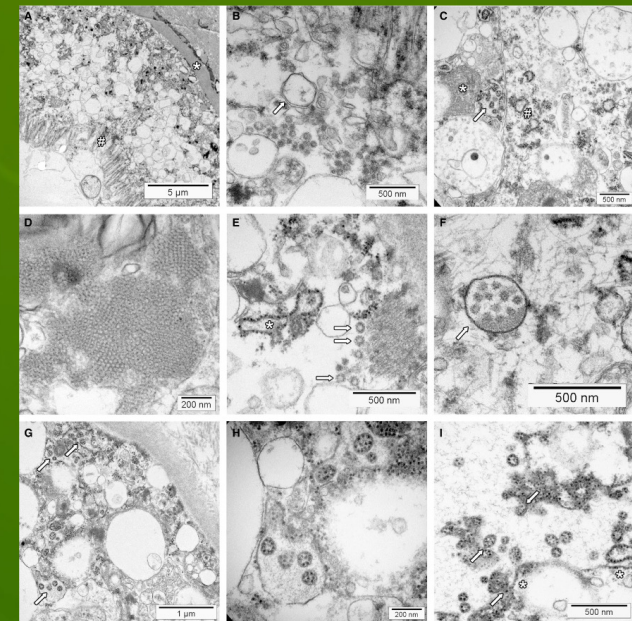


Limitations of Electron Microscopy

C. Microscopy

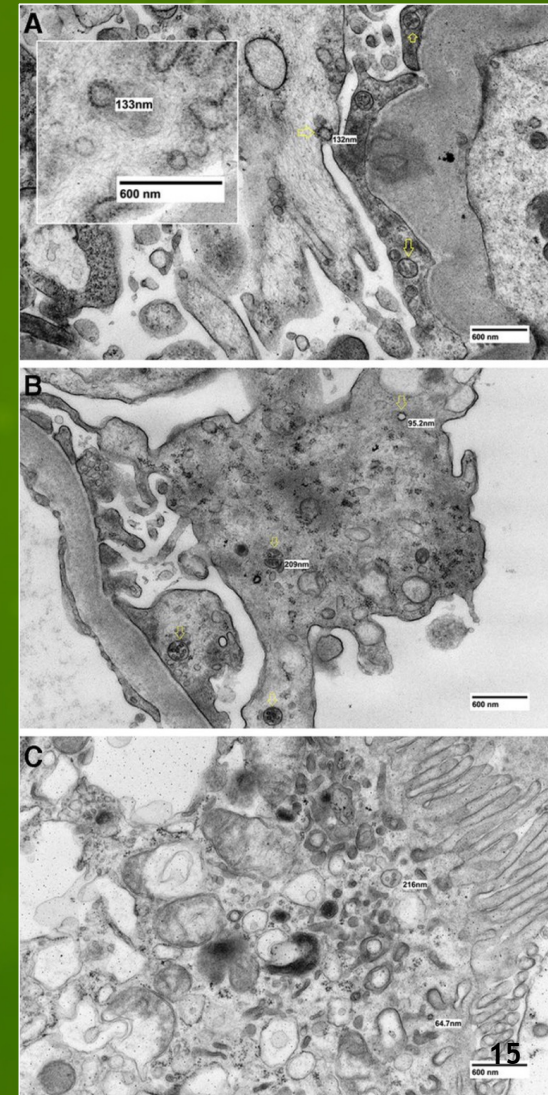
When a tissue is prepared for histology, histochemistry, electron microscopy, or immunocytochemistry, an animal is killed; the tissue is excised; it is fixed or frozen; it is embedded; it is sectioned; it is rehydrated; it is stained; it is mounted; it is radiated by light, or bombarded by electron beams. Living tissue could not survive the dehydration, low pressure, x-irradiation and electron bombardment, which occur in the electron microscope. So, heavy metal salts of osmium, tungsten, manganese, uranium or lead, are deposited on fixed tissue, and these deposits are examined. When one studies unfixed tissues in physiological media, one is looking at cells, which exchange approximately normally with their environments. In histological sections, one is examining tissue *plus* reagents used in the preparation, *minus* constituents of the tissue (including water), dissolved in or extracted by, the reagents used. The electron microscopists look at heavy metal salts, *plus* other reagents used in the preparation, *minus* substances extracted by the reagents. Virtually nothing is seen if heavy metal salts are not used for staining, as was shown by Weakley in an elegant illustration in her book, 'Beginners Handbook of Electron Microscopy', (1972). In addition, one does not see any cellular structures, which do not react with or dissolve in reagents, including ethanol and acetone.



Appearances Can Be Deceiving...

“we have observed morphologically indistinguishable inclusions within podocytes and tubular epithelial cells both in patients negative for coronavirus disease 2019 (COVID-19) as well as in renal biopsies from the pre-COVID-19 era.”

Source: (Appearances Can Be Deceiving-Viral-like Inclusions in COVID-19 Negative Renal Biopsies by Electron Microscopy. Kidney360. <https://kidney360.asnjournals.org/content/1/8/824>)



Caution in Identifying Coronaviruses by Electron Microscopy

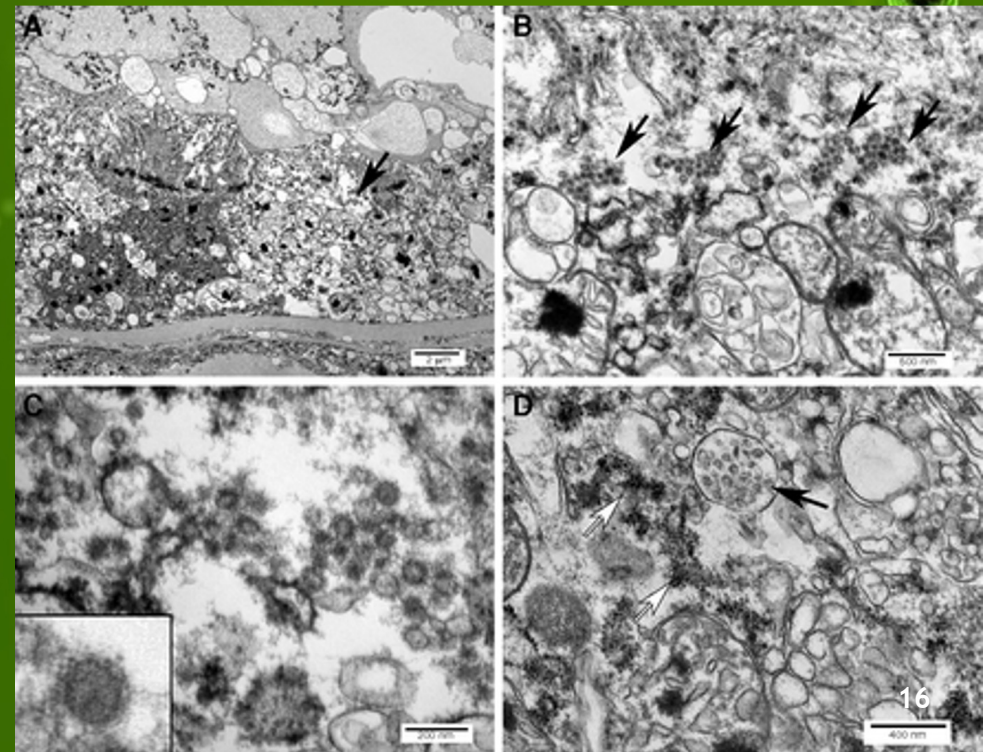
“The evidence provided in the article by Farkash *et al.*⁸ in *JASN* likewise does not confirm the presence of SARS-CoV-2 in kidney tissue.

In the article by Farkash *et al.*, 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.

Additionally, Farkash *et al.* document their findings by referring to an article by Su *et al.* 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*.

Identification of viruses is not always straightforward. 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). Care should be taken to prevent mistaking cell organelles for viral particles.”

Source: (Caution in Identifying Coronaviruses by Electron Microscopy | American Society of Nephrology. asnjournal.org)



Multivesicular Bodies Mimicking SARS-CoV-2 in Patients Without COVID-19

“Recent publications in *Kidney International* used electron microscopy (EM) to detect the virus in autopsy or biopsy specimens of the kidney. Most of the published images depicting the suspected virus are very similar, if not identical, to multivesicular bodies (MVBs). MVBs have been well-known since the 1960s and their appearance and occurrence is detailed in the classic monograph of Feroze Ghadially; however, their exact significance and function is unclear. We suspect that the EM images of SARS-CoV-2 published to date are in fact MVBs.”

Source: (Multivesicular bodies mimicking SARS-CoV-2 in patients without COVID-19 - *Kidney International*. kidney-international.org)

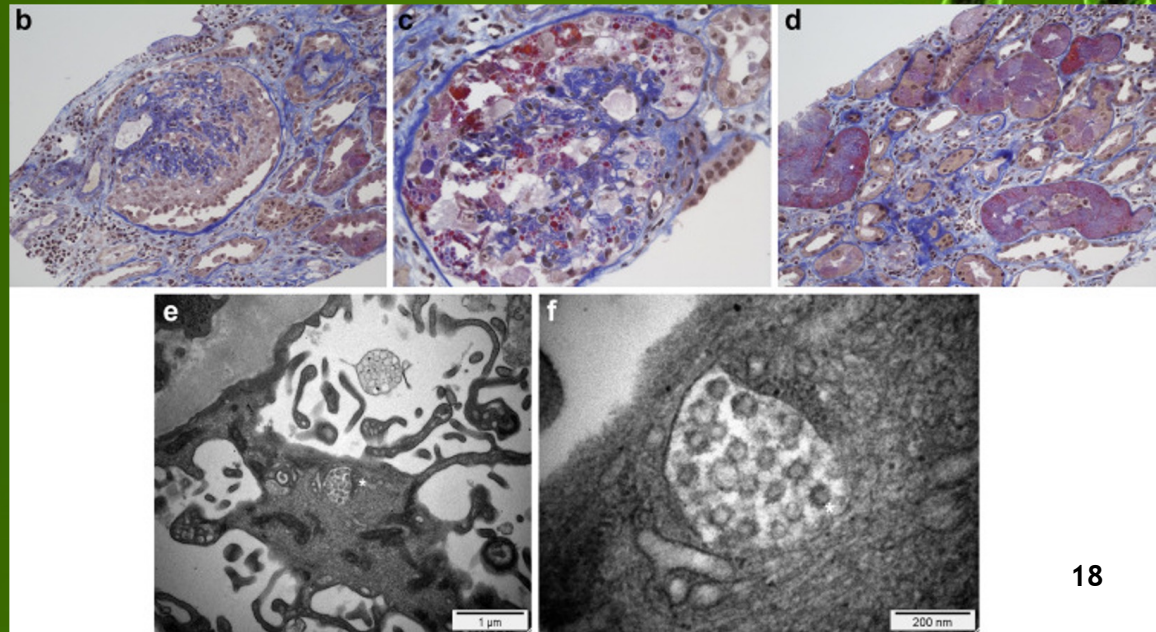


Electron microscopy of SARS-CoV-2: a challenging task

“We read with interest the Correspondence by Zsuzsanna Varga and colleagues on the possible infection of endothelial cells by SARS-CoV-2 using electron microscopic (EM) images as evidence. However, we believe the EM images in the Correspondence do not show coronavirus particles but instead show cross-sections of the rough endoplasmic reticulum (RER).”

Just recently, there have been two additional reports, in which structures that can normally be found in the cytoplasm of a cell have been misinterpreted as viral particles. EM can be a powerful tool to show evidence of infection by a virus, but care must be taken when interpreting cytoplasmic structures to correctly identify virus particles.”

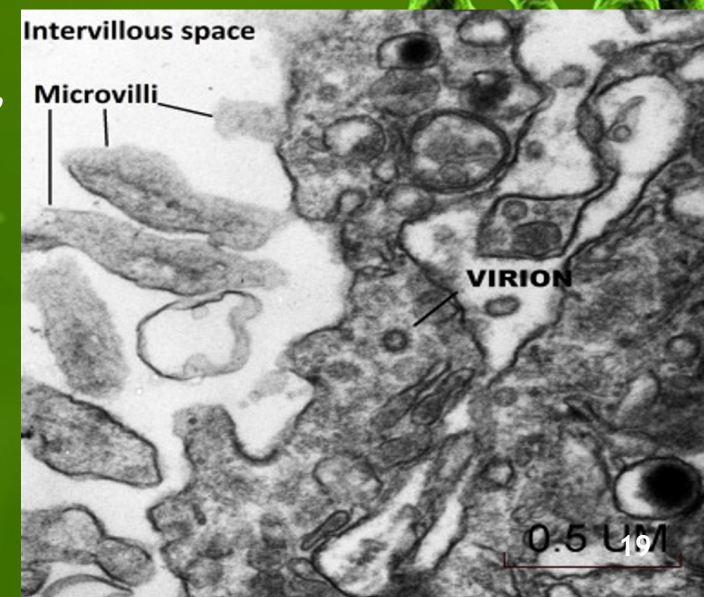
Source:
(Electron microscopy of SARS-CoV-2: a challenging task - The Lancet)



Alternative interpretation to the findings reported in visualization of SARS-CoV-2 invading the human placenta using electron microscopy

“The report of virus-like inclusions in syncytiotrophoblast is intriguing and thought-provoking. However, I respectfully offer an alternative interpretation of the data. The structures identified as SARS-CoV-2 virions look exactly like clathrin-coated pits or vesicles. Clathrin-coated vesicles are spherical structures employed by trophoblasts and other cell types to internalize cargos from the extracellular space. Coated vesicles and coated pits derive their name from the external scaffold coat composed of clathrin triskelions that decorate the surface of the structure. In transmission electron micrographs in which tissue-thin sections are stained with uranyl acetate and lead citrate, coated vesicles have an electron-dense studded surface that appears identical to the “corona” comprising the spike protein that decorates all coronaviruses, including SARS-CoV-2 virions. It is this studded surface or corona that gives the genus *Betacoronaviridae* its characteristic morphology and name.

Source: (Alternative interpretation to the findings reported in visualization of severe acute respiratory syndrome coronavirus 2 invading the human placenta using electron microscopy - American Journal of Obstetrics & Gynecology. ajog.org)

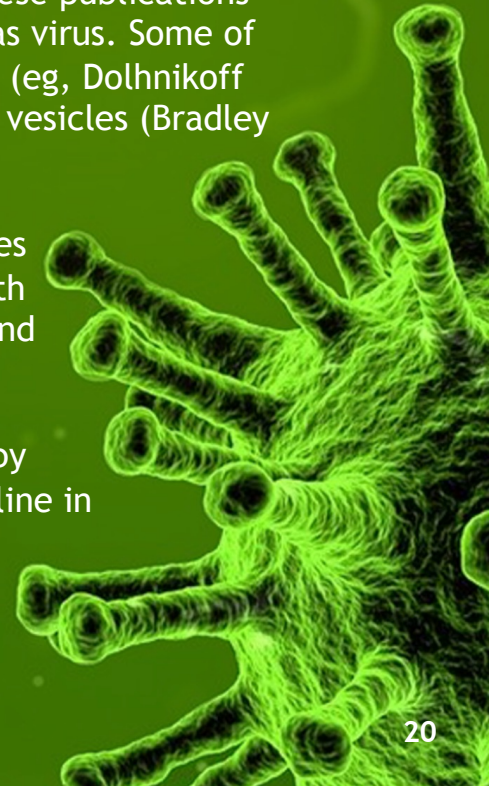


Why misinterpretation of electron micrographs in SARS-CoV-2-infected tissue goes viral

“Nevertheless, ultrastructural details in autopsy tissues have been misinterpreted as coronavirus particles in recent papers. Bradley and colleagues described ‘coronavirus-like particles’ in autopsy specimens of the ‘respiratory system, kidney, and gastrointestinal tract’, and in a case report Dolhnikoff and colleagues described ‘viral particles’ in ‘different cell types of cardiac tissue’ of a deceased child. However, the images in these publications show putative virus particles that lack sufficient ultrastructure for an unambiguous identification as virus. Some of these particles definitely represent other cellular structures, such as rough endoplasmic reticulum (eg, Dolhnikoff and colleagues,⁴ figure 3B), multivesicular bodies (Bradley and colleagues,³ figure 5C) and coated vesicles (Bradley and colleagues,³ figure 5B, G). Moreover, it is remarkable that Dolhnikoff and colleagues referred to findings, described by Tavazzi and colleagues, of ‘viral particles’ in interstitial cells, which are clearly non-viral structures, such as coated vesicles. Furthermore, Bradley and colleagues quoted publications as a reference for their virus particle identification, which, in our opinion, both identified non-coronavirus structures as coronavirus particles, as already discussed by Goldsmith and colleagues and by Miller and Brealey.

As diagnostic EM requires both specialised staff and expensive equipment, and has been replaced by other methods (eg, immunohistochemistry) in several fields of application, its use has been in decline in the past decades, resulting in irreversible loss of expertise that now becomes dramatically overt during the SARS-CoV-2 pandemic. This dilemma of diagnostic EM should alarm us all, as misleading information on the presence of SARS-CoV-2 in tissue has already made its way into the scientific literature and seems to be perpetuated.”

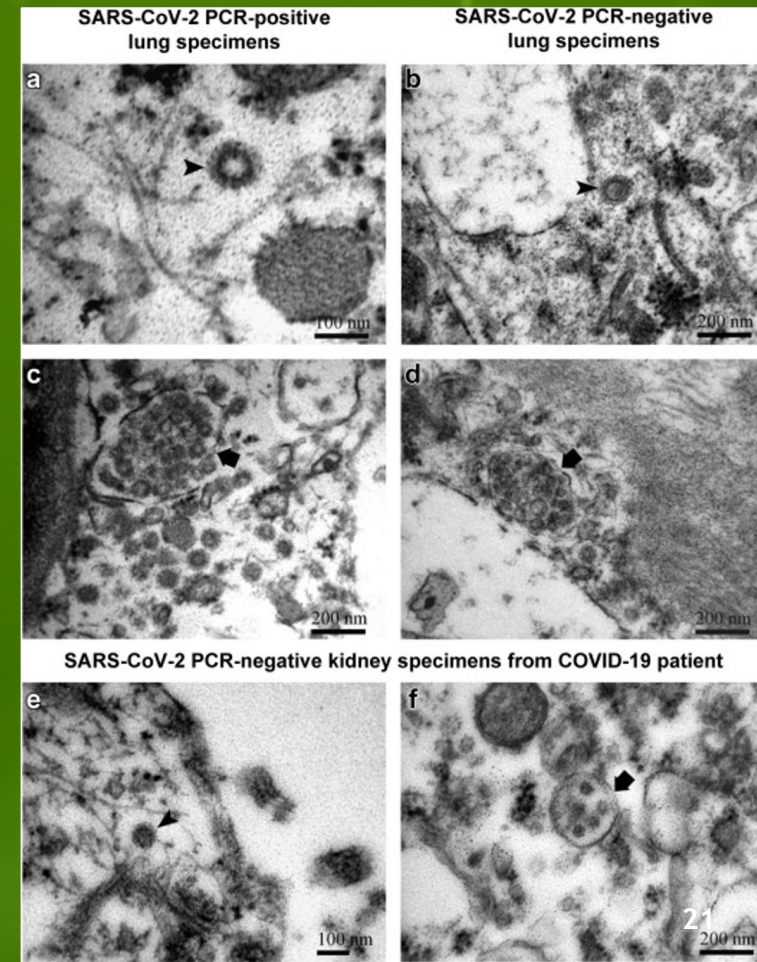
Source: (Why misinterpretation of electron micrographs in SARS-CoV-2-infected tissue goes viral - The Lancet)



SARS-CoV-2 Virions or Ubiquitous Cell Structures? Actual Dilemma in COVID-19 Era

“Figure 1 Individual vesicle with electron-dense coat (arrowhead) located freely in the cytosol of endothelial cell in lung with positive reverse-transcriptase polymerase chain reaction (RT-PCR) for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA (a) and in lung with negative RT-PCR for SARS-CoV-2 RNA (b). Note similar morphology of the 2 structures in images (a) and (b), which could be virus or coated vesicle. In view of the RT-PCR results, the observed structures might be virus in image (a) but not in image (b). Vacuole with many small vesicles inside the limiting membrane (arrow) in the cytosol of endothelial cell in lung with positive RT-PCR for SARS-CoV-2 RNA (c) and in lung with negative RT-PCR for SARS-CoV-2 RNA (d). Note again similar morphology of the 2 structures in images (c) and (d), which could be a cluster of viral particles or multivesicular bodies (MVBs) with intraluminal vesicles inside. In view of the RT-PCR results, the observed structures might be a cluster of viral particles in (c) but not in (d). (e,f) Structures resembling virions, coated vesicles or MVBs were observed in the cytosol of kidney podocytes in a SARS-CoV-2-positive patient but with negative RT-PCR for SARS-CoV-2 RNA. In view of the RT-PCR results, the presented structures are not viruses but ubiquitous coated vesicles and MVBs.”

Source: (SARS-CoV-2 Virions or Ubiquitous Cell Structures? Actual Dilemma in COVID-19 Era - Kidney International Reports. kireports.org)



The ol' "Point and Declare" method

