Continued False-Positive Detection of SARS-CoV-2 by Electron Microscopy

Carsten Dittmayer, MD,[1] and Michael Laue, PhD [2]

Dodig et al[1] show electron micrographs to document infection of skeletal muscle by SARS-CoV-2 in biopsy samples of patients with COVID-19, and report on their findings in a Brief Communication entitled "COVID-19-Associated Critical Illness Myopathy with Direct Viral Effects." The putative virus particles are termed "coronavirus-like particles" or "virus-like particles."

However, the images provided by Dodig et al[1] do not show virus particles. Most of the particles assigned as (corona)viruslike particles represent coated vesicles (Figure 1K–O and 2J–M), whereas Figure1 R shows an unidentifiable structure that does not reveal sufficient features of coronavirus particles. Confusion of coated vesicles with coronavirus happened frequently because of the clathrin coat at the outer surface of the membrane, which resembles, at first sight, the spikes of a coronavirus. However, spikes of coronaviruses show a globular head group, are less evenly distributed at the particle surface, and appear less dense than the clathrin coat of coated vesicles (Figure1). Moreover, coronavirus particles must show additional structural features such as a granular interior, representing the ribonucleoprotein, and a correct location within membrane-bound compartments.[2] The (corona)viruslike particles in Figure 1K–O and 2J–M reveal a bright interior and are localized in the cytoplasm, which is, considering the overall sufficient preservation of membrane-bound compartments shown by Dodig et al, not the correct location for coronavirus particles in infected cells.[3] Figure 1R shows a particle profile that is localized within a membrane-bound compartment, but that is too wide in diameter (>200nm) and that reveals no surface structures (ie, spikes) and a rather unusual interior granularity.

As for any other subcellular object identified by thin section electron microscopy, typical ultrastructural features must be demonstrated by using images of sufficient quality and information. Despite the publication of several comments (eg, Miller and Brealey,[3] Dittmayer et al[4]) and more extended papers (eg, Bullock et al[2]) on the difficulties of recognition of SARS-CoV-2 particles by electron microscopy in patient material, continuously more scientific papers with highly questionable coronavirus detection appear. In addition, false-positive electron microscopy data have often been used to validate other in situ virus detection methods such as immunohistochemistry and in situ hybridization, which detect virus molecules rather than the intact virus particle and can provide nonspecific results. A recent, unreviewed analysis of the literature found that at least 116 of 122 journal publications demonstrate clearly wrong subcellular structures as virus or provide only insufficient proof (ie, images) for the presence of coronaviruses.[5] To avoid further misinterpretations or false-positive results, we strictly refer to the detailed recommendations on coronavirus identification and searching strategies.[2,5]

Author Contributions

C.D. and M.L. wrote the manuscript and analyzed the data.

Potential Conflicts of Interest

Nothing to report.

[1]*Department of Neuropathology, Charité–Universitätsmedizin Berlin, Free University of Berlin and Humboldt University of Berlin, Berlin, Germany*
[2]*National Consultant Laboratory for Electron Microscopy of Infectious Pathogens, Center for Biological Threats and Special Pathogens 4, Robert Koch Institute, Berlin, Germany*

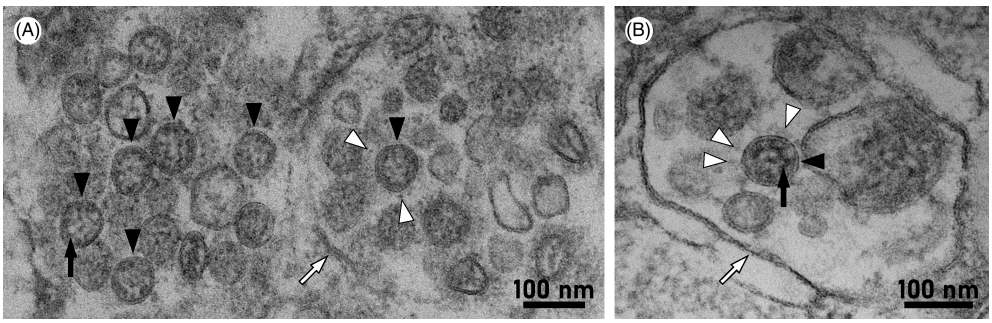

FIGURE Ultrastructure of coronavirus particles in autopsy lung. Well-preserved coronavirus (CV) particles are highlighted by black arrowheads. The most characteristic feature of CV particles in thin section electron microscopy is the electron dense appearance and granular substructure due to presence of ribonucleoprotein (*black arrows*), whereas "spikes" reveal a faint contrast with a prominent globular head (*white arrowheads*). Intracellular CV particles are enclosed in membrane-bound compartments (*white arrows*) that may be ruptured, like in image (A), due to, for example, autolysis, thus also limiting cell type assessment. See Krasemann et al.[5] for detailed recommendations of CV particle identification and information on the sample. Digitized thin sections and regions of autopsy lung are available online for open access pan-and-zoom analysis (www.nanotomy.org).[5]

Address correspondence to Dr Laue, National Consultant Laboratory for Electron Microscopy of Infectious Pathogens, Center for Biological Threats and Special Pathogens 4 (ZBS 4), Robert Koch Institute, Berlin, Germany. E-mail: lauem@rki.de

References

1. Dodig D, Tarnopolsky MA, Margeta M, et al. COVID-19-associated critical illness myopathy with direct viral effects. Ann Neurol 2022;91:568–574.
2. Bullock HA, Goldsmith CS, Zaki SR, et al. Difficulties in differentiating coronaviruses from subcellular structures in human tissues by electron microscopy. Emerg Infect Dis 2021;27:1023–1031.
3. Miller SE, Brealey JK. Visualization of putative coronavirus in kidney. Kidney Int 2020;98:231–232.
4. Dittmayer C, Meinhardt J, Radbruch H, et al. Why misinterpretation of electron micrographs in SARS-CoV-2-infected tissue goes viral. Lancet 2020;396:e64–e65.
5. Krasemann S, Dittmayer C, v Stillfried S, et al. Assessing and improving the validity of COVID-19 autopsy studies—a multicenter approach to establish essential standards for immunohistochemical and ultrastructural analyses. medRxiv 2022:https://doi.org/10.1101/2022.01.13.22269205.

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Deep Brain Stimulation for Tremor: Direct Targeting of a Novel Imaging Biomarker

Erik H. Middlebrooks, MD [1,2] Philip Tipton, MD [3] Lela Okromelidze, MD,[1] Elena Greco, MD,[1] Julio A. Mendez, MD,[1] Ryan Uitti, MD,[3] and Sanjeet S. Grewal, MD[2]

In a recent report by Neudorfer et al.,[1] a novel imaging biomarker for thalamic deep brain stimulation (DBS) in treatment of essential tremor (ET) was proposed as a hypointense thalamic area identified on fast gray matter acquisition T1 inversion recovery (FGATIR) MRI. Based on prior observations regarding the utility of identification of the dentato-rubro-thalamic tract (DRTT) in DBS for ET,[2,3] we have recently transitioned to using the same FGATIR hypointensity, which we have found to reliably correlate with DRTT in our single-subject preoperative MR tractography. Typical preoperative planning MRI is obtained on either 3T or 7T MRI. Stereotactic planning is then performed using the strategy of placing the electrode tip at the inferior border of the FGATIR hypointensity described by Neudorfer et al.[1] with middle contacts traversing this hypointense region (Fig). We report our experience with this strategy in a cohort of patients with essential tremor undergoing unilateral thalamic DBS.

Fifteen consecutive patients were included in the analysis. Implants were performed by initial electrode pass with macrostimulation intraoperatively to confirm tremor benefit and absence of side effects. Microelectrode recording was not performed.

Mean preoperative Fahn-Tolosa-Marín tremor rating score (TRS) Part A was 15.3 (range 6–25) and postoperative TRS was 5.5 (range 1–13). Postoperative follow-up was performed at a mean of 3.7 months (range 1–13 months) with a mean improvement in TRS of 64.2% (range 20–91%). There was a ≥ 50% improvement in TRS in 12/15 (80%). There were no acute stimulation-induced adverse effects.

Only one of 15 electrodes required repositioning, which ultimately was placed at the intended target on postoperative imaging (Fig) suggesting brainshift may have contributed to slight deviation of the initial pass. Additionally, postoperative imaging in the worst responder revealed an electrode position slightly more anterior than the surgical plan (Fig).

In summary, our experience using the same hypointensity on FGATIR described in Neudorfer et al.[1] for direct surgical targeting shows a high rate of tremor improvement with rare need for electrode repositioning. Mean tremor improvement using this approach was slightly greater than the combined mean improvement of the previous group of studies analyzed in Neudorfer et al.[1] (64.2 vs 57.4%) and greater than our previously reported large, multicenter cohort using traditional targeting approaches.[3] One possible reason is better consideration of single-subject anatomical variation by use of direct targeting. Additionally, since many studies have reported relatively short-term follow-up, this could also reflect shorter time to optimization of programming settings by use of direct target visualization. Given our experience with reliable colocalization of the DRTT (Fig), as defined on single-subject MR tractography, with the FGATIR hypointensity, we no longer routinely use tractography in our surgical planning for thalamic DBS in tremor. Our experience further supports the hypothesis posited in Neudorfer et al.[1] and provides a reliable, patient-specific direct targeting biomarker that is easily implemented and not reliant on more complex imaging modalities, such as MR tractography.

Author Contributions

Conception and Design of the Study: E.H.M., P.T., L.O., R.U., S.S.G. Acquisition and Analysis of Data: E.H.M., P.T., L.O., E.G., J.A.M. Drafting a Significant Portion of the Manuscript or Figures: E.H.M.

Potential Conflicts of Interest

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[1]*Department of Radiology, Mayo Clinic, Jacksonville, FL, USA*
[2]*Department of Neurosurgery, Mayo Clinic, Jacksonville, FL, USA*
[3]*Department of Neurology, Mayo Clinic, Jacksonville, FL, USA*