cohorts (Diao B, Wang C, Wang R, Feng Z, Tan Y, Wang H, et al. [2020] Human kidney is a target for novel severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2] infection. 10.1101/2020.03.04.20031120).[2] However, the same immunohistochemical was not considered specific when used by others.[3] Of note, when specific and highly sensitive molecular methods for tissue viral identification were used (*i.e.*, RNAscope[3] or real-time PCR,[4] the presence of SARS-CoV-2 RNA could not be demonstrated within renal tissue, and none of the aforementioned autopsy studies included viral RNA isolation in the kidney.

Interestingly, viral nephropathies with direct tubular infection and viral replication within epithelial cells (*i.e.*, BK virus nephropathy) are usually associated with substantial viruria due to the direct release of viral particles in the tubular lumen following cytopathic cell injury. So far, there is no solid evidence of a correlation between urinary levels of SARS-CoV-2 viral copies and the degree of kidney injury in patients with COVID-19.[5] Moreover, the presence of viral particles by pure morphologic evaluation (EM) does not demonstrate by itself a direct cytopathic effect nor a replicative potential because it only supports viral entry. More specific morphologic correlates of viral cytopathy (nuclear atypia/dysmorphology and syncytia) have not been shown yet.

Three cases of collapsing glomerulopathy during SARS-CoV-2 infection have been reported,[6] presenting with severe kidney injury and variable proteinuria: in genetically predisposed subjects, increased levels of cytokines (*i.e.*, IFNs) in patients with COVID-19 are thought to drive podocyte injury, resulting in collapsing glomerulopathy as can be observed in other viral-associated nephropathies. Although in one case, the presence of virus-like particles in podocytes was observed by EM, the hypothesized mechanism of injury does not require direct kidney infection.

Taken together, these observations suggest that caution should be used when addressing the etiology of kidney dysfunction in patients with COVID-19 because unequivocal evidence of direct viral infection of the kidney is still lacking in these severe and acutely ill patients.

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See related Letters to the Editor, "Caution in Identifying Coronaviruses by Electron Microscopy," and "Authors' Reply," on pages 2223–2224 and 2225–2226, respectively.

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Authors' Reply

We acknowledge the expertise of the letter writers and appreciate their critical comments. We agree with Delsante *et al.*[1] that there are weaknesses to each modality of detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in autopsy kidneys. Immunohistochemical staining can nonspecifically label areas of necrosis, electron microscopy relies on morphologic features only, and *in situ* hybridization and other RNA studies may be limited by autolysis or confounded by plasma contamination. Definitive proof of direct renal infection will likely require some combination of techniques, such as immunogold or serial sections with immunohistochemical and molecular detection of virus.[2,3]

We also agree with Miller and Goldsmith[4] that we cannot definitively exclude that the structures that we have reported in

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figure 3, A–C in ref. 5 are clathrin-coated vesicles (CCVs). Indeed, in the discussion we caveat our findings, noting that plasma membrane–bound structures could mimic virus. Similar structures, presumably representing CCVs, are occasionally seen in renal tubular epithelial cells in kidney biopsies from patients without coronavirus disease 2019 (COVID-19).

There are features of the structures we report that are not typical for CCVs. CCVs are not usually seen in array-like clusters, as we have observed in several COVID-19 autopsies. The structures in figure 3 in ref. 5 are uniform in size, and CCVs can show a greater heterogeneity in sizes depending on cargo and number of clathrin triskelions, with diameters of 30–200 nm.[6] There is also some heterogeneity in the reported morphology of SARS-CoV-2, and our observed structures (65- to 91-nm diameter) are closer in size to the 60- to 81-nm diameter initially reported for SARS-CoV-2 grown in Vero cells than the 80- to 140-nm diameter reported by Miller.[7,8] Nonetheless, it is possible that uniform CCVs could accumulate in epithelial cells in unusual clusters due to cytokine storm or perimortem injury.[9]

Since publication, other investigators have detected SARS-CoV-2 RNA in the kidney using *in situ* hybridization, although ultrastructural localization was not performed.[10] To adequately address the question of direct renal infection, a comprehensive and sufficiently powered autopsy case series, using multiple modalities of detection and with adequate non–COVID-19 controls, is needed.

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Early Predictors of Arteriovenous Fistula Maturation: Preoperative Arterial Diameter Alone Is Not Enough

Given the lack of high-quality evidence, the use to date of preoperative ultrasound for vascular access planning has not improved the maturation rates of the arteriovenous fistula (AVF).

Recently in *JASN*, Farrington *et al.*[1] in their retrospective analysis involving 300 catheter-dependent patients receiving a new AVF proved a linear association between preoperative vascular diameter and AVF maturation not corresponding to a single threshold value. They also found that the preoperative

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