultrafiltrate, preurine, and urine. Caution and strict adherence to rigorous criteria of coronavirus identification in the injured kidney are therefore essential to confirm the validity of the direct viral infection hypothesis.

SARS-CoV-2 penetrates cells by binding to angiotensin-converting enzyme 2 (ACE2), a homolog of angiotensin-converting enzyme [12]. The viral nucleocapsid becomes internalized into cells following proteolysis by proteases such as transmembrane serine protease 2 (TMPRSS2) and cathepsin B/L [12]. Proximal tubular epithelial cells express ACE2 and TMPRSS2 and can be infected with SARS-CoV-2 in vitro [13]. Podocytes have low levels of ACE2 and TMPRSS2 but express noncanonical receptors such as BSG/CD147, and induced pluripotent stem cells (iPSC)-derived podocyte-like cells have been successfully infected in vitro with SARS-CoV-2 [14]. Kidney cells are therefore potentially susceptible to SARS-CoV-2 infection.

Data in support of the direct viral infection hypothesis have also emerged from the literature on kidney tissue organoids (Table 1) [15]. Organoids can be generated in vitro from human embryonic stem cells (ESC) or iPSC which, in response to organ-specific molecular factors, differentiate in multiple cell lineages that self-organize according to developmental patterning programs specific for the tissue being modeled [16]. Tissue organoids mimic the microscopic anatomy, cell populations, and functions of the organ of interest and have self-renewal capacity. Organoids have been used as models of disease for drug development, tissue engineering, and regenerative medicine [15, 16].

In a recent study by Jansen et al. [17], kidney organoids obtained by mixing human iPSC-derived ureteric epithelium and metanephric mesenchyme were used to demonstrate the capacity of SARS-CoV-2 to infect the kidney. Organoids generated with this method contained nephron-like structures which stained for markers of tubular and podocyte differentiation. Infection of the organoids was confirmed by viral RNA expression, ISH, IHC, and EM. SARS-CoV-2 RNA expression was localized by ISH in podocytes and proximal tubular epithelium, whereas SARS-CoV-2 nucleocapsid protein was identified by IHC in podocytes, proximal tubular epithelium, and stromal cells. Immune-based correlative light microscopy and EM identified viral particles adhering to strict criteria of virus identification in SARS-CoV-2 nucleocapsid-positive mesenchymal cells. Viral gene expression was detected by scRNAseq in neural cells and their progenitors, proximal tubular cells, podocytes, and mesenchymal cells.

Table 1. Studies showing evidence of SARS-CoV-2 infection in kidney organoids

Authors	Type of kidney organoid	Methods of SARS-CoV-2 infection identification	Results	Cells infected	Inhibition of infection
Jansen et al. [17]	iPSC-derived	Immunofluorescence, ISH, qRT-PCR, scRNAseq, EM	Positive	Neural cells, neural progenitor cells, stromal/mesenchymal cells, proximal tubular epithelial cells, podocytes	Protease inhibitor (MAT-POS-b3e365b9-1)
Garreta et al. [18]	ESC-derived and iPSC-derived	Immunofluorescence, EM, qRT-PCR	Positive	Proximal tubular epithelial cells	ACE2 genetic deletion
Monteil et al. [19]	ESC-derived	qRT-PCR, functional virion production tested in Vero cells	Positive	Not shown	Human soluble ACE2
Wysocki et al. [20]	ESC-derived	qRT-PCR	Positive	Not shown	Human soluble ACE2
Helms et al. [21]	ESC-derived, iPSC-derived, and PKD2−/− derived	SARS-CoV-2-mNG/ immunofluorescence, functional virion production tested in Vero cells	Positive	Proximal tubular epithelial cells	ACE2 genetic deletion, LCB1 (spike binding protein)
Rahmani et al. [22]	iPSC-derived	SARS-CoV-2-mNG/ immunofluorescence, functional virion production tested in Vero cells	Positive	Proximal tubular epithelial cells	Losartan

Infected organoids showed increased interstitial collagen production by IHC and Masson trichrome staining. Collagen deposition by IHC could be ameliorated by treating organoids with a transforming growth factor β inhibitor [17]. In this study, SARS-CoV-2 nucleocapsid protein was demonstrated in autopsy kidneys by PCR and immunofluorescence though no confirmatory EM studies showing actual coronavirus particles in the kidney tubular epithelium were presented [17].

Viral particles with ultrastructural features of coronavirus were demonstrated in SARS-CoV-2-infected ESC- or iPSC-derived organoids by Garreta et al. [18]. In this study, infected cells were shown to be primarily proximal tubular epithelial cells by IHC. Kidney organoids exposed to diabetic-type culture conditions showed enhanced expression of the SARS-CoV-2 receptor ACE2 and exhibited higher viral loads compared to controls. Genetic deletion of ACE2 by CRISPR technology prevented viral infection of organoids in both control and diabetic-type culture conditions. In the same work, the authors showed that tubular epithelial cells isolated from diabetic kidney biopsies expressed higher ACE2 levels and were more susceptible to SARS-CoV-2 infection than tubular epithelial cells of nondiabetic patients [18]. In an earlier study, Monteil and coworkers [19] showed that SARS-CoV-2 infection of human vascular or kidney organoids could be inhibited by human recombinant soluble ACE2. Proof of successful infection in this study was provided by demonstrating the presence of viral RNA in the organoids by qRT-PCR and by testing the organoid supernatant for its capacity to efficiently infect target cells (Vero E6) [19]. Neutralization of SARS-CoV-2 infection with soluble ACE2 protein was also reported by Wysocki and coworkers in an ESC-derived kidney organoid model in which SARS-CoV-2 viral infection was analyzed by qRT-PCR [20].

Using genetically engineered SARS-CoV-2 expressing a fluorescent protein (mNeonGreen) and immunofluorescence staining for different cell markers, Helms et al. [21] were able to demonstrate viral infection in proximal tubular epithelial cells of kidney organoids surrounded by a monolayer of stromal and endothelial cells. The fluorescent signal localized to the organoids, whereas infection of the surrounding stromal or endothelial cells was not detected. Infected organoids produced replicating virus and showed features of cell injury and a proteomic pattern of interferon pathway upregulation resembling the urine proteomic signature of severely ill COVID-19 patients. Organoid infection was ameliorated by genetic ablation of ACE2 or peptides that interfere with SARS-CoV-2 binding to cell receptors. Rahmani et al. used a similar infection protocol with SARS-CoV-2 mNeonGreen, immunofluorescence, and scRNAseq and showed that proximal tubular epithelial cells in kidney organoids are the most susceptible renal cells to infection based on their repertoire of cell surface receptors and proteases. They also found that losartan, an ANG2 receptor blocker, attenuated the kidney organoid infection by SARS-CoV-2 [22].

Table 2. Kidney biopsy studies showing lack of evidence of SARS-CoV-2 infection in COVID-19 patients with renal dysfunction

Authors	Number of patients	Main diagnoses	Methods of SARS-CoV-2 identification	Results
Akilesh et al. [2]	17 (3 transplants)	Acute tubular injury, collapsing glomerulopathy, endothelial injury/TMA, active antibody-mediated rejection	EM, IHC, ISH	Negative
May et al. [3]	235 (38 transplants)	ATI, collapsing glomerulopathy/FSGS, minimal change disease, diabetic nephropathy, acute cellular or antibody-mediated rejection	IHC, ISH	Negative
Basic-Jukic et al. [23]	7 (all transplants)	ATI, collapsing glomerulopathy/FSGS, chronic antibody-mediated rejection, borderline acute cellular rejection	EM	Negative
Couturier et al. [24]	2	Collapsing glomerulopathy, ATI	RT-PCR	Negative
Ferlicot et al. [25]	47 (2 transplants)	ATI, collapsing glomerulopathy/FSGS, acute vascular nephropathy/TMA	EM, IHC	Negative
Gambella et al. [26]	9 (2 pediatric cases, 1 transplant)	Minimal change disease, ATI, collapsing glomerulopathy, C3 glomerulopathy	EM	Negative
Kudose et al. [27]	17 (3 transplants)	ATI, collapsing glomerulopathy, immune-mediated glomerulonephritis, acute cellular rejection, infarction	EM, IHC, ISH	Negative
Nasr et al. [28]	13	ATI, collapsing glomerulopathy	EM, ISH	Negative
Nomura et al. [29]	5 (all pediatric cases)	TMA, minimal change disease, IgA vasculitis	EM	Negative
Sharma et al. [30]	10	ATI, collapsing glomerulopathy/FSGS, TMA, pauci-immune glomerulonephritis	EM, IHC	Negative
Wu et al. [31]	6	ATI, collapsing glomerulopathy	EM, ISH	Negative

ATI, acute tubular injury.

Taken together, studies with kidney organoids support the direct viral infection hypothesis, with proximal tubular epithelial cells representing the primary target of SARS-CoV-2. However, many studies from groups across the world have found no evidence of viral infection in kidney biopsies from COVID-19 patients with renal dysfunction using rigorous ultrastructural criteria of coronavirus identification corroborated by ISH and/or IHC (Table 2) [2, 3, 23–31].

What are the reasons behind the discrepancy? Why do kidney biopsies show no evidence of SARS-CoV-2 infection while kidney organoids can be reproducibly infected with the virus? One possible explanation is that mature kidneys are resistant to infection in vivo, whereas the in vitro environment, culture conditions, mode of virus delivery, and intrinsic features of the kidney organoid model favor SARS-CoV-2 entry into susceptible cells.

Kidney organoids are a valuable model system to study the biology of SARS-CoV-2 infection, generate viral progeny, identify mechanisms of diseases that impact COVID-19, and test the efficacy of antiviral drugs. However, kidney organoids lack the architectural complexity of an adult kidney, and this feature may facilitate viral entry into cells. Kidney organoids contain clusters of endothelial-like cells [17–19] but do not have the highly specialized glomerular and peritubular capillary vasculature that plays a critical role in the function of the mature kidney. Differentiated endothelial cells do not express the ACE2 receptor or express it at low levels, are resistant to infection [32], and provide a protective in vivo barrier for SARS-CoV-2 entry into susceptible cells such as the tubular epithelium. This protective barrier is lacking in the in vitro environment of kidney organoids, where all cell types can potentially be reached by the virus which is delivered in the culture medium. Podocyte-like cells present in organoids form aggregates, but they do not have well-developed foot processes and an associated fenestrated capillary endothelium to form an intact glomerular capillary wall. The absence of circulating blood is an additional limitation, as kidney organoids may develop a hypoxic core with resulting metabolic deficiency [16]. The lack of circulating leukocytes, which play a critical role in the host innate immune defense, may weaken the tissue response to the virus and further facilitate infection. In addition, kidney organoids may contain incompletely matured cells and off-target cells of non-kidney origin which

could alter the molecular milieu, interfere, and possibly facilitate the viral infection through paracrine mechanisms [15, 17]. An additional limitation of the tissue organoid model is the variability of results. For example, in the study by Jansen et al., RNAseq studies showed viral nucleic acids in neural cells and their progenitors, tubular epithelial cells, podocytes, endothelial cells, and mesenchymal cells, whereas others have shown viral expression primarily in tubular epithelial cells [17–22]. Differences in kidney organoid maturity and the presence of off-target cell populations are related to the specific protocols and cell lines used by different laboratories [33].

Thus, kidney organoids are susceptible to SARS-CoV-2 infection whereas kidney biopsies show no evidence of infection, possibly due to protective in vivo mechanisms that are not present in the organoid cultures. The biopsy findings strongly implicate indirect mechanisms of injury in the pathogenesis of renal disease in COVID-19. For example, interferons, which can be elevated in patients with COVID-19, have been shown to synergize with APOL1 high-risk phenotypes, triggering collapsing glomerulopathy [34–36]. Similarly, TMA could be the result of inflammatory cytokine-induced endothelial injury, platelet dysfunction, and hypercoagulability [32].

Although the kidney biopsy results clearly favor indirect effects of infection, results with cell culture and organoid studies raise the possibility that under certain conditions, for example, in cases of severe COVID-19 when protective vascular and immune barriers fail, the renal parenchyma might become infected also in the in vivo environment. Notably, a study by Braun et al. [13] showed that 60% of postmortem kidney samples from patients with severe COVID-19 contained SARS-CoV-2 mRNA. In this study, they were also able to isolate infectious SARS-CoV-2 from an autopsy sample which was capable of replicating in non-human primate tubular epithelial cells [13]. In keeping with this observation, Caceres et al. [37] reported that a high SARS-CoV-2 viral load detected in the urine sediment by RT-PCR correlated with kidney injury and poor outcome, though no infective virions were found in the urine of these patients. These studies show correlation between viral load and severity of disease but lack confirmatory morphologic evidence of coronavirus particles and productive viral infection in the native kidney tissue of the patients examined. Moreover, kidney biopsy studies showing no evidence of SARS-CoV-2 infection included patients with severe COVID-19 and poor outcome [23, 25, 27–30], who presumably had high urine viral load [37]. In addition, reports of coronavirus-like particles identified in postmortem kidneys by other groups have been questioned due to the lack of rigorous criteria of virus identification, the resemblance of these particles to cytoplasmic organelles, and the presence of postmortem artifacts [8, 9]. Ultrastructural evaluation of postmortem kidneys could be significantly improved in the future by the adoption of rapid autopsy protocols [38]. The mitigation of postmortem artifacts with this approach combined with the use of rigorous EM criteria of virus identification would facilitate the distinction between putative coronavirus particles and their cellular mimics in autopsy material.

The negative biopsy findings could be explained by a transient infection of the renal parenchyma. In support of this hypothesis, a "hit and run" scenario of in vivo kidney infection has been proposed, whereby kidneys are transiently infected by SARS-CoV-2 [4] and the virus is cleared from the renal parenchyma by the time injury becomes clinically manifested and biopsies are performed [4]. There are, however, currently no data in support of this hypothesis which require further scrutiny and research. Characterizing the time course of a possible transient kidney infection will be difficult with kidney biopsies. Animal models of respiratory SARS-CoV-2 infection established during the past 2 years have significantly contributed to our understanding of COVID-19 pathogenesis and transmission dynamics [39]. Application of these models to the renal complications of SARS-CoV-2 infection may bring additional knowledge to the field and provide new insights into the pathogenesis of kidney disease in COVID-19.

While more studies are conducted to investigate whether kidneys can be infected by SARS-CoV-2 in vivo, more work is needed to better understand why kidney organoids are susceptible to the infection in the in vitro environment. Particularly important will be to develop methods that further promote the maturation and complexity of the organoids and more closely reproduce the tissue architecture and vasculature of an adult kidney.

Organoids with transcriptional signatures matching the second trimester gestational kidney and tubuloids with mature tubular structures have been developed, but their complexity is limited, and they lack a functional vascular bed [40, 41]. Although the generation of higher-level structures such as nephrons with intact glomerular capillary filter remains a challenge, microfluidic chip studies have shown that organoids exposed to perfusion shear stress develop an augmented vasculature with perfusable lumens [42]. Improved vascularization of kidney organoids was also obtained by using low oxygen levels comparable to those of a developing kidney [43] and by

implanting organoids beneath the renal capsule of rat recipients [44] or on the chorioallantoic membrane of the chick embryo [40]. In all these studies, vascularization promoted maturation of the organoids [40, 42–44]. Enhanced differentiation and maturation were also achieved by culturing kidney organoids in soft 3-D biomaterial gels [40, 45], which were reported in one study to reduce off-target cells [45]. Kidney organoids have proven to be an invaluable model to study mechanisms of SARS-CoV-2 infection, the effectiveness of antiviral drugs, and the impact of conditions such as diabetes on the infection [18, 46]. However, when compared with injured kidneys of COVID-19 patients, which show no evidence of SARS-CoV-2 on biopsy, the organoids have demonstrated to be distinctly susceptible to the infection. This discrepancy is probably related to the organoid limited maturation and architectural complexity including the lack of a functional vasculature which in vivo could act as a protective barrier to the virus. Thus, to reproduce the pathophysiology of the disease more effectively, it will be important to deliver the virus to organoid cells through a perfusable vasculature lined by differentiated endothelial cells. Additional strategies should be pursued to further promote the maturation of parenchymal and stromal cells and to provide immune cells to the organoids. Achievement of these milestones will bring organoids closer to a functioning in vitro kidney model which more closely mimics the in vivo environment of COVID-19 patients. It is possible that in the setting of a fully vascularized and differentiated kidney organoid containing immune cells, SARS-CoV-2 may not be able to cross the vascular barrier and infect tubular epithelial cells or podocytes. A vascularized organoid model could then be challenged with conditions associated with severe cases of COVID-19 such as high circulating viral load [47]. It could also be used to investigate indirect effects of the SARS-CoV-2 infection such as cytokine-mediated endothelial injury and TMA [32]. As lessons learned from kidney biopsy and organoid studies conducted to date are applied to future investigations, discrepant findings will likely be resolved toward an improved understanding of the mechanisms of kidney injury in COVID-19 and the development of more effective therapies.

Conflict of Interest Statement

The author has no conflicts of interest to disclose.

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