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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, 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.

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.

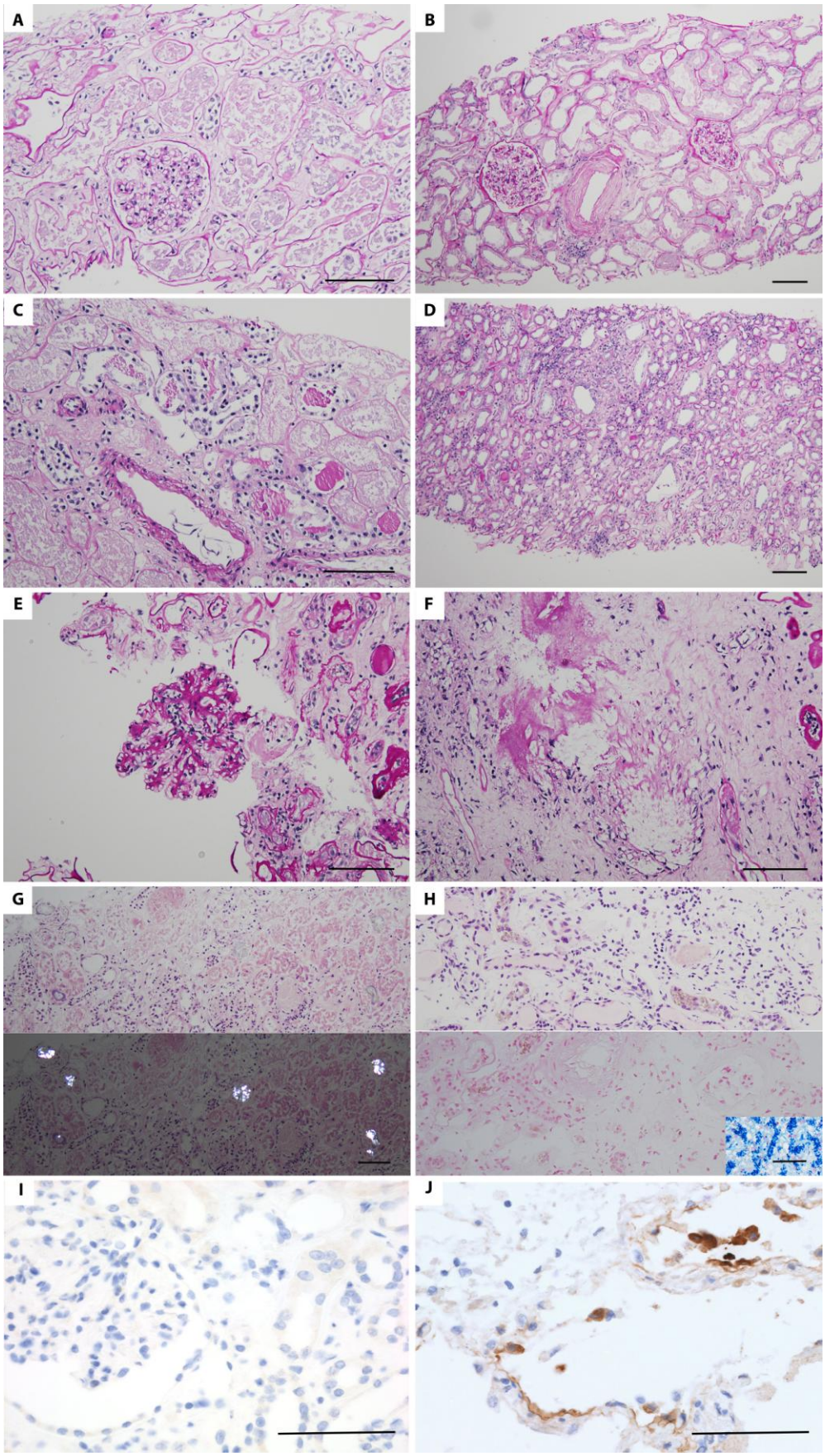
The Northwell Health Institutional Review Board approved this case series as minimal risk research using data collected as part of standard clinical practice. Autopsy consents included consent for use of pathological material in scientific research and publication. Descriptive statistics were reported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

Supplementary table 1. Clinical characteristics of the study population

Patient Number	Age (Y)	Sex	Race	BMI(kg/m2)	Prior Kidney disease	Hypertension	Diabetes	Cardiovascular disease	Creatinine, mg/dL			Urine			peak CK (U/L)	Vancomycin trough>20 (ug/ml)	IV Contrast	Bacteremia/Fungemia	vasopressor	Mechanical ventilation	RRT	COVID treatment^	Time to biopsy (hr)
									Baseline	Admission	Peak	Proteinuria mg/dl	RBC ≥ 5/hpf	WBC≥ 5/hpf									
1	58	M	H	29.7	N	N	N	N	1.1	5.5	8.9	100	Y	Y	135	Y	Y	N	Y	Y	Y	2	20
2	92	F	H	30	N	Y	N	N	0.7	5	5.01	30	N	N	250	Y	N	N	Y	Y	N	1	70
3	78	M	B	25	N	Y	Y	Y		2.6	6.15	100	Y	N	3745	Y	N	N	Y	Y	Y	1	2.5
4	49	M	H	28.4	N	N	N	N	0.5	0.51	2.64	30	Y	N		Y	N	Y	Y	Y	N	2	17
5	77	M	H	32.8	N	N	N	N	0.6	0.76	5.7	30	N	Y	420	Y	N	N	Y	Y	Y	2	2
6	72	M	H	28.5	N	Y	Y	Y	1.07	2	5.6	30	N	N	2900	Y	N	Y	Y	Y	Y	1	1.5
7	81	M	W	22.1	Y	Y	Y	Y	2.5	2.61	5.4	30	N	N	153	Y	N	N	Y	Y	Y	1	19
8	76	M	W	24.4	N	Y	N	N	1.1	1.6	4.2	100	Y	N	576	N	N	N	Y	Y	Y	2	15
9	56	M	H	28.3	N	Y	N	N	0.9	0.77	3.6	30	Y	N	508	N	N	Y	Y	Y	Y	2	19
10	76	F	W	23	N	Y	N	N	1	2.35	2.35				148	N	N		N	N	N		24
11	74	M	B	38	N	Y	N	N	1	1.6	2.7	30	Y	N	890	N	Y	N	Y	Y	N	2	12
12	54	M	B	44.4	N	Y	Y	N		2.2	11	30	N	N	4170	Y	Y	N	Y	Y	Y	2	18

* 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).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