Minerva Anestesiologica
EDIZIONI MINERVA MEDICA

ARTICLE ONLINE FIRST
This provisional PDF corresponds to the article as it appeared upon acceptance.
A copyedited and fully formatted version will be made available soon.
The final version may contain major or minor changes.

First ultrastructural autoptic findings of SARS-Cov-2 in olfactory pathways and brainstem

Gaetano BULFAMANTE, Davide CHIUMELLO, Maria Paola CANEVINI, Alberto PRIORI, Michele MAZZANTI, Stefano CENTANNI, Giovanni FELISATI

Minerva Anestesiologica 2020 May 13
DOI: 10.23736/S0375-9393.20.14772-2

Article type: Letter to the Editor (Correspondence)

© 2020 EDIZIONI MINERVA MEDICA

Article first published online: May 13, 2020
Manuscript accepted: May 8, 2020
Manuscript received: May 6, 2020

Subscription: Information about subscribing to Minerva Medica journals is online at:
http://www.minervamedica.it/en/how-to-order-journals.php
Reprints and permissions: For information about reprints and permissions send an email to:
journals.dept@minervamedica.it - journals2.dept@minervamedica.it - journals6.dept@minervamedica.it

EDIZIONI MINERVA MEDICA

COPYRIGHT© EDIZIONI MINERVA MEDICA

First ultrastructural autoptic findings of SARS-Cov-2 in olfactory pathways and brainstem

Gaetano BULFAMANTE [1 *], Davide CHIUMELLO [1], Maria Paola CANEVINI [1], Alberto PRIORI [1], Michele MAZZANTI [2], Stefano CENTANNI [1], Giovanni FELISATI [1]

1. Department of Health Sciences, University of Milan, Italy
2. Department of Bioscience, University of Milan, Italy

SARS-Cov-2 infection determines neurological signs in 36.4 % of the patients, usually starting with smell and taste disorders, later including dizziness, headache, impaired consciousness, acute cerebrovascular disease, ataxia, and seizures[1]. Respiratory dyssynergia is a distinctive neurological symptom potentially occurring in invasively ventilated patients and that can compromise the return to spontaneous breathing[2]. Hypotheses that can explain the onset of neurological signs include: a) direct viral invasion of the brain through the olfactory mucosa and the olfactory pathways; b) viral invasion via retrograde routing from the lungs through the vagal nerve to the medullary cardiorespiratory center in the brainstem[2]; c) viral invasion through the bloodstream. Conversely, some neurological signs - especially respiratory dyssynergia - could be related to hypoxia and metabolic alterations consequent to the Acute Respiratory Distress Syndrome (ARDS).

Autopsies from China reported brain hyperemia, edema and neuron degeneration[3], and SARS-CoV-2 was observed in the spinal fluid[4] and in the frontal lobe[5] of two affected patients. However, conclusive demonstrations of how SARS-CoV-2 reaches the CNS are missing, and there is no evidence from pathology of the presence of SARS-CoV-2 in the olfactory pathways and brainstem as of today, which can justify the symptoms presumably related to these structures.

Our observations are based on a 54-year-old male who was admitted with fever, anosmia, dysgeusia, headache, a possible seizure, and severe ARDS, due to SARS-CoV-2 [RT-PCR]. He was moved to the intensive care unit 10 days later, where he received mechanical ventilation, sedation, and paralysis. Upon withdrawal of sedation and paralysis the patient became profoundly agitated with severe ventilator asynchronies such as ineffective efforts and double cycling, that required deep sedation, and paralysis. The patient died after 19 days.

Autopsy was performed within three hours from death, assuring very good quality of the specimens. We performed ultrastructural analysis of three different anatomic regions: the olfactory nerve, the gyrus rectus and the brainstem at the level of the medulla oblungata. We observed severe and widespread tissue damage, involving the neurons, glia, nerve axons, and myelin sheath. Notably, we

This document is protected by international copyright laws. No additional reproduction is authorized. It is permitted for personal use to download and save only one file and print only one copy of this Article. It is not permitted to make additional copies (either sporadically or systematically, either printed or electronic) of the Article for any purpose. It is not permitted to distribute the electronic copy of the article through online internet and/or intranet file sharing systems, electronic mailing or any other means which may allow access to the Article. The use of all or any part of the Article for any Commercial Use is not permitted. The creation of derivative works from the Article is not permitted. The production of reprints for personal or commercial use is not permitted. It is not permitted to remove, cover, overlay, obscure, block, or change any copyright notices or terms of use which the Publisher may post on the Article. It is not permitted to frame or use framing techniques to enclose any trademark, logo, or other proprietary information of the Publisher.

COPYRIGHT© EDIZIONI MINERVA MEDICA

observed numerous particles referable to virions of SARS-Cov-2 (fig.1). The damages we observed were progressively more severe from the olfactory nerve to the gyrus rectus and to the brainstem. These findings support the hypothesis of a direct invasion of the virus that enters through the olfactory mucosa. The damages of the brainstem could justify the specific respiratory dyssynergia presented by this patient.

References

1. Mao L, Jin H, Wang M, Hu Y, Chen S, He Q et al. Neurologic Manifestations of Hospitalized Patients With Coronavirus Disease 2019 in Wuhan, China. JAMA Neurol. 2020 Apr 10:e201127. doi: 10.1001/jamaneurol.2020.1127.

2. Li YC, Bai WZ, Hashikawa T. The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients. J Med Virol. 2020;92:552–555.

3. National Health Commission of the People's Republic of China. Diagnosis and treatment of the novel coronavirus pneumonia (Trial version 7) [D]. Published 2020. Accessed March 3, 2020. http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989/files/ce3e6945832a438eaae415350a8ce964.pdf

4. Moriguchi T, Harii N, Goto J, Harada D, Suguwara H, Takamino J et al. A first case of meningitis/encephalitis associated with SARS-Coronavirus-2. Int J Infect Dis. 2020 Apr 3;94:55-58. doi: 10.1016/j.ijid.2020.03.062 (Epub ahead of print).

5. Paniz-Mondolfi A, Bryce C, Grimes Z, Gordon RE, Reidy J, Lednicky J et al. Central Nervous System Involvement by Severe Acute Respiratory Syndrome Coronavirus -2 (SARS-CoV-2). J Med Virol. 2020 Apr 21. doi: 10.1002/jmv.25915 (Epub ahead of print).

NOTES

Conflicts of interest: The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript

Funding: not applicable

Group name: not applicable

Contributors: not applicable

Acknowledgements: not applicable

Figure 1 legend

This document is protected by international copyright laws. No additional reproduction is authorized. It is permitted for personal use to download and save only one file and print only one copy of this Article. It is not permitted to make additional copies (either sporadically or systematically, either printed or electronic) of the Article for any purpose. It is not permitted to distribute the electronic copy of the article through online internet and/or intranet file sharing systems, electronic mailing or any other means which may allow access to the Article. The use of all or any part of the Article for any Commercial Use is not permitted. The creation of derivative works from the Article is not permitted. The production of reprints for personal or commercial use is not permitted. It is not permitted to remove, cover, overlay, obscure, block, or change any copyright notices or terms of use which the Publisher may post on the Article. It is not permitted to frame or use framing techniques to enclose any trademark, logo, or other proprietary information of the Publisher.

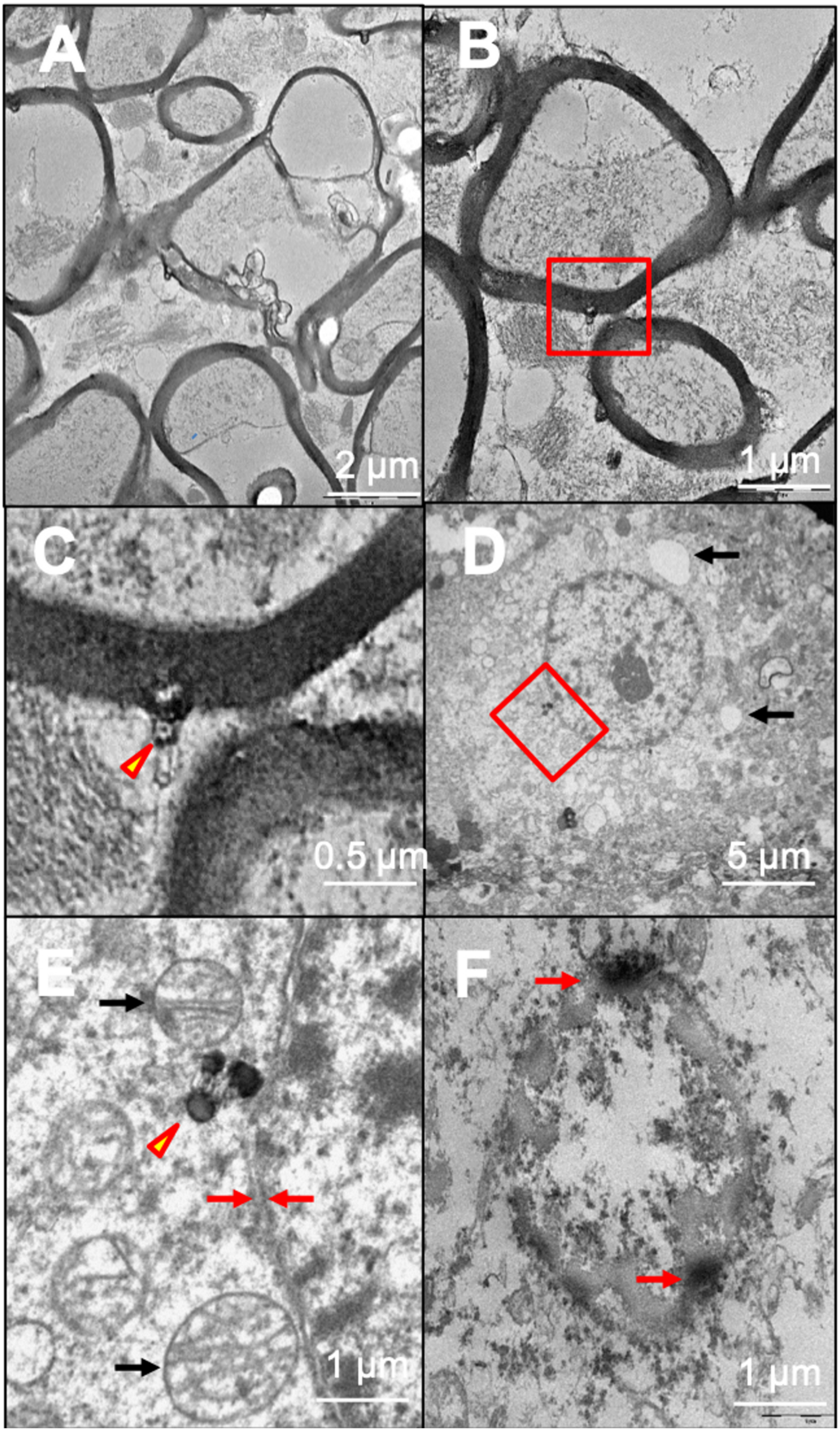
COPYRIGHT© EDIZIONI MINERVA MEDICA

Figure. 1. Neurologic damage in SARS-CoV-2 infection. Ultrastructure of the: medulla oblongata (A-C); gyrus rectus (D-E); olfactory nerve (F). **A.** Marked axonal damage at the medulla oblongata, with irregular axonal swelling and disarrangement of the myelin sheath. The damage appears to be widespread. **B.** A spherical particle with size suspicious for a viral particle (red box) is observed in the periaxonal matrix near the outer surface of a myelin sheath.

C. Magnification of the red box area in B: the spherical particle (~98 nm) has a crown shape with a dense inner core and electron-dense periphery with small external projections. A small roundish electron-dense structure is detected in the center of the particle. This morphology is compatible to that of SARS-CoV-2. **D.** The image shows a neuron of the gyrus rectus, as demonstrated also by the presence of a nucleolus at the center of the euchromatic nucleus; numerous phenomena of autophagy in the cytoplasm (arrows) suggest cell damage. **E.** Magnification of the red box area in D, showing a viral-like particle of 160 nm (arrowhead). Black arrows indicate two well preserved mitochondria; red arrows show the typical double nuclear envelope of the neuron. The good ultrastructural preservation of these organelles demonstrates adequate methods of collection and fixation, and suggests that the tissue damage is related to the viral infection. **F.** Severe tissue damage in the olfactory nerve: the oval structure is difficult to identify, and is characterized by extensive phenomena of autophagy with markedly electron-dense peripheral aggregates (arrows) (Images by Unitech NO LIMITS).

This document is protected by international copyright laws. No additional reproduction is authorized. It is permitted for personal use to download and save only one file and print only one copy of this Article. It is not permitted to make additional copies (either sporadically or systematically, either printed or electronic) of the Article for any purpose. It is not permitted to distribute the electronic copy of the article through online internet and/or intranet file sharing systems, electronic mailing or any other means which may allow access to the Article. The use of all or any part of the Article for any Commercial Use is not permitted. The creation of derivative works from the Article is not permitted. The production of reprints for personal or commercial use is not permitted. It is not permitted to remove, cover, overlay, obscure, block, or change any copyright notices or terms of use which the Publisher may post on the Article. It is not permitted to frame or use framing techniques to enclose any trademark, logo, or other proprietary information of the Publisher.

COPYRIGHT© EDIZIONI MINERVA MEDICA



This document is protected by international copyright laws. No additional reproduction is authorized. It is permitted for personal use to download and save only one file and print only one copy of this Article. It is not permitted to make additional copies (either sporadically or systematically, either printed or electronic) of the Article for any purpose. It is not permitted to distribute the electronic copy of the article through online internet and/or intranet file sharing systems, electronic mailing or any other means which may allow access to the Article. The use of all or any part of the Article for any Commercial Use is not permitted. The creation of derivative works from the Article is not permitted. The production of reprints for personal or commercial use is not permitted. It is not permitted to remove, cover, overlay, obscure, block, or change any copyright notices or terms of use which the Publisher may post on the Article. It is not permitted to frame or use framing techniques to enclose any trademark, logo, or other proprietary information of the Publisher.