Identification of coronavirus particles by electron microscopy requires demonstration of specific ultrastructural features

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To the Editor:

With interest we read the publication of Evangelou *et al*. [1], which studied SARS-CoV-2 induced senescence in severe COVID-19. Immunohistochemistry (IHC) and electron microscopy (EM) were used for *in situ* detection of SARS-CoV-2 in autopsy tissues. The authors used formalin-fixed and paraffin-embedded (FFPE) autopsy lung of COVID-19 and non-COVID-19 patients to perform re-embedding for EM and ultrastructural analysis. They report detection of SARS-CoV-2 virions within alveolar type 2 cells of representative COVID-19 cases (figure 1c in Evangelou *et al.* [1]). Three electron micrographs show putative virus particles, indicated by arrows, to document their findings.

Since the fundamental work of G.E. Palade in the middle of the last century [2], we know about the general ultrastructural features of cellular organelles, which allow their identification by EM. With a similar strategy, namely virus isolation and structural characterisation by EM, we have learnt to distinguish viruses from the structural standard inventory of cells and to categorise them into morphological groups [3]. As a consequence, any identification of a particular subcellular structure must be demonstrated by showing their typical structural features.

Evangelou *et al*. [1] did not show sufficient structural detail to allow an identification of coronavirus (CV) particles by EM. The particles indicated in figure 1c (Evangelou *et al.* [1]) as "SARS-CoV-2" and "virions" lack essential morphological features of CV particles, such as a clearly visible enveloping biomembrane, club-shaped spikes at their surface and a characteristic granular substructure of the electron dense interior which is due to presence of ribonucleoprotein [4]. The particles meet some of the required criteria, such as electron dense appearance, round to oval shape and a size that roughly matches the size described for CV particles in FFPE re-embedded material (58–108 nm [4, 5]). They also appear to be located in membrane compartments, although it is difficult to clearly identify organelles apart from the nucleus and, perhaps, the rough endoplasmic reticulum, due to limited overall structural preservation. However, these basic criteria can also be met by other, possibly artificially altered cellular organelles and particles, such as multivesicular bodies, severely altered mitochondria and invaginations of the rough endoplasmic reticulum [4].

Generally, preservation of ultrastructure is negatively affected by the FFPE re-embedding procedure applied by Evangelou *et al*. [1] and, in some cases, is insufficient to allow a reliable identification of CV. However, even in samples prepared according to this procedure, characteristic and essential structural features of CV can be demonstrated and allow the identification of CV (figure 1) [4–7]. An important additional criterion for CV identification is the presence of numerous similarly shaped CV particles within the same cell, which is especially important in structurally impaired samples. Due to the negative effect of the FFPE re-embedding procedure on the morphology of CV particles, more particles per cell are necessary to recognise all relevant features for CV identification than in samples of a conventional preparation (figure 1) [4, 5].

EM allows direct detection of morphologically intact virus particles if structural preservation is sufficient and thereby serves as a validating tool for indirect *in situ* detection methods such as IHC [5, 8]. Detailed recommendations for correct *in situ* detection of coronavirus in autopsy tissues by EM were recently compiled [5]. Positive IHC or *in situ* hybridisation signals may guide the finding of infected cells and a

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Unequivocal detection of ultrastructural features specific to organelles or viruses is required to infer their presence by electron microscopy. This article also provides reference images for the correct identification of coronavirus. https://bit.ly/3IFYDjc

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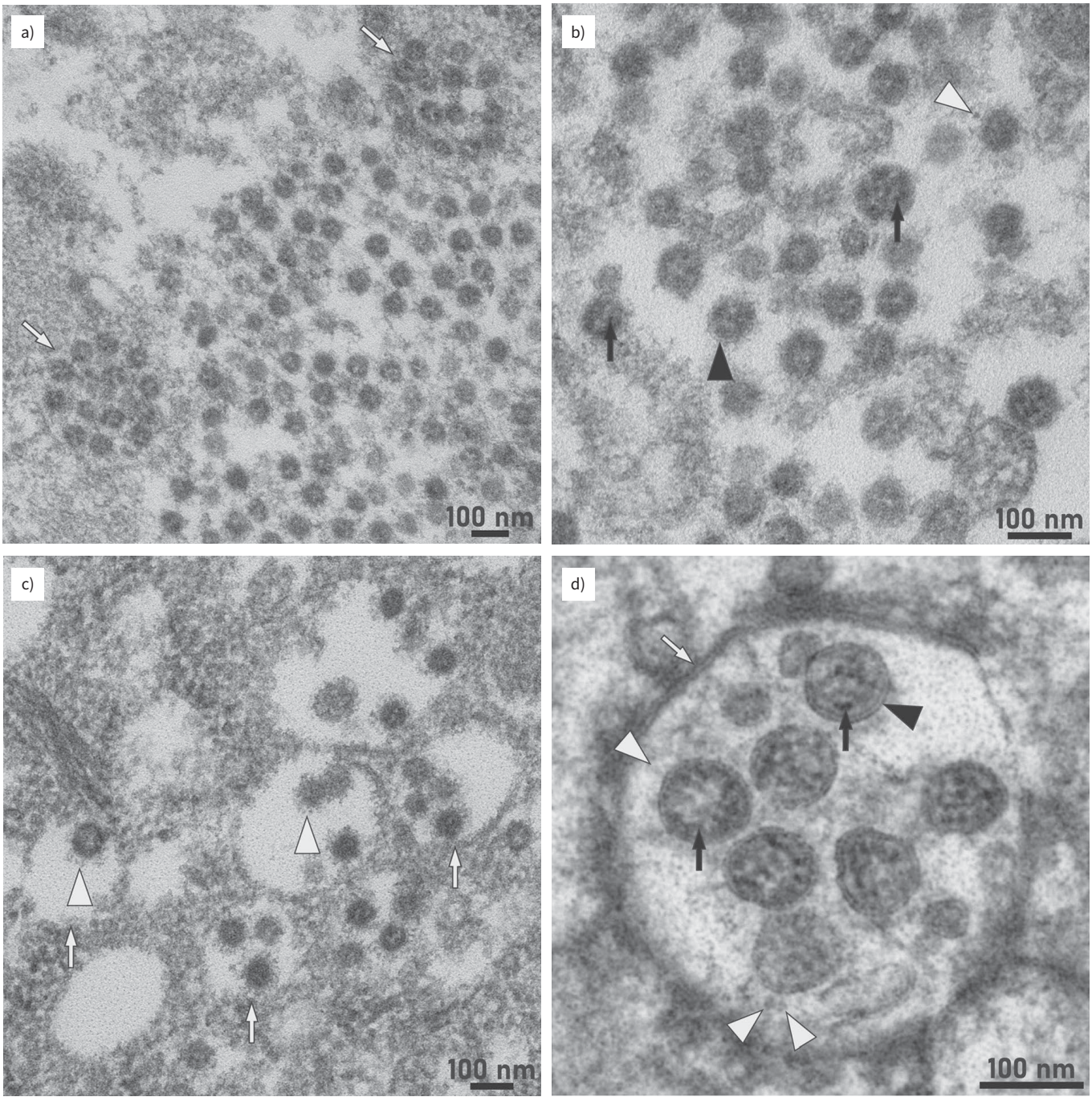

FIGURE 1 Ultrastructure of coronavirus particles in autopsy samples. Formalin-fixed and paraffin embedded autopsy olfactory mucosa, re-embedded for electron microscopy (a–c), and conventionally embedded autopsy lung of COVID-19 patients (d). Coronavirus (CV) particles can be identified due to a high number of similar particles that together fulfil all essential structural criteria [5]. Membrane compartments with multiple or single enclosed CV particles (white arrows), ribonucleoprotein (electron dense, partly granular structure: black arrows), surface projections ("spikes": white arrowheads) and biomembrane (black arrowheads). See Krasemann *et al.* [5] for further information on the specimens and detailed recommendations for sample processing and virus identification. Entirely digitised sections and regions of these samples are online available for pan-and-zoom analysis, allowing for training of finding virus particles in autopsy samples (www.nanotomy.org) [5].

sufficient number of virus particles [5]. On the other hand, since IHC signals can be false-positive, they should not be used to support the identification of insufficiently preserved structures by EM in corresponding FFPE re-embedded samples [5]. It is also necessary to note that IHC signals can only indicate the presence of virus protein, which does not necessarily imply that it is linked to a virus particle [5].

In a recently uploaded preprint, we identified 116 of 122 journal publications that misinterpreted different cellular structures as SARS-CoV-2 particles in human samples using EM, or insufficiently documented the presence of the virus [5]. Ultrastructural proof of virus in human samples is challenging [9] but achievable. However, numerous published electron micrographs showing incorrectly or insufficiently identified virus perpetuate further misinterpretations [10]. This unfortunate development increasingly hinders correct use of diagnostic EM for virus identification. Furthermore, numerous false-positive EM data were used to insufficiently validate other *in situ* detection methods such as IHC [1, 5]. Hence, the data which were used to unravel the cellular tropism of SARS-CoV-2 in patient tissues need to be critically re-evaluated [5].

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