Histopathologic and Ultrastructural Findings in Postmortem Kidney Biopsy Material in 12 Patients with AKI and COVID-19

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Since its emergence in Wuhan, China, coronavirus disease 2019 (COVID-19) has spread rapidly throughout the world. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) primarily targets the lung, but other organs may be affected, and kidney involvement is frequent. Recent data from New York City found a 36.6% incidence of AKI among 5499 hospitalized patients with COVID-19.1 Of those who developed AKI, 14.3% required RRT in this study. Among those who required mechanical ventilation, 86.9% developed AKI, and 23.2% of intubated patients required RRT.

The pathophysiology of AKI in the setting of COVID-19 infection remains to be fully elucidated. Several of the studies and patient reports focusing on renal involvement with COVID-19 published to date provide different and sometimes conflicting results.2–9 In an attempt to further characterize the pathophysiology of AKI associated with COVID-19 infection, we performed postmortem kidney biopsies in a United States urban multiracial patient population with clinically significant AKI.

Postmortem renal-limited percutaneous autopsies were offered from April 24, 2020 to May 8, 2020 at Lenox Hill Hospital in New York City for all patients >18 years with confirmed COVID-19 infection and stage 2 or 3 AKI (as defined by the Kidney Disease Improving Global Outcomes Work Group criteria). We included deceased patients if they had a diagnosis of COVID-19 infection confirmed using real-time RT-PCR testing for SARS-CoV-2 in a respiratory sample along with typical COVID-19 signs and symptoms.

We describe handling of and processing of kidney biopsy material for light and electron microscopy, immunohistochemical assays, and RNA in situ hybridization using RNAScope in Supplemental Material.

The Northwell Health Institutional Review Board approved this patient series as minimal risk research using data collected as part of standard clinical practice.

Of the 16 patients who met criteria over the time period, next of kin declined renal-limited autopsy for 3 patients, and renal tissue was not obtained for another patient. The study assessed 12 patients with COVID-19 and stage 2 or 3 AKI for whom an adequate sample of kidney tissue was obtained in postmortem biopsy. See Supplemental Table 1 for a summary of clinical data for the 12 patients.

The 12 biopsy samples had an average of 27 glomeruli (Table 1) and revealed autolysis ranging from minimal in three cases to mild or moderate in the remaining cases. All patients had a primary pathologic diagnosis of acute tubular injury with focal acute tubular necrosis, which varied from mild (with involvement of isolated tubules) to diffuse (with up to 50% epithelial necrosis). There was no evidence of GN, vasculitis, or thrombotic microangiopathy. One patient had findings compatible with mild acute interstitial nephritis, another exhibited renal oxalosis of uncertain cause, and a third had medullary urate crystal deposits. Eight patients (67%) had no significant glomerular pathology, but two had mild mesangial expansion, and one displayed early diffuse and nodular glomerulosclerosis. Two of these eight patients were diabetic; one of the patients with mild mesangial expansion had hypertension and obesity. Two biopsies had focal

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brown lipofuscin pigment in some tubular epithelial cells, but iron staining was negative. The findings are depicted in Supplemental Figure 1. Electron microscopy examination found no significant glomerular abnormalities except for increased mesangial matrix and thickening of the glomerular basement membranes in a patient with early nodular diabetic glomerulosclerosis. We observed no significant glomerular electron-dense deposits. Glomerular and tubular epithelial cells revealed various degrees of autolytic changes. We identified numerous clathrin-coated vesicles resembling viral particles that varied substantially in size from <20 to >500 nm and found multivesicular bodies in most cases. However, none of the cases exhibited definitive viral particles that would fit the description of coronavirus morphology (Figure 1).

Immunohistochemical assays for SARS-CoV-2 nucleocapsid protein were negative in all 12 cases (Supplemental Figure 1). Confirmatory in situ hybridization performed in four cases had negative results. All assays were run with a lung tissue positive control exhibiting adequate reaction (Figure 1).

This autopsy series represents one of the largest series of patients with COVID-19–associated AKI that includes renal pathology evaluation. The predominant finding in our postmortem kidney biopsies was acute tubular injury with variable degrees of acute tubular epithelial necrosis. Despite the observation that COVID-19 tends to produce a hypercoagulable state,\textsuperscript{10} we saw no evidence of thrombotic microangiopathy or vascular or capillary microthrombi in the biopsy material. Urinalysis revealed that all patients had proteinuria and that many had hematuria, but we found no significant glomerular disease or vasculitis in any cases.

None of the biopsies displayed viral cytopathic effects or necrotizing inflammatory changes. Electron microscopy did not detect viral particles characteristic of coronavirus, as previously characterized and described, despite a careful ultrastructural search in every biopsy. SARS-CoV-2 is characterized by surface projections and an outer coating formed by budding, and the individual particles are approximately 78 nm in diameter. Particles are enclosed in vacuoles within the cell or are seen budding from the cell surface, but they are not commonly seen floating freely in the cytosol.\textsuperscript{11,12} Although our ultrastructural examination of the glomerular and tubular epithelium noted many structures resembling viral particles, including multivesicular bodies and clathrin-coated vesicles, immunohistochemical tests and confirmatory in situ hybridization were negative for SARS-CoV-2. On the basis of these findings, we conclude that there is no significant viral presence in the kidney parenchyma detectable by standard means.

Our results are consistent with the fact that the SARS-CoV-2 virus has generally not been detected in urine, even in patients with viremia,\textsuperscript{14,15} and with other reports that found no immunohistochemical evidence of kidney viral infection.\textsuperscript{4,5} Similarly, patients infected during the 2002–2004 coronavirus outbreak of severe respiratory distress syndrome (SARS) who also developed AKI did not have any evidence of direct kidney viral infection.\textsuperscript{16} If virus is indeed present, it seems to be at very low levels and does not appear to be a significant contributor to renal injury.

Our findings suggest considerable similarities between COVID-19–associated AKI and sepsis-associated AKI, which also generally involves ischemic acute tubular necrosis, resulting from systemic infection and hypoperfusion. In addition, both postmortem and experimental studies of sepsis-associated AKI have observed that the pathologic findings may not fully correlate with functional findings. In our series, 42% of the patients had only mild acute tubular necrosis, defined by ≤10% necrotic tubules, but most

### Table 1. Summary of histopathologic findings

<table>
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<tr>
<th>Patient No.</th>
<th>Tubules Involved by ATN, %</th>
<th>Level of Autolysis</th>
<th>Other Findings</th>
<th>Glomeruli (Total/ Globally Sclerosed)</th>
<th>Glomerular Pathology</th>
<th>IFTA, %</th>
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<tr>
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ATN, acute tubular necrosis; IFTA, interstitial fibrosis and tubular atrophy; AAS, arterial and arteriolar sclerosis; THP, Tamm–Horsfall protein; AlN, acute interstitial nephritis; ME, mesangial expansion; Fe, iron; GS, glomerulosclerosis; PRG, protein reabsorption granule; GH, glomerular hypertrophy.
Figure 1. Electron microscopy and in situ hybridization for SARS-CoV-2 reveal no evidence of viral presence in kidney biopsy material. (A) Electron micrograph of a proximal tubule epithelial cell with many clathrin-coated vesicles aggregated in cytosol, closely resembling viral particles. Original magnification, ×10,000. The white square in (A) is shown magnified in (B) and (C). Original magnification, ×50,000 in B; ×100,000 in C. (D) Multivesicular bodies in the podocyte cytoplasm. Original magnification, ×50,000. (E) RNAscope using probes directed against SARS-CoV-2 shows absence of signal in the patient’s kidney parenchyma. Original magnification, ×200. (F) Adequate quality of tissue is confirmed by RNAscope analysis for mRNA of the housekeeping gene peptidylprolyl isomerase B using 3,3′-diaminobenzidine (brown) chromogen. Original magnification, ×200. (G) Negative control of the in situ hybridization assay. Original magnification, ×200.
of these patients required RRT. Both sepsis and COVID-19 infection are characterized by intense inflammatory dysregulation, including highly elevated cytokines, which likely play a role in the observed renal dysfunction.\textsuperscript{17,18} Additionally, sepsis is associated with microcirculatory alterations and mitochondrial dysfunction that likely exacerbate functional renal failure out of proportion to the extent of cell necrosis.\textsuperscript{17}

In conclusion, in our multiracial patient population with COVID-19–associated AKI, we found that acute tubular injury and epithelial necrosis are the predominant histopathologic findings in postmortem kidney biopsies. We saw no evidence of direct viral infection in our patients who died of COVID-19. Even if present in low levels in the kidney, SARS-CoV-2 is unlikely to cause renal injury by direct infection. The pathophysiology of AKI is most likely similar to that of septic AKI.

DISCLOSURES

All authors have nothing to disclose.

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REFERENCES

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Methods

Post mortem renal limited percutaneous autopsies were offered at Lenox Hill Hospital in New York City with consent of next of kin in all adult patients (>18 years) with confirmed COVID-19 infection and AKI (defined by The Kidney Disease Improving Global Outcomes (KDIGO) Work Group criteria as stage 2 or 3) from April 24 to May 8, 2020. Deceased patients were included if they had a diagnosis of COVID-19 infection confirmed using real-time reverse transcription polymerase chain reaction (rRT-PCR) test for SARS-CoV-2 in a nasopharyngeal, oropharyngeal, or tracheal aspirate sample, along with typical signs and symptoms of COVID-19. Stage 2 and 3 AKI were defined according to KDIGO guidelines as follows: Stage 2 AKI is an increase in serum creatinine to 2.0 to 2.9 times baseline. Stage 3 AKI is an increase in serum creatinine to 3.0 times or greater than baseline, or the initiation of renal replacement therapy.

For each patient the electronic medical records (EMR) were reviewed to extract data on patients' demographics, comorbidities, laboratory findings, treatments, and clinical course. Baseline serum creatinine level within 6 months (if available), as well as creatinine level at admission, and peak creatinine during admission were identified. Hematuria and pyuria were defined as > 5 cells / high power field on urinalysis of red blood cells and white blood cells respectively. Proteinuria was defined by at least 30 mg/dl protein on urinalysis. Further quantification of proteinuria by protein/creatinine ratio or 24 hour urine was not available.

Tissue processing

Core needle biopsy material was examined under the stereomicroscope and divided for light and electron microscopy studies. The sample for light microscopy was placed in 10% buffered formalin and processed using standard techniques; all biopsies were examined using hematoxylin eosin, periodic acid-Schiff, Jones methylamine silver, and trichrome stains. Iron stain was performed in select cases where brown intracellular pigment was identified in the tubular epithelium.

Tissue submitted for electron microscopy was fixed with 2.5 % glutaraldehyde in 0.1M cacodylate buffer, and then processed in 1% osmium tetroxide, dehydrated in an alcohol gradient, and embedded in an epoxy embedding medium. 1 micron-thick sections were cut and stained with toluidine blue stain. Thin sections were stained with uranyl acetate and lead citrate and examined with a JEOL JEM 100 CXII electron microscope in Electron Microscopy Lab at Northwell Feinstein Institute in New York. If available, in each case 2 glomeruli were examined for usual pathologic changes, as well as for the presence of viral particles; 2 cases had only a single glomerulus available for examination. Multiple tubules per case were thoroughly examined for viral particles and representative images of 5-10 tubules were recorded for each case.

Immunohistochemical assays were performed on 3 micron sections of paraffin embedded tissue. Antigen retrieval was performed using BOND Epitope Retrieval Solution 2 (prediluted, pH 9.0; ref. AR 9640) for 20 minutes at 100°C. Specimens were incubated with a primary mouse antibody directed against SARS-CoV-2 nucleocapsid protein (Clone 1C7, Bios, Woburn, MA) for 15 minutes at room temperature, followed by visualization with the Leica Bond detection kit at room temperature (Ref. DS 9800). The specimens were then counterstained with hematoxylin.
RNA in situ hybridization was performed using RNAScope probes (ACD, Newark, CA) directed against SARS-CoV-2 (cat no. 848568). A negative control probe (diaminopimelate B; DapB) to assess background signals as well as a positive control probe to the housekeeping gene peptidylprolyl isomerase B (PPIB) were included with each run. The Leica Bond RNAScope detection kit (catalog #DS9790) was utilized per manufacturer’s instructions. Tissue antigen retrieval was performed at 95°C for 15 minutes followed by incubation with RNAScope protease for 15 minutes at 40°C. Probes were added and hybridized for 3 hours at 42°C. AMP1 3,3’-diaminobenzidine (DAB) (30 min), AMP2 DAB (15 min), AMP3 DAB (30 min), AMP4 DAB (15 min), AMP5 DAB (30 min), and AMP6 DAB (15 min) were incubated followed by incubation with DAB for 20 minutes. Sections were counterstained with periodic acid-Schiff.

The Northwell Health Institutional Review Board approved this case series as minimal risk research using data collected as part of standard clinical practice. Autopsy consents included consent for use of pathological material in scientific research and publication. Descriptive statistics were reported as means (standard deviation, SD), median (interquartile range, IQR), or counts and proportions.
**Supplementary table 1.** Clinical characteristics of the study population

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<th>Sex</th>
<th>Race</th>
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<th>Prior Kidney Disease</th>
<th>Hypertension</th>
<th>Diabetes</th>
<th>Cardiovascular disease</th>
<th>Creatinine, mg/dL</th>
<th>Urine peak CK (U/L)</th>
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<th>Bacteremia/Fungemia</th>
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* Race, B: Black, H: Hispanic, W: White. BMI: body mass index. RBC: red blood cell. WBC: white blood cell. CK: creatine kinase. RRT: renal replacement therapy. ^COVID treatment, 1=hydroxychloroquine+IV steroid±azithromycin, 2=Tocilizumab+ hydroxychloroquine+IV steroid±azithromycin. Empty cell: no data available
Supplementary Figure 1 – Light microscopic findings in renal limited autopsy needle biopsy material. All scale bars represent 100 micrometers. (A) Most postmortem biopsies revealed more or less prominent autolytic changes, without other significant degenerative changes; no significant inflammation, glomerular changes, or chronic damage is seen in most cases (Periodic acid-Schiff (PAS) stain, 200x). (B) Mild acute tubular injury manifested by tubular distension and flattening of the epithelium, in the absence of viral cytopathic changes or significant interstitial inflammation (PAS, 100x). (C) The presence of cellular debris in some, predominantly distal tubules signifies acute tubular epithelial necrosis in the proximal nephron segments (PAS, 200x). (D) Mild interstitial inflammation is noted in a single case, in this image presented in the medulla (PAS, 100x). (E) Diffuse and early nodular diabetic glomerulosclerosis was seen in a single patient (PAS, 200x). (F) Focal urate crystals are seen in inner medulla of a single patient (PAS, 200x). (G) One sample revealed widespread deposition of polarizable oxalate crystals (hematoxylin and eosin (HE) stain, 100x, top – bright field, bottom – polarized). (H) Tubular epithelial cells with iron-negative brown pigment, likely lipofuscin (top – HE stain, 100x; bottom – iron stain, 100x, with positive control in lower right corner inset). (I) – Negative immunohistochemistry staining for SARS-CoV-2 nucleocapsid protein after antigen retrieval (400X). (J) Lung tissue from a known SARS-CoV-2 infected patient served as positive control for immunohistochemistry method (400X).