Caution in Identifying Coronaviruses by Electron Microscopy

We are concerned about the erroneous identification of coronavirus directly in tissues by authors using electron microscopy. Several recent articles have been published that purport to have identified severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) directly in tissue.[1–4] Most describe particles that resemble, but do not have the appearance of, coronaviruses.[5–7]

The evidence provided in the article by Farkash *et al.*[8] in *JASN* likewise does not confirm the presence of SARS-CoV-2 in kidney tissue. Coronaviruses have been carefully described in electron microscopic images of thin sections.[9–12] In these images of thin sections, coronaviruses appear as spherical structures containing black dots on the inside, which are cross-sections through the helical viral nucleocapsid (Figure 1). Coronaviruses receive their outer covering by budding into cellular membranes of the rough endoplasmic reticulum and Golgi complex forming a vacuole and are found in the intracisternal space. The spikes are seen with difficulty in thin sections of infected cells, but a "fuzz" is sometimes visible. However, note that these spikes face the inside of the vacuole and do not touch the cytoplasm of the cell. Complete virus particles can also be found at the cell surface, having been extruded when the vacuolar membrane fuses with the plasma membrane and exocytoses them; in this case, the spikes on the virions face the extracellular space, again, not the cytoplasm.

In the article by Farkash *et al.*,[8] the electron microscopic images in their Figure 3, A–C do not demonstrate coronaviruses. Rather, the structures described as virus are clathrin-coated vesicles (CCVs), normal subcellular organelles involved in intracellular transport. Figure 3A[8] is a low magnification of a dying cell with nonspecific disorganized cytoplasm with an arrow pointing to an aggregation of CCVs. Panels B and C in their Figure 3[8] show clusters of CCVs, and the inset for Figure 3C[8] shows a higher magnification. None of these spherical structures contain cross-sections through the nucleocapsid of virus particles. In addition, these CCVs are seen free in the cytoplasm, whereas coronavirus particles are found enclosed within a vacuole so that the spikes face the inside of the vacuolar contents, not the cytoplasm. Figure 3D[8] contains a multivesicular body

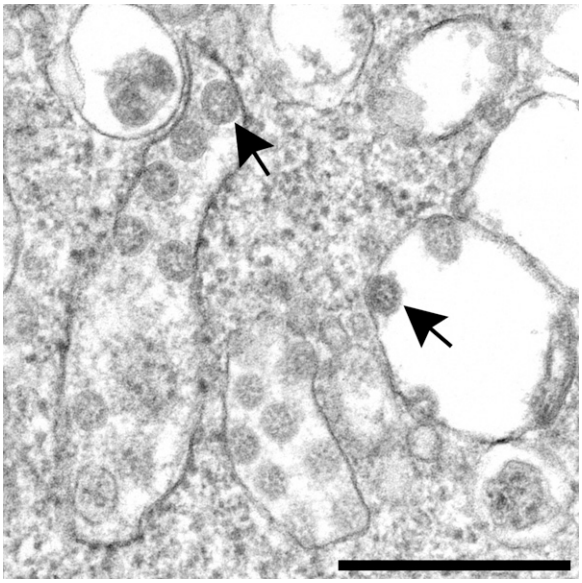

Figure 1. Severe acute respiratory syndrome coronavirus 2 isolate grown in cell culture showing numerous spherical viral particles (arrows) that are in the cisternae of the rough endoplasmic reticulum/Golgi complex area of the cell. Note the black dots on the interior of the particles, which are cross-sections through the viral nucleocapsid. Scale bar: 400 nm.

(MVB), which they have likened to double-membrane vesicles, the replication complex for coronaviruses. The structure shown in the manuscript by Farkash *et al.*[8] does not have the two tightly opposed membranes seen in double-membrane vesicles and does not have the appearance of what is shown in the reference they cite.[13] In addition, MVBs can be found in kidney tissues observed historically.[6] Moreover, MVBs are formed by invaginations of endosomes and are intermediates in trafficking for lysosomes.

Additionally, Farkash *et al.*[8] document their findings by referring to an article by Su *et al.*[2] that purports to have identified coronavirus in kidney. Likewise, that article shows only normal cell structures that, to the non-electron microscopist virologist, may resemble coronavirus. Their interpretation has been refuted in Letters to the Editor of *Kidney International*.[5,6]

Identification of viruses is not always straight forward. Consideration should be given to the mechanism of virus production, including the location inside of cells, as well as the appearance (size, shape, internal pattern of the nucleocapsid, and surface spikes).[14–16] Care should be taken to prevent mistaking cell organelles for viral particles.[17,18]

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See related Letters to the Editor, "Kidney Involvement in COVID-19: Need for Better Definitions," and "Authors' Reply," on pages 2224–2225 and 2225–2226, respectively.

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Kidney Involvement in COVID-19: Need for Better Definitions

Since the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus outbreak in China at the end of 2019, increasing interest emerged regarding renal involvement during coronavirus disease 2019 (COVID-19). AKI with or without proteinuria is described in a variable percentage of patients with COVID-19, and several reports outlined an increased mortality risk in those with AKI.

The mechanisms of renal injury during COVID-19 are difficult to study due to the interference of several coexisting factors, such as polypharmacy, hypoxia, and cytokine storm. Just like severe acute respiratory syndrome coronavirus, SARS-CoV-2 virus entry into target cells is facilitated by the presence of angiotensin-converting enzyme 2 (ACE2) expressed in respiratory cells as well as renal tubular cells and podocytes, making the hypothesis of a direct renal infection particularly intriguing.

Farkash *et al.*[1] observed renal tubular vacuolization and virus-like inclusions in tubular cells by electron microscopy (EM) in the autopsy of a patient with COVID-19. Similar EM findings together with suggestive immunohistochemical stain for SARS-CoV-2 proteins in tubules were shown in a few patients from two independent Chinese COVID-19 autopsy

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