

COVID-19–Associated Kidney Injury: A Case Series of Kidney Biopsy Findings

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ABSTRACT

Background Reports show that AKI is a common complication of severe coronavirus disease 2019 (COVID-19) in hospitalized patients. Studies have also observed proteinuria and microscopic hematuria in such patients. Although a recent autopsy series of patients who died with severe COVID-19 in China found acute tubular necrosis in the kidney, a few patient reports have also described collapsing glomerulopathy in COVID-19.

Methods We evaluated biopsied kidney samples from ten patients at our institution who had COVID-19 and clinical features of AKI, including proteinuria with or without hematuria. We documented clinical features, pathologic findings, and outcomes.

Results Our analysis included ten patients who underwent kidney biopsy (mean age: 65 years); five patients were black, three were Hispanic, and two were white. All patients had proteinuria. Eight patients had severe AKI, necessitating RRT. All biopsy samples showed varying degrees of acute tubular necrosis, and one patient had associated widespread myoglobin casts. In addition, two patients had findings of thrombotic microangiopathy, one had pauci-immune crescentic GN, and another had global as well as segmental glomerulosclerosis with features of healed collapsing glomerulopathy. Interestingly, although the patients had confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection by RT-PCR, immunohistochemical staining of kidney biopsy samples for SARS-CoV-2 was negative in all ten patients. Also, ultrastructural examination by electron microscopy showed no evidence of viral particles in the biopsy samples.

Conclusions The most common finding in our kidney biopsy samples from ten hospitalized patients with AKI and COVID-19 was acute tubular necrosis. There was no evidence of SARS-CoV-2 in the biopsied kidney tissue.

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its resulting illness coronavirus disease 19 (COVID-19) are a global pandemic with a worldwide disease burden of 4.5 million cases and 307,000 deaths as of May 15, 2020.¹ Kidney involvement has been reported to

occur frequently in SARS-CoV-2 infection. AKI among hospitalized patients with COVID-19 in the United States has recently been reported to range from 28% to 46%.^{2,3} Early reports from China found the incidence of AKI to range widely from 0.5% to 29%.^{4–11} Proteinuria has been

reported in 40%–50 % of patients, and hematuria has been reported in 11% of patients.^{2,6} AKI from COVID-19 is associated with poor outcomes and a high mortality rate.²

The mechanism of AKI in the setting of COVID-19 is unclear, but direct viral infection, cytokine-mediated injury, and ischemic/hypoxic injury have all been cited as potential causative factors. Several studies as well as patient reports focusing on the histopathologic and

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ultrastructural evaluation of kidney samples in living patients and autopsy series have been published to date with controversial and conflicting results.^{12,13} Single patient reports of kidney biopsy findings in patients with COVID-19 have shown several cases of collapsing glomerulopathy and a singular case of acute tubular necrosis (ATN).^{3,14–17}

To further understand the clinical and kidney histopathologic findings of this novel disease, we evaluated a series of ten kidney biopsy cases of patients with COVID-19 along with clinical features of AKI and proteinuria with or without hematuria.

METHODS

Ten hospitalized patients with AKI and COVID-19 underwent kidney biopsy at our institution (North Shore University Hospital, Manhasset, NY and Long Island Jewish Medical Center, New Hyde Park, NY). The decision to biopsy was at the discretion of the attending nephrologist. 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 methylene silver, and trichrome stains. In cases with suspected rhabdomyolysis, immunohistochemistry staining for myoglobin was performed (NeoGenomics Laboratories, Inc., Aliso Viejo, CA).

All ten kidney biopsies were analyzed with immunohistochemistry for SARS-CoV-2 (Arkanalabs, Little Rock, AR). All immunohistochemical assays were performed on the Leica BOND-III platform (Leica, Wetzlar, Germany) using formalin-fixed, paraffin-embedded specimens sectioned at 3 μ m onto positively charged glass slides. Immunohistochemical 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; Bioss, 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.

In some patients with suspected primary glomerular disease on the basis of clinical information, light microscopic findings, or electron microscopy changes, immunofluorescence microscopy on formalin-fixed, paraffin-embedded tissue was performed; the sections were pretreated with protease solutions for antigen retrieval and incubated with antibodies specific for the heavy chains of IgG, IgA, and IgM; κ - and λ -light chains; C3; C1q; albumin; and fibrin-related antigens.

Tissue submitted for electron microscopy was fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, then processed in 1% osmium tetroxide, dehydrated in an alcohol gradient, and embedded in an epoxy embedding medium. One-micrometer-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 Laboratory at Northwell Feinstein Institute in New York. If available, two glomeruli of each case were examined for usual pathologic changes as well as for the presence of viral particles. Available proximal and distal tubules were thoroughly examined for the presence of viral particles in each case.

The Northwell Health Institutional Review Board approved this study as minimal risk research using data collected for routine clinical practice and waived the requirement for informed consent.

RESULTS

SARS-CoV-2 infection was confirmed in all patients by RT-PCR assay at our center. The mean age of the patients who were biopsied was 65 years. Half of the patients were women. Of ten patients in this group, five patients were black, three patients were Hispanic, and two were

Significance Statement

AKI is a known complication of severe COVID-19. Autopsy series of patients who died with COVID-19 reported acute tubular necrosis in the kidney, and a few patient reports noted the presence of collapsing glomerulopathy in patients with COVID-19. The authors describe clinical and kidney histopathologic findings for ten hospitalized patients with COVID-19 and AKI who underwent kidney biopsy. All patients had proteinuria, and eight required dialysis. Biopsy samples showed varying degrees of acute tubular necrosis in all patients; one patient also had associated myoglobin cast nephropathy. Pathologic analysis of biopsied kidneys showed no immunohistochemical evidence of severe acute respiratory syndrome coronavirus 2, and ultrastructural examination found no viral particles. This case series reveals acute tubular necrosis as the most common kidney biopsy finding in patients with COVID-19 and severe AKI.

white. A majority of the patients had a history of diabetes mellitus (DMII), hypertension (HTN), or both. All patients biopsied had varying degree of proteinuria, and six patients had hematuria. Of note, four patients had kidney biopsies in the intensive care unit (ICU) setting when intubated and on pressor support. Mean peak serum creatinine (Scr) was 6.6 mg/dl. Some patients were noted to have large kidneys (12–15 cm) on sonogram. Eight patients had severe AKI requiring RRT. All ten kidney biopsies showed varying degrees of ATN (Figure 1A). Immunofluorescence microscopy was performed on select patients on the basis of clinical presentation and/or light microscopy findings. Two biopsies had findings of thrombotic microangiopathy (TMA), and one showed focal sclerosis with features of healing collapsing glomerulopathy in isolated glomeruli. One patient had pauci-immune crescentic GN. All biopsies showed negative immunohistochemistry staining for SARS-CoV-2. Table 1 describes demographic data, pertinent clinical information, kidney sonogram details and biopsy diagnosis of all ten patients. Table 2 provides treatment details for both SARS-CoV-2 infection and AKI. Table 3 summarizes the

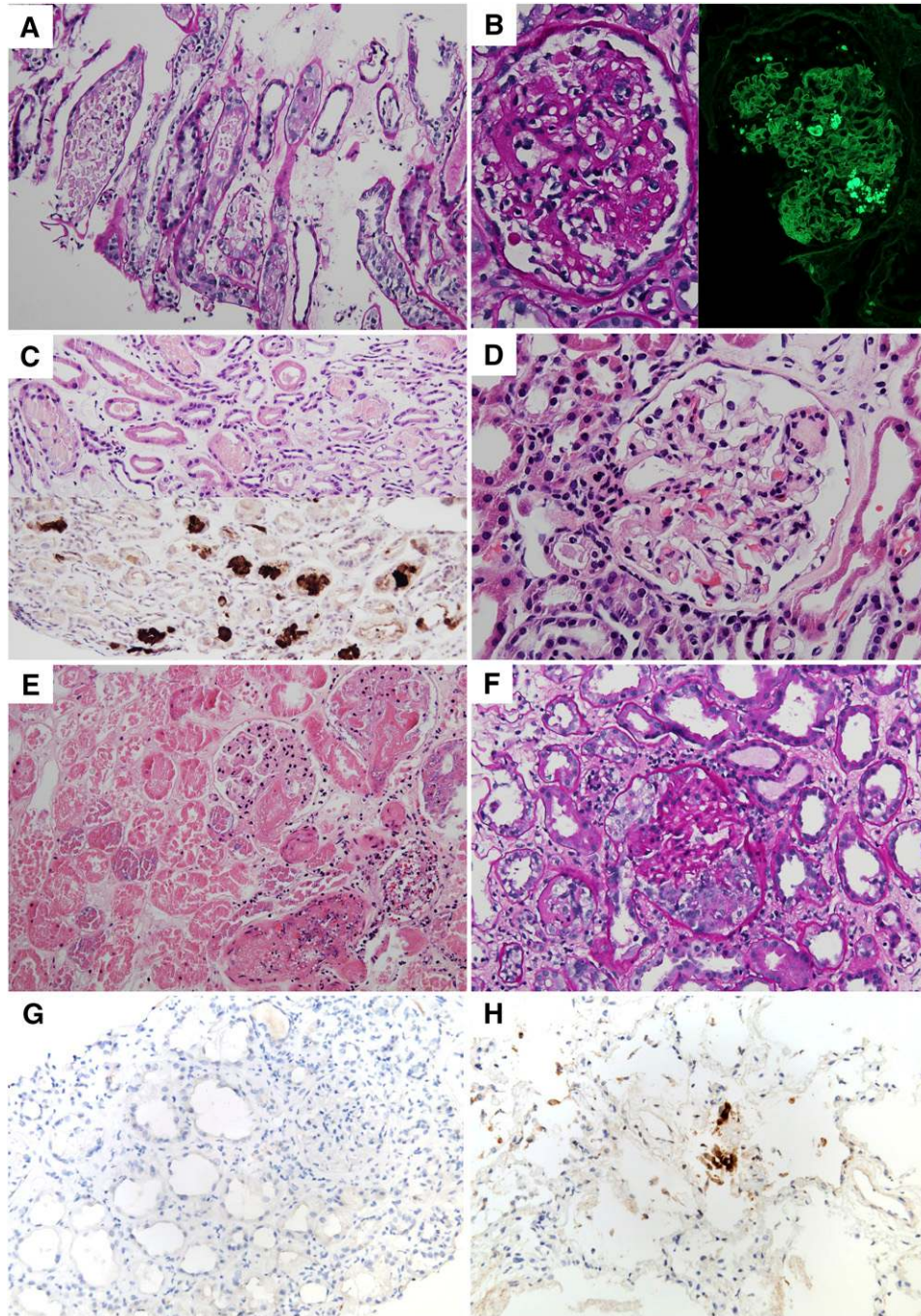


Figure 1. A variety of kidney histopathological findings seen in our patients with COVID-19 and AKI. (A) ATN is often manifested by accumulation of cellular debris in lumens of distal tubules (periodic acid–Schiff [PAS]: $\times 200$). (B) Segmental glomerulosclerosis with features of healing collapse and protein reabsorption granules in podocytes (left, PAS; right, FITC IgG immunofluorescence stain: $\times 400$). (C) Red-brown casts in tubules in the patient with rhabdomyolysis, staining positively for myoglobin stain (upper, hematoxylin and eosin [H&E]; lower, myoglobin immunohistochemistry stain: $\times 200$). (D) Diffuse and early nodular diabetic glomerulosclerosis (H&E, $\times 400$). (E) Diffuse cortical necrosis in a patient with severe TMA (H&E, $\times 200$). (F) Cellular crescent in a glomerulus and surrounding acute tubular injury with flattening of the tubular epithelium in a patient with ANCA disease (PAS, $\times 200$). (G) Representative section of negative immunohistochemistry staining for SARS-CoV-2 nucleocapsid protein after antigen retrieval ($\times 200$). (H) Lung tissue as positive control for immunohistochemistry staining for SARS-CoV-2 ($\times 200$).

Table 1. Demographic data, pertinent clinical information, kidney sonogram details, and biopsy diagnosis of ten patients with COVID-19 and AKI

Clinical Data	Patient No.									
	1	2	3	4	5	6	7	8	9	10
Age, yr	77	60	62	69	76	45	69	64	59	69
Sex	F	M	M	M	F	F	F	M	M	F
Race	AA	H	H	H	C	AA	C	AA	AA	AA
DMII/HTN present	HTN	DMII, HTN	DMII	HTN	DMII/HTN	HTN	None	None	HTN	HTN
CKD status	No history of CKD	No history of CKD	No history of CKD	No history of CKD	No history of CKD	No history of CKD	No history of CKD	No history of CKD	Unknown	No history of CKD
Admission Scr, mg/dl	8.1	0.8	1.2	0.9	1.0	7.4	0.7	7.8	4.5	1.9
AKI stage at the time of biopsy (KDIGO criteria)	Stage 3	Stage 3	Stage 3	Stage 3	Stage 3	Stage 3	Stage 3	Stage 3	Stage 3	NA
Proteinuria, g/d	1.5	4.7	NA (urine dipstick: 300 mg/dl)	2.4	0.9	5.7	1.4	5.0	2.8	7.6
Hematuria, yes/no	No	Yes	Yes	Yes	No	Yes	No	Yes	Yes	No
Pertinent serologies/laboratory values	None	CK>90,000, high LDH, low platelets	IgGλ band (weak)	Positive anticardiolipin Ab (IgM)	None	High LDH, low haptoglobin	High LDH, low haptoglobin	+MPO titer	Serum κ-λ ratio: 10.69	None
Vasopressor use at time of AKI	No	Yes	Yes	Yes	No	No	Yes	No	No	No
Peak Scr, mg/dl	8.3	13.1	6.3	5.8	4.4	8.4	4.3	7.8	6.0	2.0
Risk factors for AKI (other than COVID-19)	None	Hypotension	Hypotension	Hypotension + vancomycin	Vancomycin + piperacillin/tazobactam	Gemcitabine	Hypotension	None	Hypertensive emergency	None
Kidney size, cm, right/left	13/11.8	15/13.7	12.2/14.7	NA	15/14.6	9.8/10.3	9.6/9.3	11/11.3	8.7/8.7	11.3/11.4
Indications of kidney biopsy	Severe AKI, significant proteinuria	Severe AKI, nephrotic-range proteinuria, elevated CK levels and laboratory findings suggestive of TMA	Severe AKI, proteinuria	Severe AKI, significant proteinuria, and positive anticardiolipin Ab	Severe AKI, proteinuria	Severe AKI, nephrotic-range proteinuria and laboratory findings suggestive of TMA	Severe AKI, significant proteinuria and laboratory findings suggestive of TMA	Sever AKI, nephrotic-range proteinuria, and +MPO Ab	Severe AKI, significant proteinuria, and elevated serum κ-λ ratio	Nephrotic-range proteinuria and AKI
Complications of kidney biopsy	None	None	None	None	None	Perinephric and retroperitoneal hematoma	None	None	None	None
Kidney biopsy diagnosis	ATN, features of healed collapse	ATN + myoglobin casts	ATN	ATN + chronic sclerosis	ATN + early diabetic changes	ATN + TMA	TMA + cortical necrosis	Crescentic GN, ATN	ATN + chronic sclerosis	ATN + chronic sclerosis
Clinical outcome of the AKI	Patient needed dialysis; AKI subsequently improved and patient came off dialysis	Dialysis dependent, patient died	Dialysis dependent, patient died	Dialysis dependent, patient died	No improvement in kidney function; however, patient did not require dialysis during hospital stay	Dialysis dependent, patient died	Dialysis dependent, patient died	Patient needed dialysis, but AKI improved with corticosteroids therapy; patient subsequently came off dialysis	Kidney function improved as outpatient, but patient deemed CKD	Kidney function remained stable during hospital stay

F, woman; M, man; AA, black; H, Hispanic; C, white; KDIGO, Kidney Disease Improving Global Outcomes; NA, not available/applicable; MPO, myeloperoxidase.

kidney pathology findings. Supplemental Table 1 summarizes the inflammatory marker details of each patient. Figures 1 and 2 describe the various pathologies noted in all ten patients.

Patient 1

A 77-year-old black woman with a history of HTN, cardiac disease, and atrial fibrillation presented with fatigue and shortness of breath. On admission, she required 6 L of oxygen by nasal cannula to maintain an oxygen saturation of 96%. Her home medication included losartan 100 mg once a day. On admission, her laboratory data revealed a BUN of 108 mg/dl and Scr of 8.15 mg/dl; her baseline Scr was 1.0 mg/dl 4 months prior to admission. Her urinalysis revealed 600 mg/dl protein and no hematuria. A spot urine protein-creatinine (UP/C) ratio was 1.5 g/g creatinine. Fractional excretion of sodium (FeNa) was 0.9%. She remained anuric for 2 days and required dialysis.

Pathology Findings

A kidney biopsy was performed in the third week of her admission that showed widespread ATN. There was also global and segmental glomerulosclerosis with features of healed collapse in isolated glomeruli. Prominent protein reabsorption granules were seen in some podocytes and some tubular epithelial cells (Figure 1B). Lastly, moderate tubular atrophy and interstitial fibrosis were noted.

Clinical Follow-Up

The patient received a short course of steroids for her pulmonary disease with clinical improvement. Other causes of collapsing GN, such as parvovirus B19, HIV, and autoimmune diseases, were ruled out. Kidney function subsequently improved and she was able to come off dialysis. Serum creatinine was 3.0 mg/dl at the time of discharge.

Patient 2

A 60-year-old Hispanic man with uncontrolled DMII, HTN, and hyperlipidemia presented to the hospital with fever, cough, and fatigue. He was in acute respiratory failure on presentation and required a

non-rebreather mask to maintain an oxygen saturation >94%. Laboratory tests on admission showed a BUN of 11 mg/dl, Scr of 0.8 mg/dl, serum lactate dehydrogenase (LDH) of 343 U/L, and serum creatinine kinase (CK) of 380 U/L. Urinalysis revealed 100 mg/dl protein and 7 red blood cells/high power field (RBCs/hpf). Spot UP/C ratio was 4.7 g/g creatinine. Over the next few days, his oxygenation progressively worsened, leading to intubation and transfer to the ICU. In addition, he developed acute rhabdomyolysis, with his CK rising to 92,000 U/L, thought to be secondary to propofol. He developed multiorgan failure and needed pressor support. He sustained AKI at the time, necessitating dialysis. His laboratory values also showed rising LDH, low platelets, worsening anemia, undetectable haptoglobin, and several schistocytes on the peripheral smear.

Pathology Findings

The kidney biopsy revealed widespread ATN with necrotic cellular debris in many tubules, some of red-brown color. Immunohistochemistry staining for myoglobin revealed numerous myoglobin-positive casts within the tubules (Figure 1C). Glomeruli did not reveal significant pathologic findings; signs of TMA or acute interstitial nephritis (AIN) were not seen in this biopsy.

Clinical Follow-Up

The patient died from multiorgan failure.

Patient 3

A 62-year-old Hispanic man with a history of DMII presented with cough, shortness of breath, and rapidly developed acute respiratory failure, necessitating ICU transfer. His admission laboratory values showed a BUN of 19 mg/dl, Scr of 1.22 mg/dl, and an elevated C-reactive protein level. Urinalysis showed 8 RBCs/hpf, protein was 300 mg/dl, and specific gravity was 1.032. He was initiated on vancomycin and piperacillin/tazobactam. Over the next few days, his clinical status worsened, and the patient developed multiorgan failure requiring pressor support and AKI necessitating dialysis. Vancomycin trough remained between 15 and 20 μ g/ml. His CK was in normal range.

Pathology Findings

The kidney biopsy showed moderate ATN with no evidence of AIN or TMA.

Clinical Follow-Up

The patient died from multiorgan failure.

Patient 4

A 69-year-old Hispanic man with HTN presented with cough, shortness of breath, and chest pain. He was in acute respiratory failure at the time of arrival, requiring 10 L oxygen to maintain an oxygen saturation of >96%. Admission laboratory values showed a BUN of 11 mg/dl and an Scr of 0.9 mg/dl. He progressively worsened, requiring intubation and pressor support. He developed AKI at the time of ICU admission, necessitating dialysis. Urinalysis showed 30 mg/dl protein and hematuria with 14 RBCs/hpf. Spot UP/C ratio was 2.4 g/g creatinine. FeNa was 0.9%. He was subsequently initiated on vancomycin and meropenem for worsening sepsis.

Pathology Findings

The kidney biopsy showed moderate ATN with no evidence of AIN or TMA.

Clinical Follow-Up

The patient died from multiorgan failure.

Patient 5

A 76-year-old white woman with DMII, HTN, asthma, and a recent diagnosis of COVID-19 at an outside facility was admitted to our center with hypoxia. Her hospital course was complicated by development of diabetic ketoacidosis in the setting of sacral decubitus ulcer infection. She was treated with vancomycin and piperacillin/tazobactam. On admission, her Scr was 1.0 mg/dl. During the course of her stay, she developed worsening renal function, with Scr increasing to a peak of 4.4 mg/dl. Urinalysis showed proteinuria with no hematuria. Her spot UP/C ratio was 0.9 g/g creatinine. FeNa was 5.7%. Her vancomycin trough level at the time of AKI was 17 μ g/ml.

Pathology Findings

The kidney biopsy revealed moderate ATN. Glomeruli were slightly enlarged and

Table 2. Treatment given for SARS-CoV-2 infection and AKI

Patient No.	Need for RRT	Steroid Use/Indication	COVID-19–Specific Treatment	Kidney Disease–Specific Treatment
1	Yes	Yes/COVID-19	Hydroxychloroquine	None
2	Yes	Yes/COVID-19	Hydroxychloroquine	None
3	Yes	Yes/COVID-19	Azithromycin Hydroxychloroquine	None
4	Yes	Yes/COVID-19	Anakinra Convalescent plasma Hydroxychloroquine Anakinra Convalescent plasma	None
5	No	No	None	None
6	Yes	Yes/autoimmune hemolytic anemia	None	Rituximab (for gemcitabine-induced TMA)
7	Yes	Yes/COVID-19	Hydroxychloroquine Tocilizumab Steroids Convalescent plasma	Eculizumab
8	Yes	Yes/vasculitis	Hydroxychloroquine Tocilizumab Convalescent plasma	Intravenous methylprednisolone daily for three days
9	No	No	None	None
10	Yes (at an outside hospital)	Yes/acute gout	None	None

revealed mild diffuse and early nodular diabetic glomerulosclerosis, with occasional Kimmelstiel–Wilson nodules (Figure 1D).

Clinical Follow-Up

There was no improvement in kidney function however patient did not require dialysis during her hospital stay.

Patient 6

A 45-year-old black woman with recurrent metastatic cervical squamous cell cancer presented to the hospital with symptomatic anemia. Her past medical history was significant for peritoneal carcinomatosis, pulmonary embolism, and HTN. In addition, she had a history of bilateral hydronephrosis with placement of nephrostomy tubes. She had recently received gemcitabine and radiation for her cancer. Although she was positive for COVID-19, she did not have pulmonary manifestations and did not require oxygen support. Admission laboratory values revealed a hemoglobin of 4.8 mg/dl, platelet count of 91 thousand/ μ l, BUN of 113 mg/dl, Scr of 7.4 mg/dl, LDH of 916 U/L, and haptoglobin <20 mg/dl. Urine dipstick analysis showed 600 mg/dl protein and >50 RBCs/hpf. Spot UP/C ratio was 5.7 g/g

creatinine. She was also noted to be Coombs IgG positive. She was initiated on steroids for autoimmune hemolytic anemia and also received one dose of rituximab. AKI worsened, requiring initiation of dialysis, and a kidney biopsy was performed. Further testing revealed a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 activity of 28.8%. Complement C3 and C4 and total hemolytic complement were within normal range. Lupus and antiphospholipid testing were negative.

Pathology Findings

Kidney biopsy showed acute TMA with remodeling of glomerular capillary loops and crosslinked fibrin aggregated in glomerular capillaries on electron microscopy. There was also moderate ATN and focal interstitial edema and inflammation.

Clinical Follow-Up

The patient remained dialysis dependent until her death in the hospital.

Patient 7

A 69-year-old white woman with asthma presented with cough and shortness of breath. She was in significant respiratory distress and required a non-rebreather

mask to maintain an oxygen saturation >94%. Admission laboratory values showed a BUN of 8 mg/dl and an Scr of 0.7 mg/dl. She progressively worsened, necessitating mechanical ventilation and vasopressors. During this time, she developed severe AKI needing dialysis. Her laboratory values revealed a hemoglobin of 6.3 g/dl, platelet count of 58 thousand/ μ l, LDH of 990 U/L, and a haptoglobin of <20 mg/dl. Urinalysis showed proteinuria and no hematuria. Spot UP/C ratio was 1.4 g/g creatinine. Complement testing revealed a low factor H complement antigen and elevated plasma C3 and SC5b-9 levels, suggesting an activation of the alternative complement pathway. After a multidisciplinary meeting, the patient received eculizumab. This patient has also been described in a recent publication.¹⁸

Pathology Findings

A kidney biopsy showed diffuse coagulative cortical necrosis as seen in severe TMA (Figure 1E). The tissue sample revealed very little viable parenchyma.

Clinical Follow-Up

The patient developed worsening shock and multiorgan failure and died.

Table 3. Kidney pathology findings in COVID-19 and AKI

Patient No.	Main Pathology Finding	Glomeruli (Total/Globally Sclerosed)	IFTA, %	AAS	Electron Microscopy
1	ATN and FSGS/healed collapse	29/4	30	Severe	Moderate segmental effacement of podocyte foot processes
2	ATN with myoglobin cast nephropathy	2/1	20	Moderate	No significant findings
3	ATN	3/1	15	Severe	No significant findings
4	ATN	8/3	15	Mild	No significant findings
5	ATN and early nodular GS	31/9	20	Moderate	Mesangial expansion, thick GBMs
6	ATN and TMA	1/0	Mild	Moderate	Fibrin thrombi, subendothelial widening, endothelial injury
7	Cortical necrosis and TMA	22/0	10	Severe	Fibrin thrombi, subendothelial widening, endothelial injury
8	Crescentic GN and ATN	9/0	10	Mild	Subendothelial widening, endothelial injury
9	ATN on advanced chronic changes	17/12	50	Severe	Thin GBMs (221 nm)
10	ATN on advanced chronic changes	14/10	50	Moderate	Subendothelial widening, endothelial injury

IFTA, interstitial fibrosis and tubular atrophy; AAS, arterial and arteriolar sclerosis; GS, glomerulosclerosis; GBM, glomerular basement membrane.

Patient 8

A 64-year-old black man with a history of cryptogenic organizing pneumonia presented to the hospital with shortness of breath. He was in acute respiratory failure, requiring supplemental oxygen via a non-rebreather mask. Admission laboratory values showed a BUN of 124 mg/dl, Scr of 7.8 mg/dl, and urinalysis with 55 RBCs/hpf and spot UP/C ratio of 5 g/g creatinine. Serologies were positive for perinuclear ANCA (titer 1:320), antimyeloperoxidase antibody (titer 32.5 U), antinuclear antibody (titer 1:160), antidouble-stranded DNA antibody (112 IU/ml), and antiribonucleoprotein antibody (1.1 Antibody Index). Complement levels 3 and 4 were within normal limits. He was initiated on dialysis.

Pathology Findings

Kidney biopsy showed pauci-immune crescentic GN with cellular crescents in 40% of glomeruli, fibrocellular crescents in 30% of glomeruli, and moderate ATN (Figure 1F).

Clinical Follow-Up

The patient received intravenous methylprednisolone for 3 days. In addition, he received convalescent plasma therapy and tocilizumab as part of antiviral treatment. Kidney function subsequently improved and he was able to come off dialysis. Serum creatinine was 3.5 mg/dl at time of discharge.

Patient 9

A 59-year-old black man with history of congestive heart failure and HTN presented to the hospital for productive cough and dyspnea on exertion for 2 weeks. The patient was found to be in hypertensive emergency (207/109 mm Hg) as well as volume overload in the emergency room and received intravenous diuretic therapy. On admission, Scr was elevated at 4.5 mg/dl. Urinalysis showed 100 mg/dl protein and >50 RBCs/hpf. FeNa was 7%, and spot UP/C ratio was 2.8 g/g creatinine.

Pathology Findings

A kidney biopsy showed advanced chronic changes with 70% global glomerulosclerosis, 50% interstitial fibrosis

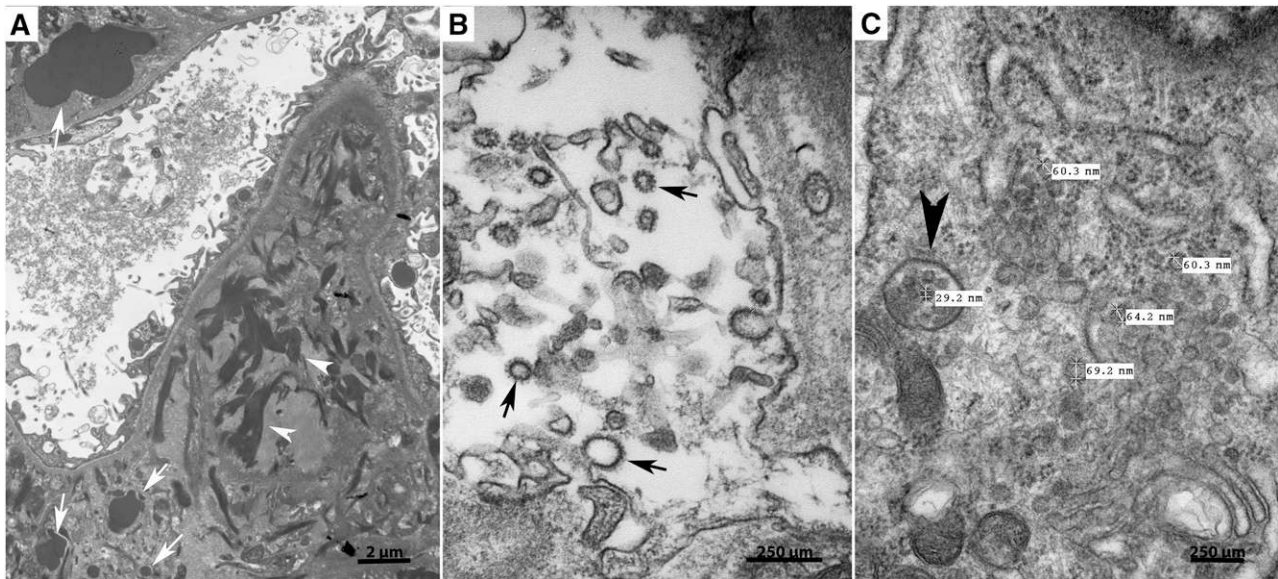


Figure 2. Ultrastructural examination confirmed the kidney pathological diagnosis in some patients but showed no convincing evidence of viral presence in any of our cases. (A) Electron micrograph showing acute TMA in a patient with gemcitabine exposure; endocapillary space often contains aggregates of crosslinked fibrin (white arrowheads) and fragmented red blood cells (white arrows). Original magnification, $\times 5,000$. (B) Ultrastructural detail of podocyte cytoplasm with clathrin-coated vesicles (black arrows). Original magnification, $\times 40,000$. (C) Multivesicular bodies (black arrowhead) in epithelial cell cytoplasm have been often confused with viral particles. Original magnification, $\times 50,000$.

and tubular atrophy, and severe vascular sclerosis. There was also mild ATN.

Clinical Follow-Up

The patient was deemed to have mild ATN superimposed on advanced CKD. He was discharged with an Scr of 5.7 mg/dl. Scr decreased to 5.36 mg/dl on first outpatient follow-up visit.

Patient 10

A 69-year-old black woman with HTN, hyperlipidemia, and a recent outpatient diagnosis of COVID-19 was admitted for acute gout. She had AKI at an outside hospital requiring dialysis; however, her kidney function improved, and dialysis was discontinued. On admission, her Scr was 1.9 mg/dl and remained stable. Urinalysis showed 600 mg/dl protein and no hematuria. Spot UP/C ratio was 7.6 g/g creatinine.

Pathology Findings

The kidney biopsy showed mild ATN superimposed on advanced chronic changes with sclerosis in 70% of the glomeruli. Ultrastructural findings of glomerular endothelial cell injury with subendothelial

widening were also noted in this patient.

Clinical Follow-Up

Kidney function remained stable during hospital stay, and the patient was discharged with outpatient follow-up.

DISCUSSION

Early reports of COVID-19 showed a low incidence of AKI (0.5%–15%). Hirsch *et al.*² reviewed health records of close to 5000 patients at our institution and found the incidence of AKI to be 37%. Etiologies of AKI in patients with COVID-19 are variable and range from prerenal azotemia to tubular injury secondary to ischemic insult or toxin (*e.g.*, myoglobin in rhabdomyolysis). Our current knowledge of kidney histopathologic findings in the AKI associated with COVID-19 comes from autopsy data from China as well as sparing patient reports from around the world. An autopsy series from China reported that 9 of 26 patients had AKI primarily characterized by diffuse proximal tubular

injury and that some had frank necrosis and ultrastructural and immunohistochemistry evidence of direct viral infection of the kidney.¹² The correlation of these findings with AKI was not clearly discernible in all patients. The first living patient with COVID-19 who underwent a kidney biopsy was a noncritically ill black woman with prior comorbidities (including CKD) who developed severe AKI requiring dialysis. Her kidney biopsy revealed collapsing glomerulopathy with presence of endothelial tubuloreticular inclusions.¹⁴ A high-IFN state induced by viral infection in the setting of APOL-1 high-risk status was hypothesized and no evidence for virus infection in the kidney was established on the basis of *in situ* RNA hybridization and ultrastructural examination. Subsequently, several cases have reported collapsing glomerulopathy as the most common finding on kidney biopsy with COVID-19.^{15,16} In clinical practice however, ATN seems to be the most common cause of AKI. Detailed data on clinical features and laboratory markers in patients with AKI and COVID-19 are lacking. We report the clinical characteristics

and kidney biopsy findings in patients with AKI in the setting of COVID-19 and postulate various mechanisms in play.

All ten kidney biopsies showed tubular injury of varying degree, frequently with frank epithelial necrosis and cellular debris in the tubular lumens. Three of these patients had a clear trigger for the AKI, namely profound hemodynamic instability and rhabdomyolysis. In addition, these patients had severe acute lung injury that is associated with elevated Angiotensin II and decreased Angiotensin 1–7A levels.¹⁹ Studies have shown that the onset of COVID-19–associated AKI is temporally linked to systemic collapse, and mechanical ventilation with vasopressor support was temporally related to the development of AKI.² In another article describing COVID-19–associated AKI, 66% of patients had hemodynamic instability; hence, the likely diagnosis was thought to be ischemic ATN.³ Furthermore, the current autopsy data also support evidence of ATN.¹²

One of our patients had kidney biopsy findings of myoglobin casts in addition to ATN. This correlated with the clinical findings of rhabdomyolysis with CK levels >92,000 and exposure to vasopressor medications. Rhabdomyolysis has been reported to be a contributing factor in the development of AKI in patients with COVID-19.¹²

There were two patients in our group in whom there was no clear trigger for ATN in terms of hemodynamic instability, medications, or pigment nephropathy. In addition, their COVID-19 disease was not severe, and the oxygen requirements were minimal.

The possible mechanisms in play here are intriguing. Some have suggested renal tropism of SARS-CoV-2.¹³ Using tissue microdissection from six patients who underwent autopsy, SARS-CoV-2 viral load was identified in kidney compartments, and three of six patients had a detectable viral load with preferential targeting of glomerular cells. The authors also detected viral RNA and protein using *in situ* hybridization and indirect immunofluorescence in a single patient. In the autopsy series from

Wuhan, China, three of six tested patients also showed positive granular staining in a nuclear or cytoplasmic pattern in tubular epithelium by immunofluorescence studies.¹² The significance of this finding remains to be further elucidated. In our series, we found ATN as the most common kidney biopsy finding, with ischemia as the most common trigger. Other causes could include toxic sepsis–associated state, angiotensin-converting enzyme 2 inhibition, antibiotics, and rhabdomyolysis. Viral staining for SARS-CoV-2 by immunohistochemistry was negative in all ten patients, with adequate reaction in the positive control tissue, suggesting that there is no significant viral presence in any of our kidney biopsy samples. We cannot, however, completely dismiss the possibility of the viral presence at concentration levels below the threshold of detection.

Several studies and patient reports also suggested the presence of viral particles in podocytes and tubule epithelial cells on the basis of electron microscopy findings in their tissue.^{12,20,21} Consistent confirmation by immunohistochemical staining or *in situ* hybridization is often lacking in these studies. Moreover, the identity of viral-like particles in some publications has been questioned by others because numerous intracellular components exhibit viral-like morphology, including clathrin-coated vesicles and multivesicular bodies.^{22,23} We have performed a thorough ultrastructural search for viral particles in all of our samples and identified numerous viral-like particles in all of them (Figure 2); however, we did not detect structures that convincingly fit description and features of coronavirus particles previously described in the literature.²⁴ The identity of viral-like particles remains highly controversial in the literature at this time.

The role of cytokine-induced epithelial injury is interesting, particularly in patients of African ancestry with a carrier state of the kidney disease high-risk variants of APOL1 gene. Presence of high-risk variants of the APOL1 gene confers a much higher risk of collapsing glomerulopathy.^{25,26} It has been shown that collapsing glomerulopathy develops

in response to cytokine injury often related to high IFN states, such as various viral infections and IFN treatment. At least three reports of collapsing glomerulopathy in AKI with COVID-19 were found to be homozygous for the G1 allele.^{14,16,20} In our patient series, we have a single patient with features of healed collapse in isolated glomeruli; however, this patient's APOL1 status is currently unknown.

We have two patients with TMA associated with COVID-19. One of the patients had a history of gemcitabine use; association of gemcitabine exposure with TMA has been well established in the literature.^{27–29} The second patient likely had an underlying complement-mediated disorder.¹⁸ Severe COVID-19 has been associated with hypercoagulability. There have been autopsy reports demonstrating microthrombi in various organs, including the lung.³⁰ Endothelial cells with high expression of angiotensin-converting enzyme 2 are target cells of COVID-19 that can result in endothelial cell dysfunction. There have been recent data on COVID-19 infection that suggest activation of alternate and lectin pathway.³¹ It is possible that COVID-19 infection served as a “second hit” in patients predisposed to TMA from gemcitabine use and an underlying complement defect.¹⁸

Our patient with pauci-immune crescentic GN and ANCA-positive serologies had no clinical prodrome of vasculitis. His kidney function and other systemic symptoms of COVID-19 started improving after treatment with pulse dose steroids for 3 days.

On the basis of our findings, COVID-19 affects the kidneys in the tubular, vascular, and glomerular compartments. Although ATN is the most common kidney biopsy finding in our series, collapsing glomerulopathy is likely the most common glomerular disease as seen in other publications.^{14–17,20} Vasculitis and TMA are rare and can be a result of “second hit” triggers in patients with predisposition. Of note, all of our biopsies were “for-cause” biopsies and therefore, may not represent the full spectrum of kidney disease in COVID-19.

Despite the high prevalence of hematuria and proteinuria, kidney biopsy

analysis in patients with COVID-19–associated AKI showed varying degree of ATN. Some patients with ATN had clearly identifiable insults, whereas in others, the cause was not identified. COVID-19 with its associated hyperinflammatory state and cytokine storm predisposes to tubular injury and may in addition unmask conditions, such as ANCA vasculitis. Coagulopathy has been a hallmark of this disease, and that combined with low renal perfusion or additional triggers, such as medications or complement pathway abnormalities, can lead to TMA. Lastly, we did not find evidence of significant viral presence in the kidney tissue by immunohistochemistry and ultrastructural examination, suggesting that direct viral infection is not the main cause of AKI in our patients with active COVID-19.

DISCLOSURES

K.D. Jhaveri serves as a consultant for Astex Pharmaceuticals and Natera. All remaining authors have nothing to disclose.

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SUPPLEMENTAL MATERIAL

This article contains the following supplemental material online at <http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2020050699/-/DCSupplemental>.

Supplemental Table 1. Inflammatory markers in patients with COVID-19 on admission and at time of AKI onset.

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