

## letters to the editor

3. Goldsmith CS, Tatti KM, Ksiazek TG, et al. Ultrastructural characterization of SARS coronavirus. *Emerg Infect Dis.* 2004;10:320–326.
4. Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. In: Maier HJ, Bickerton E, Britton P, eds. *Coronaviruses: Methods and Protocols.* New York, NY: Springer; 2015:1–23; 1282.
5. Miller SE. Diagnosis of viral infection by electron microscopy. In: Lennette EH, Lennette DA, Lennette ET, eds. *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections.* Washington, DC: American Public Health Association; 1995:35–76.
6. Ghadially FN. *Ultrastructural Pathology of the Cell and Matrix.* 4th ed Vol. 2. Boston, MA: Butterworth-Heinemann; 1997.
7. Miller SE. Detection and identification of viruses by electron microscopy. *J Electron Microscop Tech.* 1986;4:265–301.
8. Miller SE. Problems and pitfalls in diagnostic electron microscopy. *Microsc Microanal.* 2012;18(suppl 2):172–173.
9. Calomeni E, Satoskar A, Ayoub I, et al. Multivesicular bodies mimicking SARS-CoV-2 in patients without COVID-19. *Kidney Int.* 2020;98:233–234.
10. Haguenu F. “Viruslike” particles as observed with the electron microscope. In: Dalton AJ, Haguenu F, eds. *Ultrastructure of Animal Viruses and Bacteriophages.* Waltham, MA: Academic Press; 1973:391–397.
11. Goldsmith CS, Miller SE, Martines RB, et al. Electron microscopy of SARS-CoV-2: a challenging task. *Lancet.* 2020;395:e99.

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*Kidney International* (2020) **98**, 231–232; <https://doi.org/10.1016/j.kint.2020.05.004>

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**The authors reply:** We thank Drs. Miller and Brealey<sup>1</sup> for their comments and fully acknowledge their expertise in the field of electron microscopy. We also acknowledge our uncertainties regarding the exact nature of the particles seen in the podocytes in our patient’s kidney biopsy, and we were cautious in the interpretation of these findings. Following Drs. Miller and Brealey’s comments,<sup>1</sup> we have modified our letter before its final publication in the journal to further underline that these particles may correspond to nonviral entities.

However, the particles detected in our patient’s biopsy are rather similar to the ones reported in the first documentation of severe acute respiratory syndrome coronavirus 2.<sup>2</sup> Besides, the appearance of intracellular viral inclusions appears to be quite variable from one publication to another.<sup>3,4</sup> To our opinion, it remains, therefore, possible that the particles observed in our patient are of viral origin. Nevertheless, we totally agree with Drs. Miller and Brealey<sup>1</sup> that the definite proof for the presence of viral inclusions in cells requires an immunostaining with specific antibodies, whether in cultured cells or in tissue samples.

Our knowledge of coronavirus disease 2019 is rapidly evolving and caution is of the utmost importance.



1. Miller SE, Brealey JK. Visualization of putative coronavirus in kidney. *Kidney Int.* 2020;98:231–232.
2. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med.* 2020;382:727–733.
3. Su H, Yang M, Wan C, et al. Renal histopathological analysis of 26 postmortem findings of patients with COVID-19 in China. *Kidney Int.* 2020;98:219–227.
4. Varga Z, Flammer AJ, Steiger P, et al. Endothelial cell infection and endotheliitis in COVID-19. *Lancet.* 2020;395:1417–1418.

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*Kidney International* (2020) **98**, 232; <https://doi.org/10.1016/j.kint.2020.05.002>

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**The authors reply:** We have carefully read and considered the letter from Prof. Miller and Dr. Brealey,<sup>1</sup> distinguished experts of electron microscopy (EM), and appreciate that they pointed out the limitations of our study.<sup>2</sup>



We agree with Miller and Brealey’s point and recognize that there are inherent difficulties in discrimination of cellular vesicles from viral particles solely by morphological evidence, especially in routine EM processing of autopsy tissues. These conditions differ markedly from the *in vitro* negative staining of body fluids or cell culture, which are the techniques usually utilized for optimal visualization of viral structure. However, EM is still an essential tool and a front-line evaluation method in the search for unknown pathogens in outbreaks or epidemics. For example, the causative agents of the outbreak of severe acute respiratory syndrome (SARS) in China in 2003 and human monkey pox in the United States in 2003 were both first identified by EM. In addition, with our immunofluorescence staining for SARS-coronavirus (CoV) nuclear protein as we presented in our paper (Figure 3d)<sup>2</sup> and the recent publications of ultrastructural feature of SARS-CoV-2,<sup>3,4</sup> we consider the structures as possible, but not definitively proven, CoV. We have therefore prudently changed the description in the preprint version of our article of “viral particle” to “coronavirus-like particle.” Ideally, immuno-EM or *in situ* hybridization studies to assess local protein or RNA levels of CoV will further clarify the possibility of direct kidney parenchymal infection. Such a combination of ultrastructural images and molecular data could then definitively identify viral-like particles as SARS-CoV-2.

1. Miller SE, Brealey JK. Visualization of putative coronavirus in kidney. *Kidney Int.* 2020;98:231–232.