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Collapsing glomerulopathy in a COVID-19 patient

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To the editor The first available reports indicate that renal involvement is relatively frequent in patients with novel coronavirus disease 2019 (COVID-19) due to the emerging severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Up to 43% of patients present with proteinuria (including 10% with heavy proteinuria), 11% with hematuria, and 3.5% to 5% with acute kidney injury.[1,2] Both proteinuria and acute kidney injury are associated with increased mortality.[1] However, the exact mechanisms underlying renal injury in patients with COVID-19 are unclear as renal pathology data are lacking.

We report on a 63-year-old black male patient who developed acute kidney injury in the setting of COVID-19. He had a history of hypertension treated with atenolol, nifedipine, and olmesartan. He initially presented with intense fatigue, high-grade fever (39.7 °C), and respiratory distress (respiratory rate, 36 breaths/min; arterial blood O_2 saturation, 86%) requiring O_2 supplementation (4 l/min). On admission, his serum creatinine was 1.2 mg/dl. He was diagnosed with COVID-19 based on a positive reverse transcriptase–polymerase chain reaction test for SARS-CoV-2 in a nasopharyngeal swab sample.

Shortly after his admission, he developed oliguria and rapidly progressive acute kidney injury (Kidney Disease: Improving Global Outcomes stage 3) with a serum creatinine of 4.4 mg/dl at day 4 (Figure 1a). Laboratory tests showed an increase in the C-reactive protein level, lymphopenia, increased D-dimers serum level, hypoalbuminemia, massive proteinuria (5 g/l consisting of 50% of albumin), and reduced sodium urinary excretion (sodium excretion fraction: 0.4%). The patient had no episodes of hypotension and remained hypertensive for most of his hospital stay. His respiratory condition gradually improved and the O_2 supplementation was decreased (0.5 l/min at day 8). Serum levels of a range of cytokines, including interleukin-6, were normal. However, the further increase of C-reactive protein serum levels was associated with systemic complement activation (soluble C5b-9, Bb fragment) and worsening of acute kidney injury with a serum creatinine peaking at 8.4 mg/dl at day 8 (Figure 1a). The patient did not receive SARS-CoV-2–specific experimental treatment (protease inhibitors, remdesivir, and hydroxychloroquine) or any nephrotoxic drug.

A kidney biopsy was performed at day 8. Light microscopy examination disclosed 2 main features: severe collapsing focal segmental glomerulosclerosis (FSGS) (Figure 1b and c) and acute tubular necrosis (Figure 1d) without any significant interstitial inflammation. The immunofluorescence study revealed no significant immune deposits (including anti-C5b-9 staining). A reverse transcriptase–polymerase chain reaction for SARS-CoV-2 in RNA extracted from a frozen biopsy tissue sample was negative (reverse transcriptase–polymerase chain reaction was similarly negative in blood) (Figure 1a). Electron microscopy study (Figure 1e and f) disclosed, in the podocyte cytoplasm, vacuoles containing numerous spherical particles that may correspond to viral inclusion bodies reported with SARS-CoV-2.[3]

Further work-up showed that the patient was homozygous for the at-risk apolipoprotein A (APOL1) G1 variant (A342G and I348M). No specific treatment was implemented. The patient maintained a urinary output and did not require dialysis. Renal function subsequently improved and proteinuria decreased (Figure 1a). The patient was discharged on day 17 with a serum creatinine of 5.5 mg/dl and persistent proteinuria (1.8 g/l).

To the best of our knowledge, this is the first description of the pathological features of renal injury in the setting of COVID-19 outside autopsies series. The most striking finding in our patient was the collapsing FSGS. This finding suggests that FSGS could account for the heavy proteinuria reported in a significant proportion of patients with COVID-19.[1] The collapsing FSGS is a known complication of other viral infections, in particular, HIV,[4] as well as cytomegalovirus[5] and parvovirus B19.[6] For HIV a direct toxic viral effect on podocytes has been documented.[7] The receptor for SARS-CoV-2, membrane-bound angiotensin-converting enzyme 2, is expressed on podocytes.[8,9] However, polymerase chain reaction for SARS-CoV-2 was negative in kidney biopsy samples, but the technique has a notoriously low rate of detection in nonrespiratory samples (including blood and urine),[10] and the quality of the extracted RNA material was poor. Besides, electron microscopy findings in our patient do not provide definite proof of the presence of SARS-CoV-2 in podocytes, and one cannot exclude that the detected vesicles in podocytes may correspond to nonviral particles. Collapsing FSGS, with or without acute tubular necrosis, can also complicate the course of hemophagocytic syndrome,[11] a disorder characterized by an increased release of a wide range of cytokines. In our patient, normal levels of cytokines, in particular interleukin-6, while inflammation markers were still increased, plead against this hypothesis. However, a potential virus-driven intrarenal cytokines release cannot be excluded.

Besides, our patient is of African origin and is homozygous for the APOL1 at-risk G1 variant. This variant may have contributed to the pathogenesis of collapsing FSGS, because APOL1 is a recognized risk factor for the development collapsing FSGS in HIV and non-HIV patients.[4]

In our patient, acute tubular necrosis developed in the absence of hemodynamic compromise or severe pulmonary involvement. This suggests that tubular injury in COVID-19 patients, unlike that seen in coronavirus-associated SARS,[12] is not predominantly ischemic. The possible underlying mechanisms are a direct viral toxicity on tubular cells that also harbor angiotensin-converting enzyme 2 or a cytokine-mediated tubular damage. In addition, the initial heavy

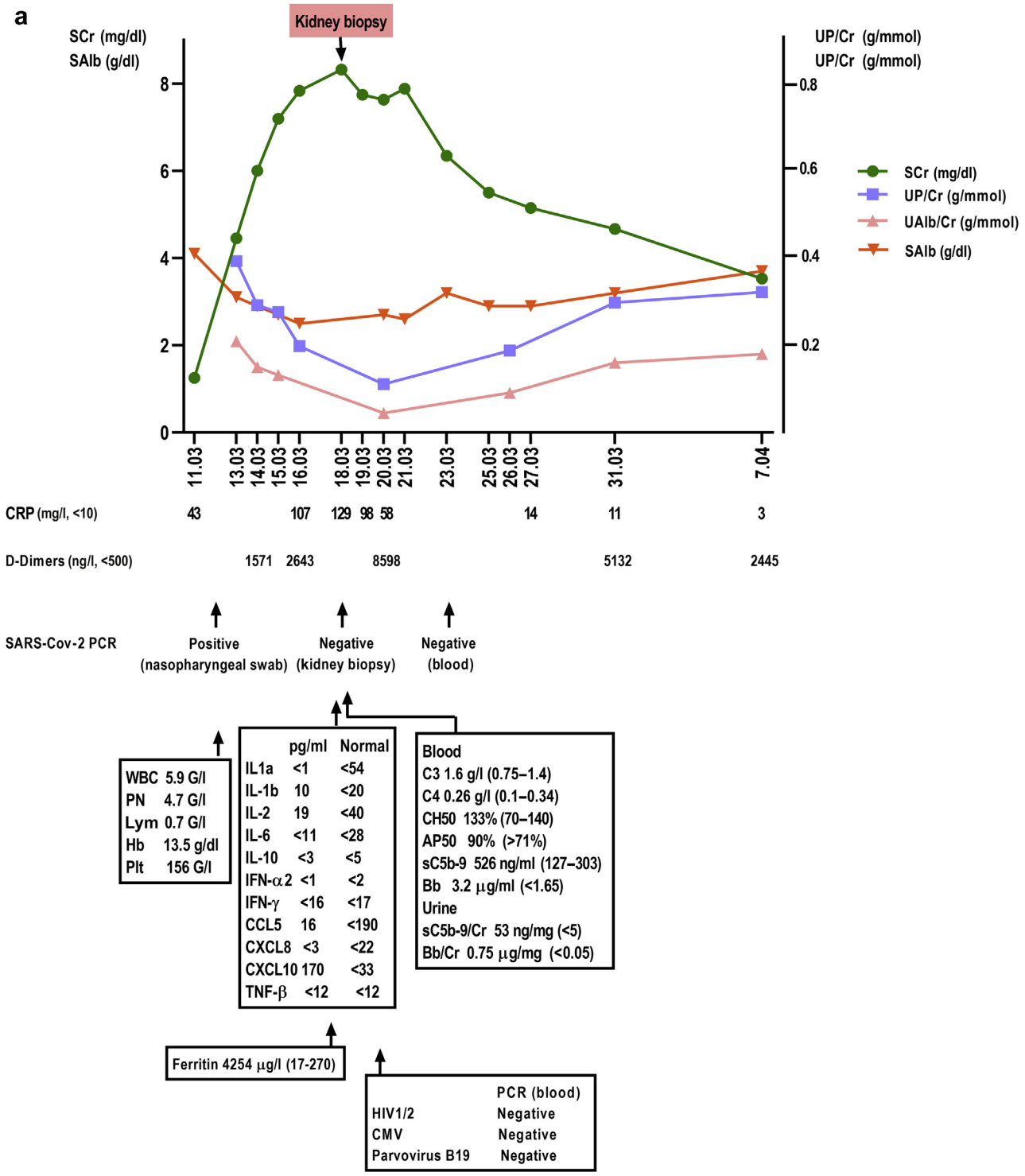

Figure 1 | A 63-year-old black male patient was admitted for acute respiratory distress associated with novel coronavirus disease 2019. (**a**) The main laboratory results for this patient are shown. He rapidly developed acute kidney injury without hemodynamic compromise. His respiratory status improved but inflammatory syndrome persisted and renal function further deteriorated. (Continued).

proteinuria in our patient may have also contributed to tubular necrosis.

Overall, in contrast to lung injury, kidney injury in COVID-19 does not appear to include a predominant inflammatory component. This observation suggests that collapsing FSGS, potentially resulting from a direct viral effect on podocytes, probably belongs to the spectrum of COVID-19–associated renal involvement.

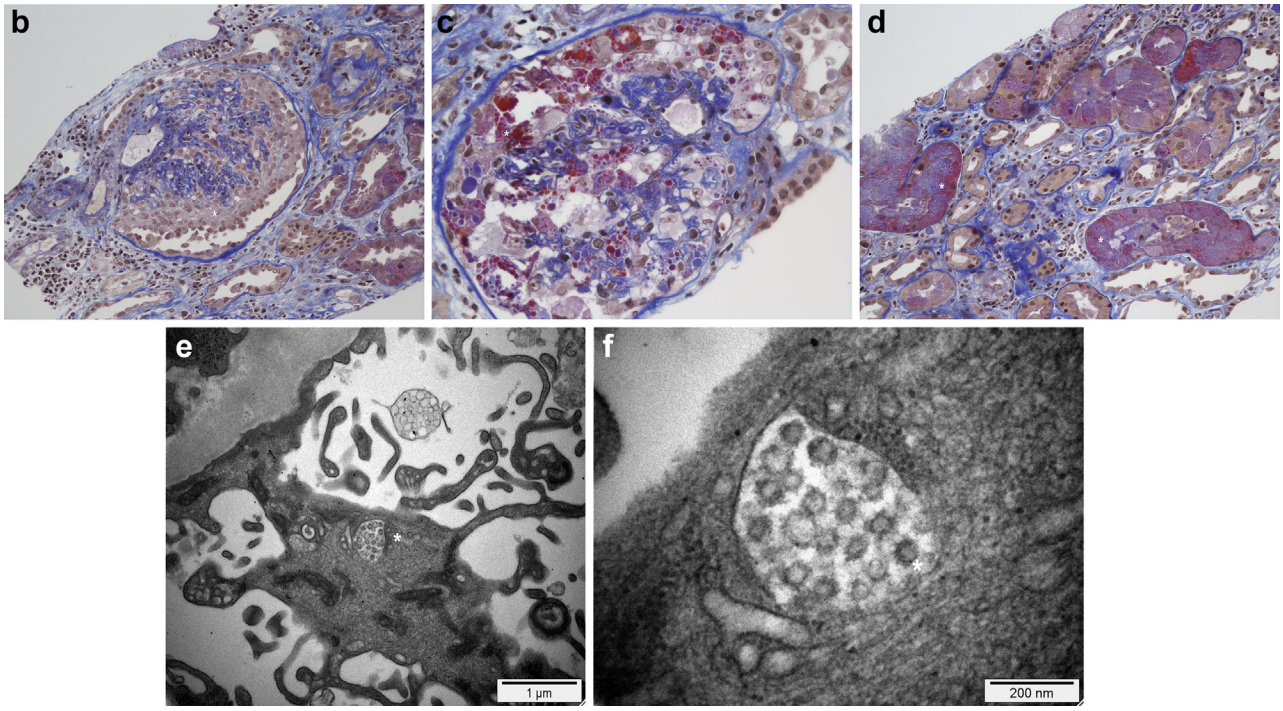

Figure 1 | (Continued) (**b–d**) Illustrative images of his kidney biopsy are shown. Light microscopy study (Masson's trichrome stain, original magnification [**b,d**] ×200 and [**c**] ×400) showed the following: first, a severe collapsing glomerulopathy (focal segmental glomerulosclerosis) characterized by (**b,c**) the global collapse of shrinking capillary loops and the detachment from the basement membrane of (**b**) hypertrophic, proliferating podocytes (or "cobblestone pattern," [asterisk]), which contained numerous (**c**) protein reabsorption vacuoles (asterisk). (**d**) Second, acute tubular lesions with focal tubular necrosis, dilatation, and the presence of intratubular reabsorption vacuoles (asterisks), reflecting the heavy proteinuria. Immunofluorescence study did not show any significant immune deposits. (**e,f**) Electron microscopy study (original magnification [**e**] ×15,000 and [**f**] ×73,000) disclosed within the podocytes cytoplasm vacuoles containing numerous (**e**) spherical particles (asterisk) measuring between 50 to 110 nm and surrounded by (**f**) spikes measuring 9 to 10 nm ("solar corona" [asterisk]). These particles may correspond to viral inclusion bodies reported with the emerging severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[3] AP50, alternative pathway activity 50%; Bb, Bb fragment; CCL, CC chemokine ligand; CH50, hemolytic complement activity 50%; CMV, cytomegalovirus; CXCL, CXC chemokine ligand; CRP, C-reactive protein; G, $\times 10^9$; Hb, hemoglobin; IFN, interferon; IL, interleukin; Lym, lymphocytes; PCR, polymerase chain reaction; Plt, platelet count; PN, polynuclear neutrophils; SAlb, serum albumin; sC5b-9, soluble C5b-9; SCr, serum creatinine; TNF-β, tumor necrosis factor-β; UAlb/Cr, urinary albumin over creatinine ratio; UP/Cr, urinary protein over creatinine ratio; WBC, white blood cell count. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

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Visualization of putative coronavirus in kidney

To the editors: We read with concern the articles that report the presence of coronavirus in kidney based on electron microscopic evidence.[1,2] Neither article, in fact, demonstrates the presence of coronavirus in the kidney. Su *et al.*[1] show purported virus particles in the cytoplasm of kidney tubular epithelium and podocytes. These structures are not viral particles, but rather clathrin-coated vesicles, normal cell organelles involved in intracellular transport. The objects in their Figure 2a and b (∼60 nm) are somewhat smaller than coronaviruses (∼80 to 140+ nm), but more importantly, their "spikes" (peplomers) are in contact with the cytosol, as are those on clathrin-coated vesicles; the larger particle in Figure 2d also has spikes that are touching the cytosol and does not have dense dots inside the particles corresponding to the coiled nucleocapsid, cut in cross section. Coronaviruses, on the other hand, have their projections either facing the extracellular space between cells or the space inside vacuoles within the cells.[3–5] This phenomenon is due to the fact that coronaviruses receive their outer covering by budding into or on cellular membranes, thereby forming intracellular vacuoles with the viral projections in contact with the vacuolar content, not the cytosol. During assembly, viral structural proteins are incorporated into the endoplasmic reticulum–Golgi complex of the infected cell, and viral RNA, packaged with another protein, buds into these membranes, forming a membrane-bound sac containing mature virions; the spikes are on the outside of the virion, but inside the vacuole and not in direct contact with the cytosol (Figure 1). These virions get out of the cell by exocytosis when the vacuole membrane fuses with the plasma membrane and opens its contents to the outside; thus, complete virions with peplomers are seen within the cell inside the membrane container (sequestered from the cytosol) and outside of cells, frequently still attached to the opened vacuolar membrane that has fused with the plasma membrane. The particles shown in electron micrographs in the article by Su *et al.*[1] have their spikes in contact with the cytoplasmic fluid, like endocytotic vesicles, that is, clathrin-coated vesicles (see Plate 523, Figures 3–5, pp. 1214–1215 in Ghadially[6]; Figure 18c and d in Miller[7]; and Miller[8]).

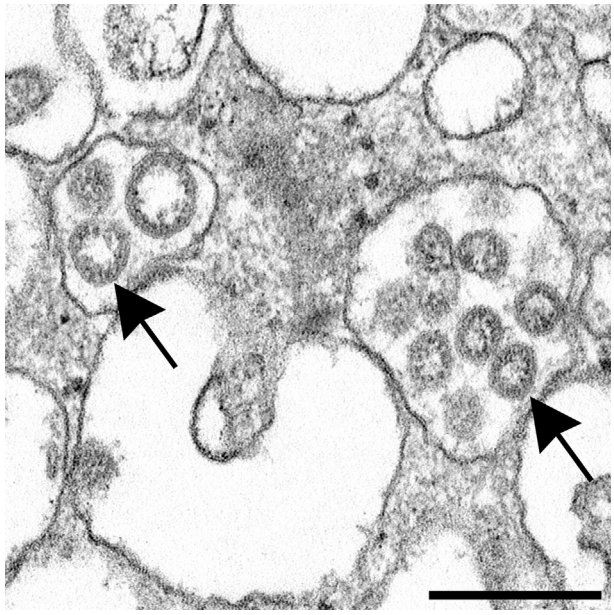

Figure 1 | Electron microscopic image of an isolate of severe acute respiratory syndrome coronavirus 2 seen here inside vacuoles (arrows). Note the dense membrane coat around the viral particles. This micrograph is of viral particles in a cell culture inoculated with infected patient nasopharyngeal and oropharyngeal fluids. Bar = 200 nm. Image provided by Cynthia S. Goldsmith, Centers for Disease Control and Prevention. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

Likewise, the particles in Kissling *et al.*[2] are not coronaviruses. While they are inside a vacuole, have spikes, and are approximately the correct size, they do not have the uniform appearance of virus particles with a membrane outer covering and dots inside indicating the nucleocapsid.[3–5] These objects are inside a vesicle called a multivesicular body (see Plates 277–278, pp. 632–634 in Ghadially[6]; Calomeni *et al.*[9]; and Figure 3, p. 393 in Haguenau[10]). The article by Kissling *et al.*[2] is concerning, as electron microscopy is the only alleged evidence presented in support of the suggestion that coronaviruses are actually present in this kidney tissue; all other tests for coronavirus in kidney were negative. These micrographs do not support the statement that the particles are indeed viruses.

Knowledge of virus morphology and morphogenesis, as well as of cellular architecture, is necessary to distinguish viral pathogens from normal subcellular organelles. This distinction is frequently difficult, because numerous cellular components can masquerade as viruses.[7–11]

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