

# SARS-CoV-2 identification in lungs, heart and kidney specimens by transmission and scanning electron microscopy

M. PESARESI<sup>1</sup>, F. PIRANI<sup>1</sup>, A. TAGLIABRACCI<sup>1</sup>, M. VALSECCHI<sup>1</sup>,  
A.D. PROCOPIO<sup>2</sup>, F.P. BUSARDÒ<sup>1</sup>, L. GRACIOTTI<sup>2</sup>

<sup>1</sup>Department of Excellence of Biomedical Sciences and Public Health, University "Politecnica delle Marche" of Ancona, Ancona, Italy

<sup>2</sup>Department of Clinical and Molecular Sciences, University "Politecnica delle Marche" of Ancona, Ancona, Italy

**Abstract.** From two COVID-19-related deaths, samples of lung, heart and kidney were collected and processed for Transmission and Scanning Electron Microscopy (TEM and SEM) with the aim of identifying the virus.

Virions of SARS-CoV-2 were found in all tissues by TEM and SEM, corroborating the hypothesis that the virus enters the cells of different organs. This is the first report identifying SARS-CoV-2 in different human tissues by TEM and SEM.

*Key Words:*

COVID-19, Transmission electron microscopy, Scanning electron microscopy.

## Introduction

After SARS-CoV-2 outbreak in Italy, the virus has been spreading quickly. As of May 2, 2020, over 28.2 thousand COVID-19-related deaths were reported by the national authorities<sup>1</sup>.

Two individuals, a 67-year-old male (case 1) and an 84-year-old female (case 2), died at home in the Marche region – where 911 COVID-19 fatalities have been reported to date – following fever, cough, and respiratory distress. A throat swab was performed within two hours of death in one case and during the autopsy in the second case, and SARS-CoV-2 infection was confirmed in both cases by Real-Time Polymerase Chain Reaction (RT-PCR). A complete autopsy was performed within 36 hours of death, and SARS-CoV-2 was also identified in heart samples by RT-PCR.

Lung, heart and kidney samples were processed for Transmission and Scanning Electron Microscopy (TEM and SEM) with the aim of identifying the virus.

## Materials and Methods

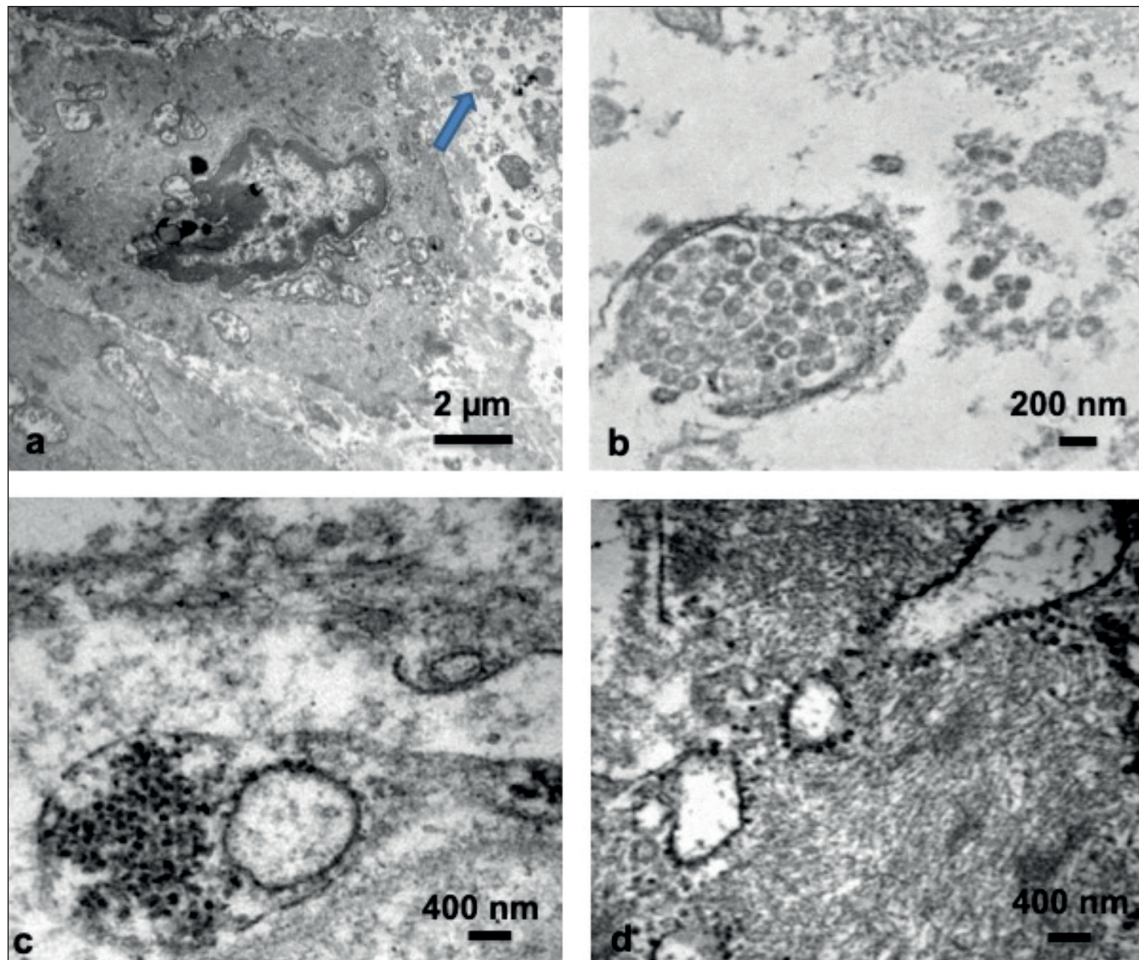
Specimens were processed as previously described<sup>2</sup>. Briefly, samples were fixed in 2% glutaraldehyde/2% paraformaldehyde in 0.1 mol/L phosphate buffer overnight at 4°C, post-fixed in 1% osmium tetroxide for 1 hour, dehydrated in Acetone series and embedded in an Epon-Araldite mixture. Thin sections (60 nm) were stained with lead citrate, then imaged at 80 KV using a Philips CM10 microscope (FEI-Thermo Fisher Scientific, Waltham, MA, USA). For SEM, fixed samples were dehydrated in Ethyl Alcohol series, chemically dried using Hexamethyldisilazane (HMDS), then mounted on aluminum stubs, coated with gold and imaged at 10 KV using a Zeiss Supra 40 Scanning Microscope.

Samples were first observed under a light microscope: only slight autolysis was noticed, and cells maintained their overall structure.

## Results

Different sections for each sample were examined by TEM. We found the presence of virions and viral particles in the lung of case 1 (Figure 1). Virions contained into a vesicle located in the extracellular compartment in the proximity of a lung smooth muscle cell are shown in Figures 1a and 1b. Intracellular viral particles were seen mainly located into vesicles or associated to internal membrane (Figure 1 c, d).

In case 2, we observed the presence of viral particles also in the cardiomyocytes adjacent to myofibrils (Figure 2 a, b) and in the renal tissue in the epithelial tubular cell (Figure 2d,e) by TEM analysis. These findings were confirmed for both tissues by SEM analysis (Figure 2 c and 2 f, g).



**Figure 1.** Lung samples of case 1 by TEM analysis. The arrow points the site containing viral particles (a). TEM micrographs showing viral particles located into vesicles or associated to internal membranes (c,d).

The difference noticed in the viral morphology by TEM can be attributed to a different virus activation/replication state or to the degeneration of the tissue and cytolytic activity due to the release of lysosomal content.

### Discussion

We demonstrated the presence of SARS-CoV-2 in lung, renal and heart tissues by SEM and TEM, thereby corroborating the hypothesis that the virus enters the cells of different organs. To the best of our knowledge, SARS-CoV-2 has been

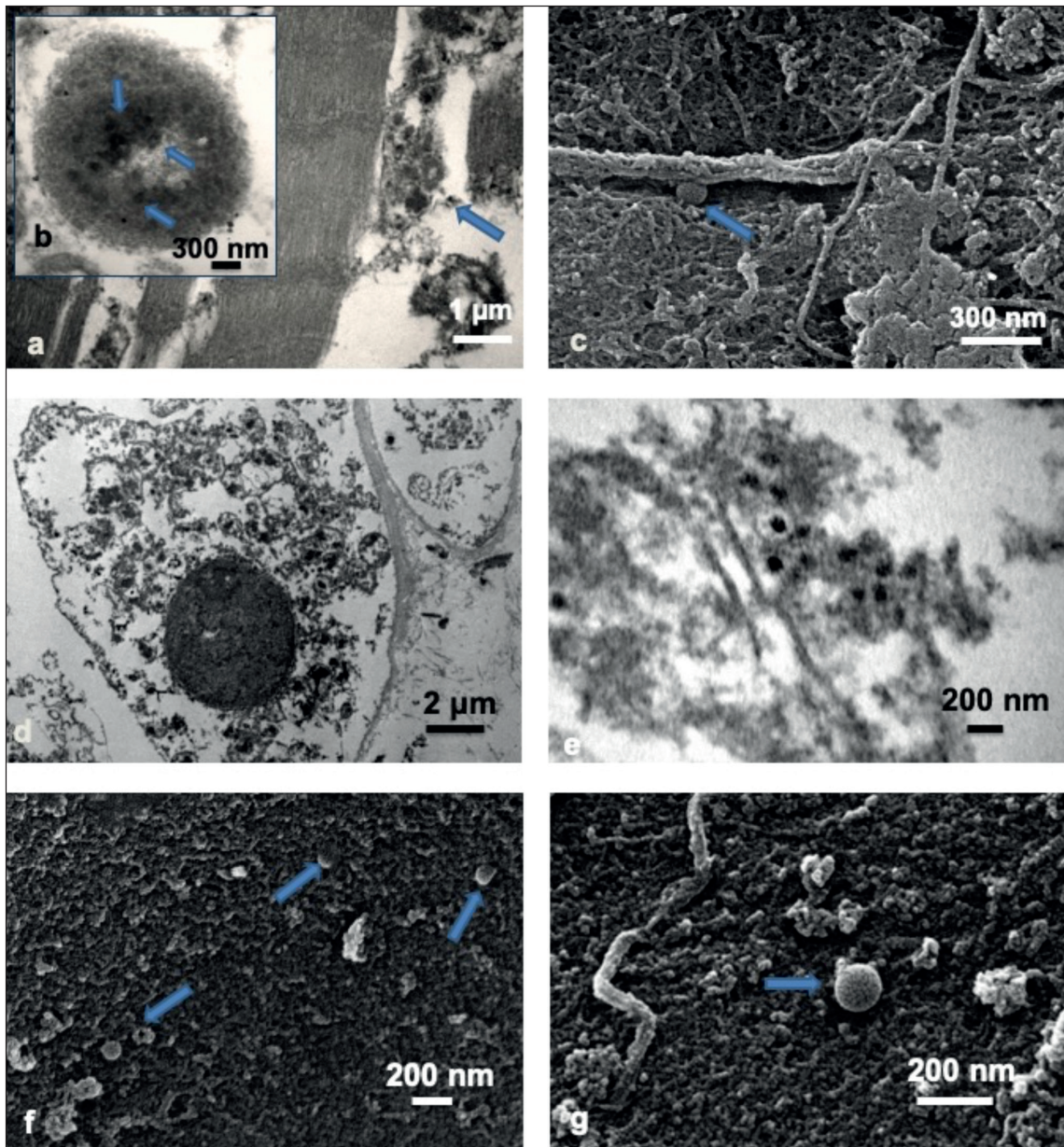
identified only in lung tissues by TEM<sup>3</sup>, and this is the first report identifying SARS-CoV-2 also in heart and renal human tissues by TEM and SEM.

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### Conflict of Interests

The Authors declare that they have no conflict of interests.



**Figure 2.** TEM micrographs of myocardium (a,b). The arrows point the sites containing viral particles (a) shown at higher magnification in (b). SEM image showing a viral particle located into a myofibril (c). TEM micrograph of renal tissue (d,e), showing viral particles. SEM images of SARS-CoV-2 located on the surface of a membrane (f,g).

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