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Vascular and glial changes in the brain of COVID-19 patients

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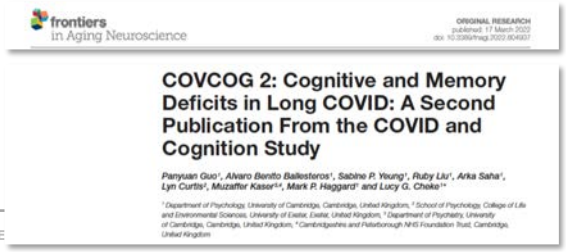
Background

- Coronavirus disease (COVID-19) is an infectious disease caused by the SARS-CoV-2 virus (severe acute respiratory syndrome coronavirus 2).
- Infected patients have symptoms not only in the acute phase, but also after recovery from the initial infection.
- „Long COVID", is defined as the continuation or development of new symptoms 3 months after the initial SARS-CoV-2 infection, with these symptoms lasting for at least 2 months.
- Most prevalent symptoms reported by „long COVID" patients include: fatigue (64%), dyspnea (40%), depression (38%), arthralgia (24,3%), headache (21%), and insomnia (20%).


frontiers in Aging Neuroscience

ORIGINAL RESEARCH

COVCOG 2: Cognitive and Memory Deficits in Long COVID: A Second Publication From the COVID and Cognition Study

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Background

- Changes in neurotransmitter levels, inflammation, substrate metabolism/ availability are potential contributors to fatigue after COVID-19 infection.
- Despite significant efforts to explain the pathogenic mechanisms of fatigue, current knowledge is limited.
- In the CNS, a multitude of changes have been described associated with SARS-CoV-2 infection, such as microglial activation, perivascular lymphocyte cuffing, hypoxic-ischemic changes, microthrombosis, infarcts or hemorrhages.

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Objective / Patients

- We sought to assess the CNS vascular basement membranes (vBM) and surrounding perivascular astrocytes for morphological changes, in COVID-19 cases versus control brain tissue, as well as to evaluate their water-buffering capabilities (AQP4 water pore expression);
- Additionally, we intended to explore the presence of the SARS-CoV-2 antigens by IHC on lung tissue and CNS.

- N=14 patients with confirmed SARS-CoV-2 infection (RT-PCR on lung tissue), collected at the National Institute of Legal Medicine Mina Minovici (Bucharest) between June 2020 – November 2020; COVID-19 was the initial cause of death. Cases with large intraparenchymal bleedings were not considered.
- N=4 control patients that died from non-CNS and non-respiratory related causes (neurodegenerative brain bank archive at the Laboratory for Microscopic Morphology and Immunology from the UMF Craiova)

* Study approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova (no. 209/08.12.2021)

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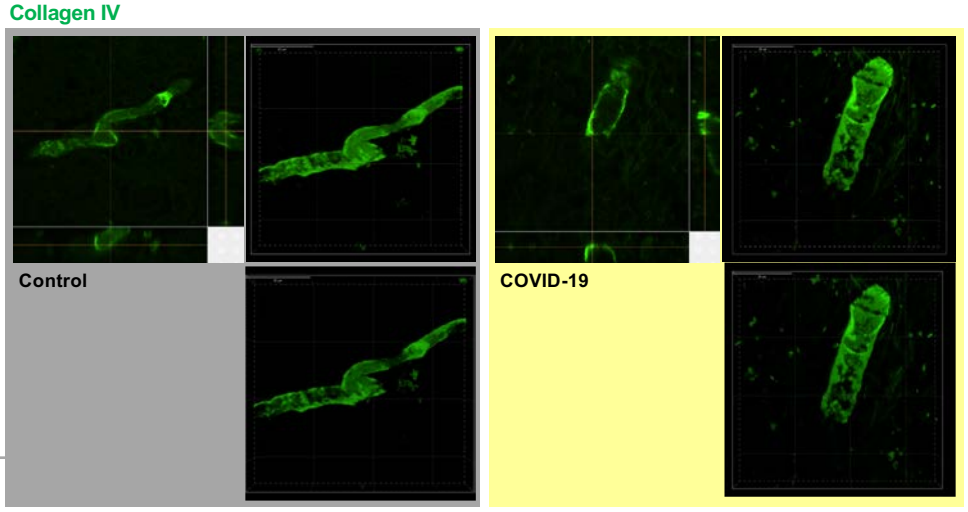
Methodology

- Is there any morphological difference of the vascular basement membranes between COVID-19 and control patients?

Collagen IV



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Methodology

- Archived paraffin CNS tissue (superior frontal lobes) and lung;
- Simple and double immunohistochemistry, enzymatic and fluorescent:
- An anti-spike peptide antibody that was run also in a peptide competition assay to ascertain its specificity;
- High resolution fluorescence microscopy and deconvolution (Nikon 90i, Nikon NIS-Elements);
- Multispectral unmixing for both transmitted light and fluorescence microscopy (Nuance FX, Perkin Elmer);
- Fractal analysis for assessing the "smoothness" of the vascular basement membranes (Image ProPlus 7, Media Cybernetics).
- Colocalization analysis (Image ProPlus 7).

Antibodies utilized in this study

Target	Species	Clone	Dilution	Code, Producer
Aquaporin 4	Rabbit	Polyclonal	1:1.000	sc-20812; Santa Cruz, Heidelberg, Germany
Cytokeratin 7	Mouse	OV-TL 12/30	1:50	M7018; Dako, Glostrup, Denmark
Collagen IV	Mouse	IgG1k	1:50	M0785; Dako
GFAP	Rabbit	Polyclonal	1:10.000	Z0334; Dako
GFAP	Mouse	IgG1k	1:1.000	M0761; Dako
SARS-CoV-2 spike glycoprotein	Rabbit	Polyclonal	1:1.000 (1µg/ml)	ab272504; Abcam plc, Cambridge, UK
* SARS-CoV-2 spike glycoprotein peptide	Human	Blocking peptide	1:20 (10µg/ml)	ab273063; Abcam
Tight Junction Protein 1 (TJP1)	Rabbit	Polyclonal	1:1000	NBP1-85047; Novus Biologicals, Centennial, USA

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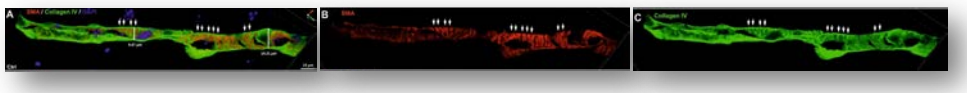
A focus on capillaries…

The main objective of our study was to observe the vascular wall in capillaries, were the BBB exists:

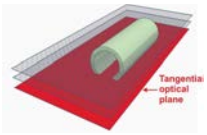
1. We choose basement membranes (Col IV) and not endothelia (CD31), as tightly packed red blood cells will mold the endothelium over their surface:

2. We needed to select capillaries only as basement membranes embrace smooth muscle cells in arterioles and venules:



3. We considered only vessels ≤ 9µm as viewed for Col IV staining*:

4. We viewed longitudinal vessels in tangential sections for BM staining:



*Saiga R et al. Sci Rep, 2021, 11(1):11768.
Ding R et al. Brain Pathol, 2020, 30(6):1087-1101.

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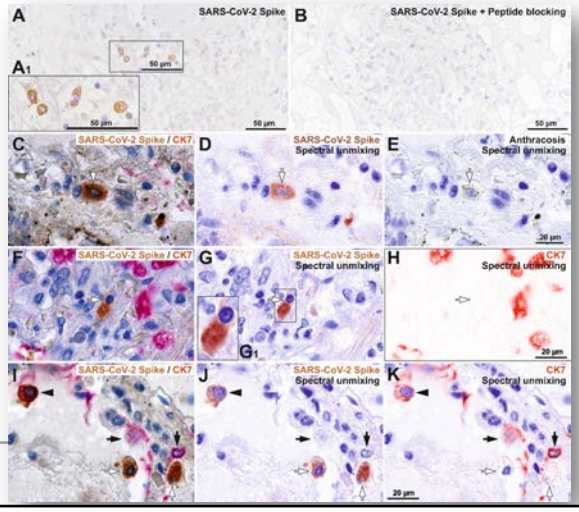
RESULTS: Lung SARS-CoV-2 spike protein expression

- COVID-19 patients lungs presented productive / proliferative pneumonia with accumulation of admixed mononuclear, epithelial and fibroblastic cells, collapsed alveolar spaces, stasis and thrombosis, hemorrhage, denudation of pneumocytes.

- **Our SARS-CoV-2 antibody is highly specific for its designated peptide!**

- **Most of the SARS-CoV-2 spike glycoprotein was expressed in:**
  - **mononuclear cells,**
  - **non-squamous pneumocyte-II-like cells;**
  - **on occasion in plasma cells.**

- **No expression was noticed for any of the brain tissue samples!**



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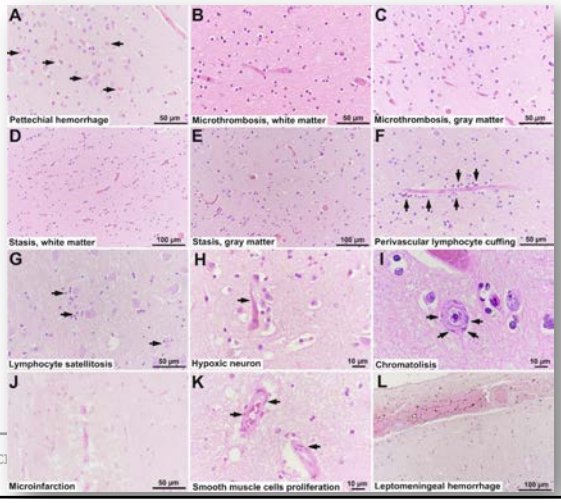
General brain histopathology

- In most COVID-19 cases, there was oedema, stasis / thrombosis, petechial red blood cells extravasate, lymphocyte perivascular cuffing and perineuronal satelitosis, and on occasion, chromatolysis, disorganization of Nissl bodies, microinfarctions and smooth muscle cells proliferations.



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Main lung/CNS histopathological findings

TABLE 1 Patients included in the study

Patient no.	Group	Age	Gender	Days from admission to death	Lung reactivity for SARS-CoV-2 spike glycoprotein	Brain pathology: Stasis	SMC proliferation	Hypoxic neurons	Microinfarcts	Haemosiderin deposition	Microthrombosis	Petechial haemorrhages	Perivascular lymphocyte cuffing	Monocyte/lymph cell interstitial infiltrates
1	SARS-CoV-2	43	M	3	+++	+++	+	++	–	–	+++	+	++	+
2	SARS-CoV-2	39	M	4	++	+	–	+	–	–	+	+	–	+
3	SARS-CoV-2	77	M	4	+	++	–	+	–	–	++	–	+	–
4	SARS-CoV-2	58	M	4	++	+++	++	+	–	++ (perivascular)	+++	+	++	++
5	SARS-CoV-2	58	F	6	+	–	+	+	–	–	–	++	+	++
6	SARS-CoV-2	71	F	6	+	+	+	++	–	–	+	++	+	–
7	SARS-CoV-2	57	M	13	++	+++	–	+	–	+ (perivascular)	+++	+	+	–
8	SARS-CoV-2	75	F	9	++	++	–	++	–	–	++	+	–	–
9	SARS-CoV-2	87	M	1	+	+	–	+	–	–	+	–	+	+
10	SARS-CoV-2	70	F	24	+	+	+	+	–	–	+	+	+	+
11	SARS-CoV-2	52	M	5	+	+	–	+	–	–	+	+	–	–
12	SARS-CoV-2	74	M	0	+	+++	+	++	+	+	++	++	++	++
13	SARS-CoV-2	58	M	11	+	+	+	+	–	–	+	+	–	–
14	SARS-CoV-2	88	F	3	+++	++	++	+	–	–	++	–	+	+
15	Control (lung tumour)	64	M	–	–	+	–	+		–	+	–	–	+
16	Control (digestive tumour)	69	F	–	–	–	–	–	–	–	–	–	+	+
17	Control (digestive tumour)	75	M	–	NA	+	–	+	–	–	–	–	–	–
18	Control (digestive tumour)	63	M	–	–	–	–	+	–	–	–	–	–	+

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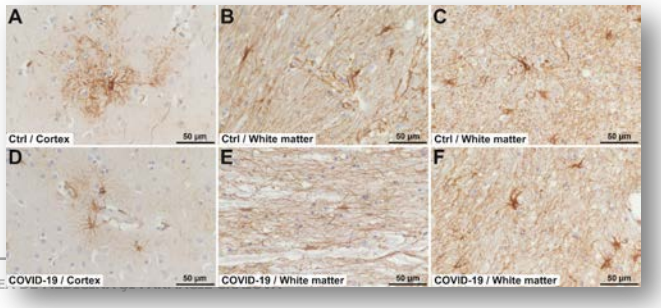
Inconspicuous gliotic levels

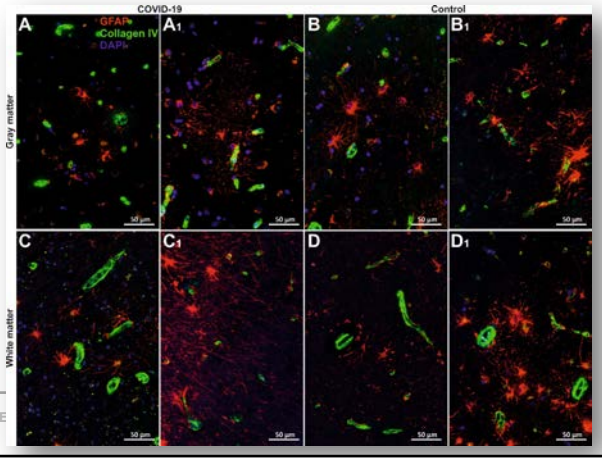
- The gray matter of both Control and COVID-19 cases showed foci of mainly protoplasmatic astrocytes grouped around blood vessels;
- In the white matter, fibrous astrocytes were present with a relative monomorphic morphology, and in both controls and COVID-19 patients we could identify on occasion groups of activated-like astrocytes;
- No gemistocytic foci and no clasmatodendrocytes could be identified in any of the cases.

GFAP



Astrocytes' end-feets and basement membranes

- COVID-19 cases showed a tendency for lower overall GFAP expression for both the cortex and white matter compared to control cases
- Also, for all patients there was a tendency for lower gliosis in cortical areas and higher GFAP expression in the white matter.



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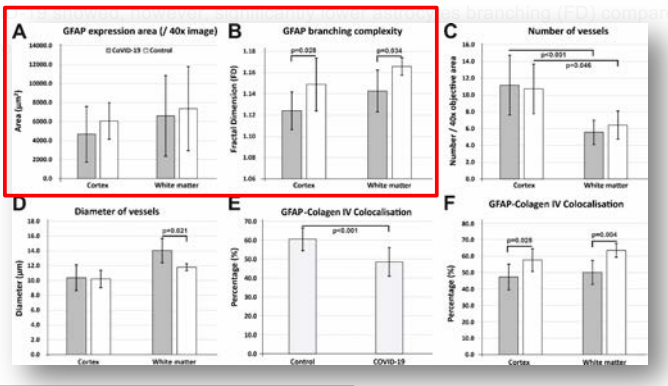
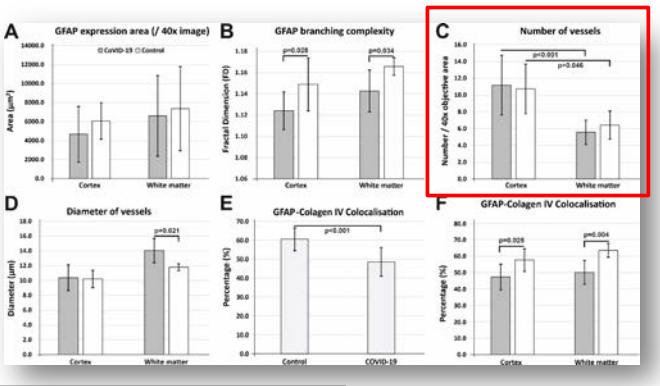


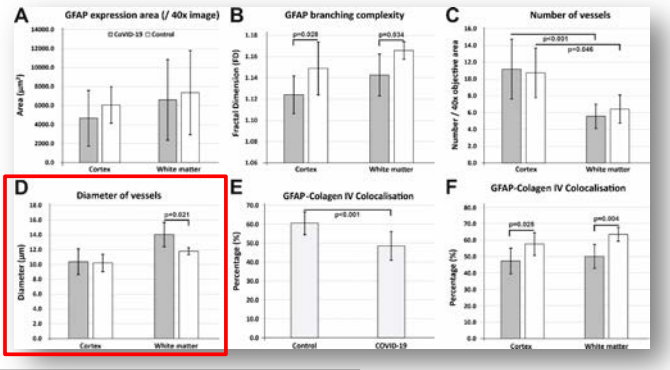
Astrocytes' end-feets and basement membranes

- Direct counting revealed no difference regarding the vessel densities, for both cortical and subcortical areas;
- There were significant differences of the vascular densities between the cortex and white matter, for both COVID-19 and control cases, as expected in the normal CNS.



Astrocytes' end-feets and basement membranes

- Measurements of the maximum vessel diameters revealed significantly lower diameters for cortex areas compared to white matter for both COVID-19 and control cases.
- When comparing the two pathological states, only for the white matter there significantly larger blood vessels in COVID-19 patients compared to control cases.



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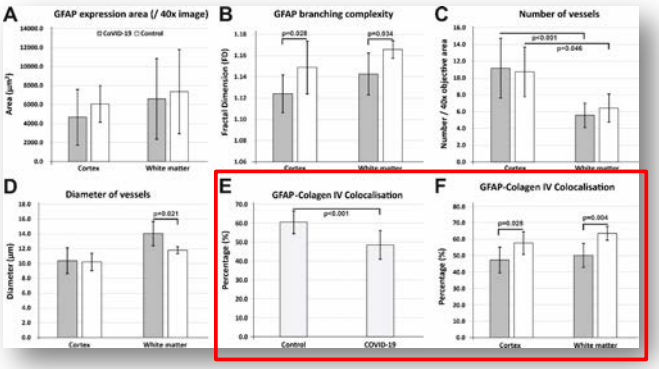
Astrocytes' end-feets and basement membranes

- Astrocyte end-feet coverage of the vascular basement membrane has been evaluated next, as the GFAP/collagen IV colocalization coefficients on 5µm perivascular ROIs.
- There was a clear-cut decrease of astrocytic coverage of the blood vessel walls for COVID-19 cases compared to control patients, for both WM and GM.



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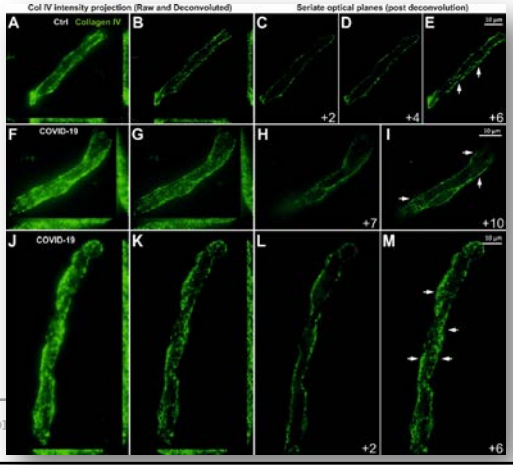
Morphology of the basement membranes

- We observed subtle variations in the irregularities (asperities) of the BM of the blood vessels, for both COVID-19 and control cases;
- We aimed to assess these irregularities on tangentially longitudinally-cut vessels by Fractal Dimension Analysis (FD).



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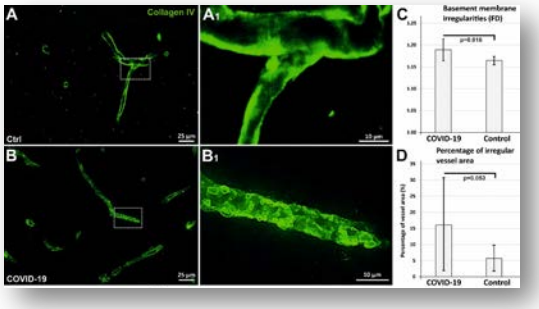
Morphology of the basement membranes

Although the FD of the BM irregularities showed a much larger variability for COVID-19 patients (FD=1.189±0.025), there was a clear-cut increase in the irregularity of the BM in these patients compared to the control group (FD=1.164±0.009), p=0.016.



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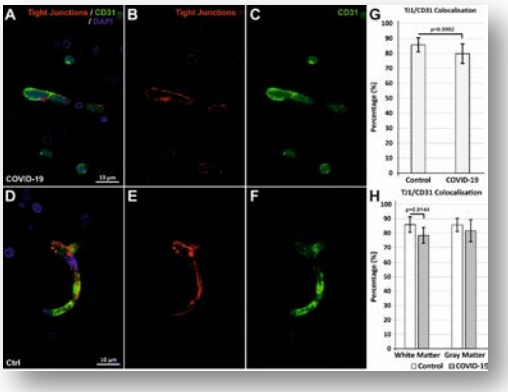
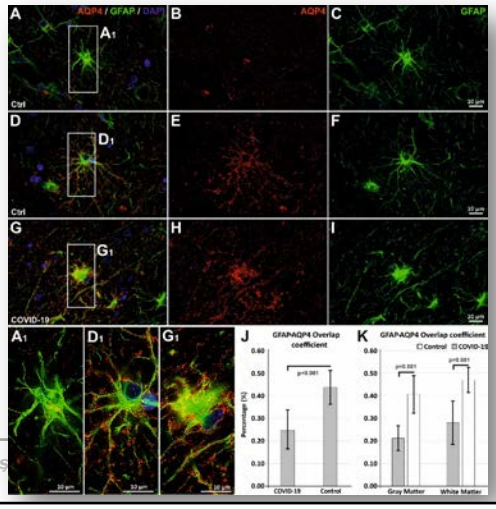
Evaluation of the endothelial tight junctions

Analysis of CD31/TJ1 overlapping coefficients showed, that the co-expression of TJ1 in endothelial cells showed an overall decrease for COVID-19 patients compared to controls, and especially for the WM areas.



AQP4 / GFAP colocalization – functional reflection?

- AQP4 is expressed not only in the astrocyte end-feets, but also alongside their full surface, including the cell bodies, for both groups of patents;
- Semiquantitative expression analysis revealed an abrupt drop for COVID-19 patients compared to controls, for WM and GM.



CONCLUSIONS

- No SARS-Cov-2 expression in the CNS in our casuistry!!
- We have also showed that AQ4 expression is reduced in the astrocytes of these patients, while we could not identify the SARS-COV-2 spike glycoprotein by immunohistochemistry in their brain tissue.
- We have utilized for the first time FD analysis to show that astrocytes decrease in complexity and reduce their coverage of the blood vessel walls in COVID-19 patients, and the blood vessel BM are more irregular in these patients, suggesting subtle but important alteration of the BBB that might greatly increase the aggregability and ischemia/hypoxia conditions.
- The plethora of non-specific CNS signs and symptoms, for COVID-19 patients during and after the cessation of the disease, suggests long lasting functional or even morphological changes, and thus, it is of outmost importance to assess the BBB structure and function in the following months and years in COVID-19 surviving patients.

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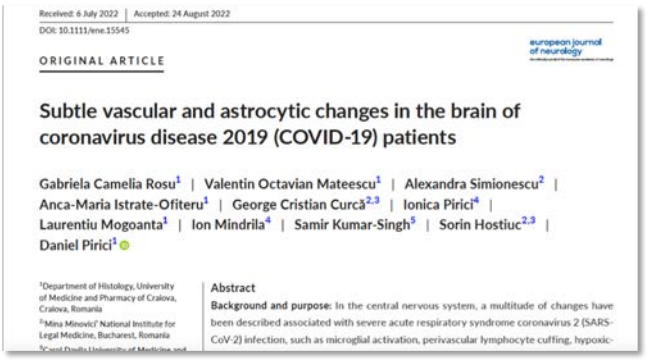
Thank you!



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ORIGINAL ARTICLE

european journal of neurology

Subtle vascular and astrocytic changes in the brain of coronavirus disease 2019 (COVID-19) patients

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Abstract

Background and purpose: In the central nervous system, a multitude of changes have been described associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, such as microglial activation, perivascular lymphocyte cuffing, hypoxic-