Ultrastructural Evidence for Direct Renal Infection with SARS-CoV-2

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ABSTRACT

Background A significant fraction of patients with coronavirus disease 2019 (COVID-19) display abnormalities in renal function. Retrospective studies of patients hospitalized with COVID-19 in Wuhan, China, report an incidence of 3%–7% progressing to ARF, a marker of poor prognosis. The cause of the renal failure in COVID-19 is unknown, but one hypothesized mechanism is direct renal infection by the causative virus, SARS-CoV-2.

Methods We performed an autopsy on a single patient who died of COVID-19 after open repair of an aortic dissection, complicated by hypoxic respiratory failure and oliguric renal failure. We used light and electron microscopy to examine renal tissue for evidence of SARS-CoV-2 within renal cells.

Results Light microscopy of proximal tubules showed geographic isometric vacuolization, corresponding to a focus of tubules with abundant intracellular viral arrays. Individual viruses averaged 76 μm in diameter and had an envelope studded with crown-like, electron-dense spikes. Vacuoles contained double-membrane vesicles suggestive of partially assembled virus.

Conclusions The presence of viral particles in the renal tubular epithelium that were morphologically identical to SARS-CoV-2, and with viral arrays and other features of virus assembly, provide evidence of a productive direct infection of the kidney by SARS-CoV-2. This finding offers confirmatory evidence that direct renal infection occurs in the setting of AKI in COVID-19. However, the frequency and clinical significance of direct infection in COVID-19 is unclear. Tubular isometric vacuolization observed with light microscopy, which correlates with double-membrane vesicles containing vacuoles observed with electronic microscopy, may be a useful histologic marker for active SARS-CoV-2 infection in kidney biopsy or autopsy specimens.

The association between coronavirus disease 2019 (COVID-19) and ARF is an evolving area of study. Renal dysfunction is common in COVID-19, with a sizeable fraction of patients presenting with proteinuria or elevated BUN.1,2 A minority of patients progress to more severe renal disease; retrospective studies of patients hospitalized with COVID-19 in Wuhan, China, report an incidence of ARF ranging from 3% to 7%.3–5 Early data from Italy suggests a correlation between kidney injury and overall disease severity, with renal failure up to four times more common in patients in intensive care units relative to hospitalized patients not requiring intensive care unit admission.6 Renal failure in COVID-19 appears to be an independent risk factor for death in hospitalized patients, with reported adjusted hazard ratios of 3.5 (95% CI, 1.50 to 8.27) and 4.7 (95% CI, 2.55 to 8.75) for stage 2 and 3 AKI, respectively.1 Preexisting renal disease may also present an increased risk for poor outcome, with an adjusted hazard ratio of 2.0 (95% CI, 1.32 to 3.15) for hospitalized patients. Various mechanisms for kidney injury in COVID-19 have been proposed, including ischemic injury owning to multiorgan failure, inflammatory injury secondary to cytokine storm, and direct infection.2,8 Immunohistochemical and PCR-based approaches are possible approaches to demonstrate direct renal infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), but both could be confounded by either filtration of circulating viral antigens or incidental detection of nucleic acids in plasma. Detection of virus within renal cells by electron microscopy has been proposed as a straightforward approach to demonstrate direct renal involvement.

CLINICAL PRESENTATION

A 53-year-old male with a history of obesity, hyperlipidemia, and a recent sick...
contact presented with an acute type A aortic dissection, extending from the sinotubular junction to the iliac bifurcation, with compression of the right renal artery. On angiogram, renal arteries had a beaded appearance suspicious for fibromuscular dysplasia. The dissection was repaired via median sternotomy with ascending and hemiaortic replacement, and the patient was discharged to intensive care on mechanical ventilation. His postoperative course was notable for leukocytosis (white blood cell count of 14,000–18,000 per μl) and persistently elevated fraction of inspired oxygen requirements. On the fourth postoperative day he was successfully extubated but remained oxygen-dependent on high-flow nasal cannula and Bilevel Positive Airway Pressure.

On the sixth postoperative day, the patient’s hypoxemia progressed, requiring reintubation as well as inhaled nitrous oxide. A chest x-ray showed bilateral patchy opacities, a PCR respiratory viral panel was negative, and a locally performed RT-PCR was positive for SARS-CoV-2. He was initially treated with hydroxychloroquine 600 mg twice daily, then 200 mg three times daily for 3 days. In addition, he enrolled in a randomized, controlled trial of sarilumab (IL-6 inhibitor), although it is not known whether he received therapy or placebo. Despite therapy, the patient developed multiorgan dysfunction, with mildly elevated liver enzymes and new-onset diabetes. On the 11th postoperative day, his respiratory and renal status worsened with progressive hypoxemia and acidosis recalcitrant to intravenous bicarbonate.

The patient’s renal function closely mirrored his respiratory function (Figure 1). Initially, his eGFR declined acutely after the cardiopulmonary resuscitation. From 42 ml/min per 1.73 m² on admission to 29 ml/min per 1.73 m². In the setting of diuresis with furosemide and metolazone on postoperative day 4, his eGFR further declined to 17 ml/min per 1.73 m². His renal function briefly improved (eGFR 22 ml/min per 1.73 m²), but subsequently deteriorated on postoperative day 6, simultaneous with the development of acute respiratory distress. Over the course of his illness, his urine output remained between 2 and 4 L per day, but on the 11th postoperative day he became acutely oliguric (100 ml/d), and continuous RRT was initiated in the setting of acidosis and declining respiratory status.

The patient died of cardiac arrest on the 12th postoperative day, and an autopsy was performed in a negative pressure suite, with renal tissue taken for light and electron microscopy. Because of the risk of infectious aerosols, the autopsy was limited to the chest and abdomen, and routine protective equipment (N95 respirator, gown, double gloves, and eye protection) was supplemented with face shields and disposable full-body suits.

**RESULTS**

At autopsy, the lungs showed gross and microscopic evidence of diffuse acute alveolar damage with well-formed hyaline membranes, edema, and early acute bronchopneumonia, consistent with early reports of patient deaths after SARS-CoV-2 infection. The left and right kidneys weighed 230 g and 240 g, respectively, and were grossly unremarkable. Light microscopic examination of formalin-fixed paraffin sections of the kidneys showed mild autolysis, with no definitive nuclear inclusions (not shown). There was no evidence of glomerulitis, FSGS, or a tubulointerstitial inflammatory infiltrate. Of note, some of the toluidine blue–stained epoxy sections showed focal tubular isometric vacuolization (Figure 2A). Other epoxy sections showed mild-to-moderate necrosis and karyolysis more typical for ischemic injury/autolysis (Figure 2B).

Ultrastructural analysis, limited to the kidney, revealed abundant viral forms within tubular epithelial cells in one of three examined blocks, correlating directly to the areas of isometric vacuolization (Figure 3A). Individual viruses ranged in size from 65 to 91 nm (mean 76 nm). Mature-appearing viruses were predominantly located within the cytoplasm, focally organized into small arrays (Figure 3B). Viral particles were composed of cores with intermediate electron density, surrounded by an envelope studded with abundant crown-like, electron-dense spikes (Figure 3C). Adjacent vacuoles contained abundant ovoid double-membrane vesicles associated with rough endoplasmic reticulum, representing possible viral assembly (Figure 3D). Examined glomeruli and vascular endothelial cells were negative for virus, and coronavirus was not identified in tubular epithelium with more conventional injury and necrosis by light microscopy.

**DISCUSSION**

There are currently multiple plausible mechanisms for renal injury in COVID-19, including ischemic injury, cytokine storm, and direct infection. Previous research has established a potential mechanism for kidney infection by SARS-CoV-2. The receptor for SARS-CoV-2 cellular entry is angiotensin-converting enzyme 2, which is present at high concentrations in the brush borders of renal tubular epithelial cells, and at lower levels in glomerular and vascular endothelial cells. In vitro experiments have demonstrated SARS-CoV-2
viral infection and replication within Vero cells, a primate kidney epithelial cell line.\textsuperscript{10,11} In humans, SARS-CoV-2 was initially detected by RT-PCR in the urine of a minority of patients with COVID-19; although other studies show no detectible urine shedding.\textsuperscript{12,13} Recently, Su \textit{et al.}\textsuperscript{14} demonstrated coronavirus by electron microscopy in the renal tubular epithelium of seven of 26 patients with COVID-19 at autopsy.

Electron micrographs from this autopsy confirm this finding and provide strong evidence for direct infection. The crown-like morphology is similar to previously published images, and the viral diameter is within the reported 70–90 \textmu m size range of SARS-CoV-2.\textsuperscript{10} The organization of intracellular viruses into arrays is suggestive of intracellular manufacture and assembly. The presence of double-membrane vesicles with possible viral assembly near the rough endoplasmic reticulum in this case is analogous to the proposed mechanism for viral assembly of SARS-CoV-1.\textsuperscript{15} Finally, vacuolation of epithelial cells has been reported after infection with SARS-CoV-1, similar to the isometric vacuoles in the proximal tubular epithelium in this patient.\textsuperscript{16} The isometric vacuoles by light microscopy correlate with double-membrane vesicles containing vacuoles by electron microscopy and were also noted in a recent autopsy series.\textsuperscript{14} Isometric vacuolization may be a helpful diagnostic clue for the presence of direct renal infection, but this association needs to be confirmed in a larger series using immunohistochemistry or electron microscopy.

There are notable caveats to the findings in this case report. First, although

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1}
\caption{Kidney function recrudescence with diagnosis of COVID-19. The patient’s eGFR over the course of hospitalization, measured from admission for aortic repair on day 0. eGFR calculated by the Chronic Kidney Disease Epidemiology Collaboration equation. CRRT, continuous RRT.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{Proximal tubular isometric vacuolization by light microscopy correlates with SARS-CoV-2 infection. (A) Toluidine blue–stained epoxy section with extensive isometric vacuolization of proximal tubular epithelial cells, corresponding to location of intracellular virus by electron microscopy. (B) Tubular epithelial cells from a section showing mild autolysis and no vacuolization. Electron microscopy showed no virus present in this section. Both images at \times 400 magnification.}
\end{figure}
this renal tissue showed only mild post-
mortem change by light microscopy, there is still significant ultrastructural
injury. Disrupted plasma membrane-
bound organelles could mimic viral
structures. Second, direct kidney infec-
tion may be an uncommon occurrence
in patients with COVID-19 and renal
dysfunction. Indeed, there was no detect-
ible intrarenal virus by electron micros-
copy in one additional autopsy of a
patient with COVID-19, although that
patient had nonoliguric stage 2 AKI
and interpretation was limited by exten-
sive autolysis. Most importantly, the
identification of direct infection by
SARS-CoV-2 does not preclude other
mechanisms of renal injury in COVID-
19. In this case, coronavirus was only
identified in a subset of tubules, and di-
rect infection is likely either an incidental
finding or a contributing cause of kid-
ney injury. This patient’s renal failure
was likely multifactorial, with preexisting
ischemic renal injury complicated by
progressive hypoxemia and focal viral
infection.

This autopsy report is illustrative of
the increased morbidity and mortality
associated with renal failure and
COVID-19. Additional studies are
needed to determine the frequency of di-
rect renal infection in patients with
COVID-19, and whether there is an as-
sociation with viral titers in plasma or
with preexisting renal comorbidities.

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REFERENCES

Dong L, et al.: Kidney disease is associated
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There are two locations where the dimensions are listed incorrectly in μm when they should be in nm. The first is in the abstract: “averaged 76 μm in diameter” should be “averaged 76 nm in diameter.” The second is in the Discussion: “within the reported 70–90 μm size range of SARS-CoV-2” should be “within the reported 70–90 nm size range of SARS-CoV-2.”