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Electron microscopic investigations in COVID-19: not all crowns are coronas

To the editor: Renal involvement, in the form of acute kidney injury, hematuria, and/or proteinuria, is common in patients with coronavirus disease 2019 (COVID-19).[1] Postmortem renal histology has shown acute tubular injury, microvascular thrombi, and inflammation[2–5]; collapsing focal and segmental glomerulosclerosis has been reported in live patient biopsies.[6] The pathogenesis of renal injury remains unclear. Direct viral cytopathic injury is possible, due to expression of viral receptor angiotensin-converting enzyme 2 (ACE2) on tubular epithelial cells. Indirect immunologic and/or prothrombotic infection-related effects may also be at play. Using electron microscopy, putative virions have been described in tubular epithelial cells,[2,4,5] endothelial cells,[3] and podocytes.[6] We performed electron microscopy on 3 biopsies from live patients with COVID-

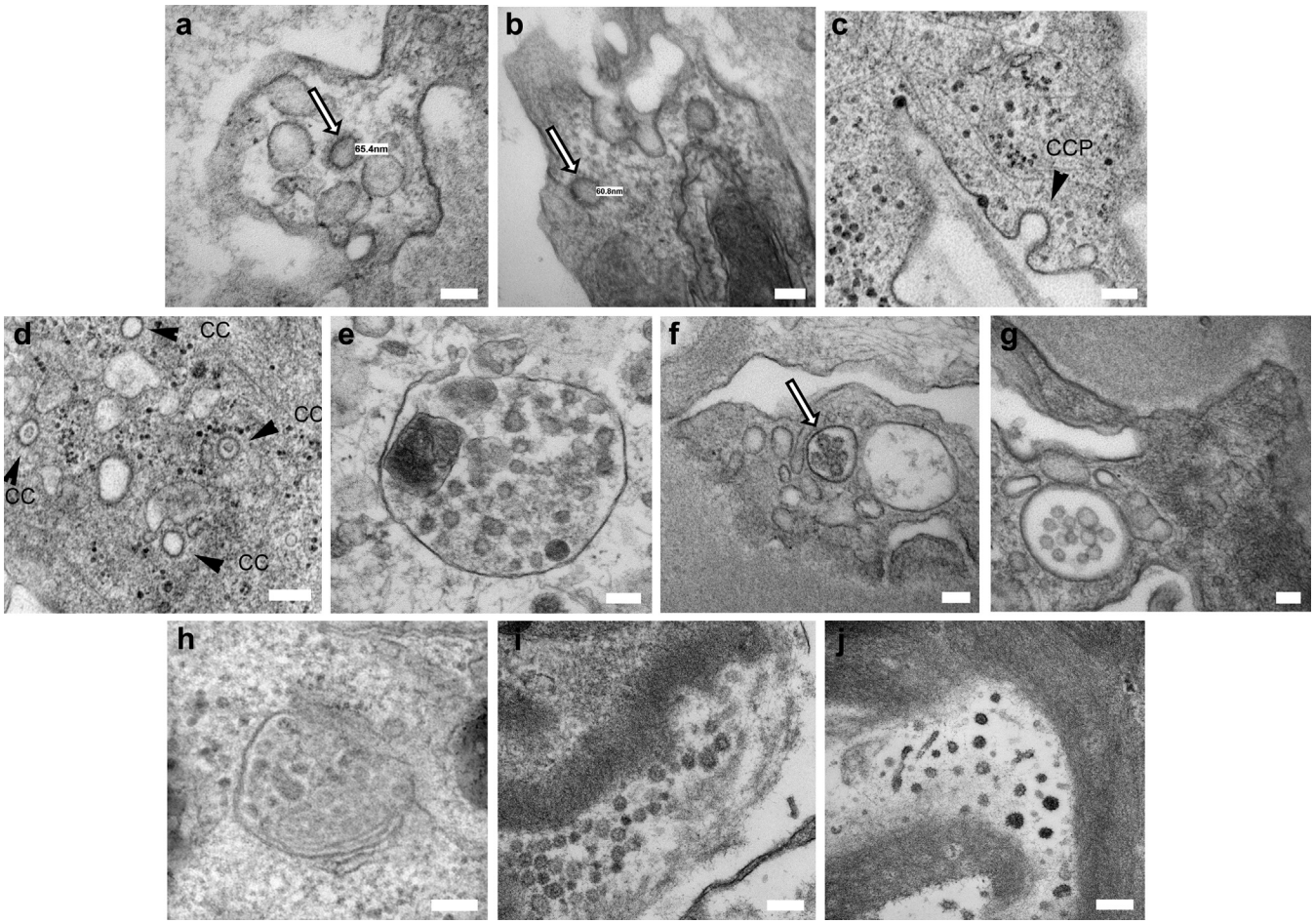

Figure 1 | (a) Transplant patient 2 weeks post-positive nasal swab for severe acute respiratory syndrome coronavirus 2 (SARS-CoV2), with graft dysfunction and borderline for T cell–mediated rejection on biopsy; endothelial cell containing a clathin-coated vesicle with a "corona," 65 nm in diameter (white arrow; bar = 100 nm, original magnification ×20,000). (**b**) Transplant patient biopsied in August 2019 with graft dysfunction and borderline for T cell–mediated rejection on biopsy, showing an endothelial cell containing identical structures (white arrow; bar = 100 nm; original magnification ×20,000). (**c**) Clathrin-coated pits (CCP; arrowhead) at the plasma membrane (renal proximal tubular epithelial cells; bar = 100 nm). (**d**) Clathrin-coated intracytoplasmic vesicles (CC; arrowheads; renal proximal tubular epithelial cells; bar = 100 nm). (**e**) Native renal biopsy from a patient with coronavirus disease 2019 (COVID-19) and collapsing focal and segmental glomerulosclerosis; podocyte containing a microvesicular body/autophagosome (bar = 100 nm, original magnification ×98,000). (**f**) Same patient as (**a**); microvesicular body in a podocyte (white arrow; bar = 100 nm, original magnification ×15,000). (**g**) Same patient as (**b**); microvesicular body in a podocyte (bar = 100 nm, original magnification ×15,000). (**h**) Microvesicular body typical for adult human cell lines in culture (HeLa cells; bar = 200 nm). (**i**) Same patient as (**e**); extracellular structures along the glomerular basement membrane with "corona" 42 to 70 nm in diameter, likely either extruded microvesicles or degenerate microvilli (bar = 200 nm, original magnification ×68,000). (**j**) Patient with a native renal biopsy from December 2019 showing extracellular structures similar to those in (**i**) in an ischemic glomerulus (bar = 200 nm, original magnification ×68,000). To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

19, from different centers, and found images similar to those reported in the literature (Figure 1a, e, f, and i). Consultation among renal pathologists, electron microscopists, and virologists led to the conclusion that the intracellular structures represented clathrin-coated vesicles and microvesicular bodies, whereas the extracellular structures represented extruded microvesicles from microvesicular bodies and degenerate microvilli (Figure 1c, d, and h). Examination of biopsies taken in 2019, preceding the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), revealed identical structures (Figure 1b, g, and j). Microvesicular bodies and clathrin-coated vesicles are both part of the endosomal pathway. Microvesicular bodies may fuse with lysosomes and autophagosomes, leading to variable appearances. Clathrin-coated vesicles arise from clathrin-coated pits; their clathrin coat resembles a crown on electron microscopy. Electron microscopy has an important role to play in elucidating the pathogenesis of COVID-19, along with identification of viral RNA or proteins, but images need to show features that are clearly distinct from viral look-a-like subcellular structures.

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Am I a coronavirus?

To the editor: The paper by Su *et al.* analyzes renal pathologic findings in the kidneys of 26 patients that underwent postmortem exam to understand the anatomic basis of kidney disease in the setting of fatal coronavirus disease 2019 (COVID-19).[1] The authors report the finding of viral particles in the kidney of COVID-19 patients and speculate that direct infection of the kidney by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus causes kidney disease.

Several findings within the manuscript by Su *et al.*[1] are presented as definite evidence of specific disease processes without considering alternative explanations. For example, acute tubular injury is reported in all cases, including in patients with normal renal function. Discerning acute tubular injury from postmortem changes is notoriously problematic, as autolysis can mimic and mask acute tubular injury.[2] Infiltration of inflammatory cells in an arcuate artery is highlighted in a micrograph in which characteristic features of muscular arteries, such as elastic lamina or defined muscular layers, are not apparent (Figure 1d in Su *et al.*[1]). Distension of small blood vessels by red blood cells is referred to as obstruction, when it may simply represent congestion. Isolated fibrin clots are interpreted as evidence of severe endothelial injury but could also be due to coagulopathy. Most importantly, small vesicular structures identified by electron microscopy are described as viral particles without consideration of other interpretations.

Cells have organelles that can mimic the structure of viral particles, and accurate interpretation of electron micrographs requires integration of morphology and biology. The virus inside renal tubular epithelial cells and podocytes that Su *et al.*[1] describe is shown as free particles in the cytoplasm, and not within membrane-bound organelles as would be expected for coronavirus based on *in vitro* studies and the rare examples of *in vivo* coronavirus infections reported prior to the current pandemic.[3–5] There is no explanation for why the virus seen by Su *et al.*[1] breaks this paradigm, which raises important questions about their interpretation of the micrographs. Cells have many structures comparable in size to the coronavirus, with varying degrees of electron-dense material surrounding and inside these structures. Notable examples include coated vesicles that are responsible for moving cargo into cells and between membrane-bound organelles (e.g., clathrin-coated vesicles and coatamer-coated vesicles).

To support their interpretation of the electron micrographs, Su *et al.*[1] present immunofluorescence studies performed on sections of formalin-fixed and paraffin-embedded tissue. The distribution and quality of the positive anti-nucleocapsid protein staining bears striking resemblance to lipofuscin autofluorescence.[6] Controls were reported to stain as expected, but no images of the controls