Intensive Care Medicine

LETTER TO THE EDITOR Un-edited accepted proof

Pericyte alteration sheds light on micro-vasculopathy in COVID-19 infection

Nathalie Cardot-Leccia* [1], MD, Thomas Hubiche* [2], MD, Jean Dellamonica [3], MD, PhD, Fanny Burel-Vandenbos [1], MD, PhD, Thierry Passeron [2,4], MD, PhD
* Equal contribution

[1] Université Côte d'Azur. CHU Nice, Department of Pathology. Nice, France
[2] Université Côte d'Azur. CHU Nice, Department of Dermatology. Nice, France
[3] Université Côte d'Azur. UPR 01, UR2CA, CHU Nice, Medical Intensive Care Unit. Nice, France
[4] Université Côte d'Azur. INSERM. U1065, C3M. Nice, France

DOI: 10.1007/s00134-020-06147-7

Corresponding author:
Thierry Passeron, MD, PhD,
Centre Hospitalier Universitaire de Nice,
Service de Dermatologie
151, Route de Saint Antoine de Ginestière,
Hôpital Archet 2,
06200 Nice, France
Email: passeron@unice.fr

Conflicts of interest: None
Key words: COVID-19; SARS-CoV-2; Micro-vasculopathy; Pericytes

Intensive Care Medicine

LETTER TO THE EDITOR Un-edited accepted proof

Dear Editor,

Understanding the mechanisms involved in SARS-CoV-2 infection is crucial to provide more efficient therapeutic approaches. Here, we report the histological patterns of skin and lung post-mortem analysis in patients hospitalized in intensive care unit that revealed a micro-vasculopathy secondary to pericyte alteration.

After family consent, we performed in-depth histological analysis, with a special focus on micro-vascularization, of post-mortem biopsies from COVID-19 positive patients hospitalized in intensive care unit (lung biopsies were taken from 2 patients and skin biopsies from 4 patients). The normal lung tissue used as control was from a 16-year-old patient cured for pneumothorax. Detailed methods are available in Supplementary Appendix.

As previously reported, we observed in the lung, patterns of diffuse alveolar damage (DAD) including exudative and proliferative changes. Interestingly, walls of venules and alveolar capillaries were thickened as compared to normal pulmonary vessels (Supplementary Figures 1A, B, C). We did not observe any thrombi, and CD34 immunostaining showed no alteration of endothelial cells of those venules and capillaries (Supplementary Figure 1D). At the contrary, pericytes were dramatically decreased in alveolar capillaries in COVID+ lung while they were abundant in normal parenchyma (Figure 1A, B). Cleaved caspase 3 immunostaining revealed apoptosis of pericytes (Figure 1C). The thickening of venules and capillaries and the loss of pericytes were observed not only in DAD territories, but also in non-inflammatory areas. Biopsies of apparent normal skin of the same two patients and two additional ones, also showed a mild thickening of small sized vessel wall in superficial dermis without inflammation (Supplementary Figure 1E). In the skin, we did not observe a pericyte loss but a mild hyperplasia of pericytes was noted (Supplementary Figure 1F, G).

Cardot-Leccia N. et al. **Pericyte alteration sheds light on micro-vasculopathy in COVID-19 infection.** ***Intensive Care Medicine*** **(2020);** DOI: 10.1007/s00134-020-06147-7

There are increasing data supporting a vascular involvement in COVID-19 patients. MRI performed in patients with neurologic signs revealed perfusion abnormalities in 100% of cases[1]. Dual-energy CT performed in COVID-19 pneumonia showed profound vascular and perfusion abnormalities without pulmonary emboli[2]. So far, studies emphasized thrombotic events, or searched for endothelial cell alteration[3]. The lack of thrombi in our samples might be explained by the curative anticoagulation that our patients received. Sprouting and intussusceptive angiogenesis along with disruption of intercellular junctions, cell swelling, and a loss of contact with the basal membrane are the hallmarks of COVID-19 infected lungs[3]. One of the key roles of pericytes is to maintain endothelial integrity. Moreover, their loss or detachment promotes endothelial cell sprouting and intussusception. Two recent single cells analyses demonstrated that endothelial cells have a very weak expression of angiotensin-converting enzyme 2 (ACE2), while pericytes are among the cells that have the highest expression of ACE2[4, 5]. More than a direct viral effect on endothelial cells or perivascular inflammation, the profound decrease of pericytes and observed apoptosis strongly suggest that the alteration of pericytes by a direct effect of SARS-CoV-2 could be the initial trigger of the micro-vasculopathyCOVIDCOVID. COVIDThese findings are of critical importance as they could explain the systemic manifestations reported with COVID-19 infection and foster new therapeutic approaches, such as prostacycline to target the micro-vasculopathy.

Cardot-Leccia N. et al. **Pericyte alteration sheds light on micro-vasculopathy in COVID-19 infection.** ***Intensive Care Medicine*** **(2020);** DOI: 10.1007/s00134-020-06147-7

Author Contributions: Drs Leccia, Hubiche and Passeron had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Concept and design: Leccia, Hubiche, Burel-Vandebos, Passeron.

Acquisition, analysis, or interpretation of data: All authors.

Drafting of the manuscript: Leccia, Hubiche, Passeron.

Critical revision of the manuscript for important intellectual content: Dellamonica, Burel-Vandenbos, Passeron

Statistical analysis: NA.

Administrative, technical, or material support: Burel-Vandenbos, Passeron

Supervision: Dellamonica, Burel-Vandenbos, Passeron.

Conflict of Interest Disclosures: The authors declare no conflict of interest.

Funding/Support: Centre Hospitalier Universitaire de Nice, France

Role of the Funder/Sponsor: The funders/sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contributions: We thank for their help and feedback Georges Leftheriotis, MD, PhD, Pascal Giordana, MD, and Véréna Fassbender, MD, from the Department of Vascular Medicine; Florence Le Duff, MD, Christine Chiaverini, MD, PhD, and Aurélia Palladini, MD, from the department of Dermatology, Barbara Seitz-Polski, MD, PhD, from the department of Immunology, Valérie Giordanengo, MD, PhD, and Géraldine Gonfrier, MD, , from the department of Virology, Damien Ambrosetti, MD, PhD from the department of Pathology, Johan Courjon, MD, and Véronique Mondain, MD, from the department of Immunology and Bérengère Dadone-Montaudié, MD, PhD, from the Laboratory of Solid Tumors Genetics.

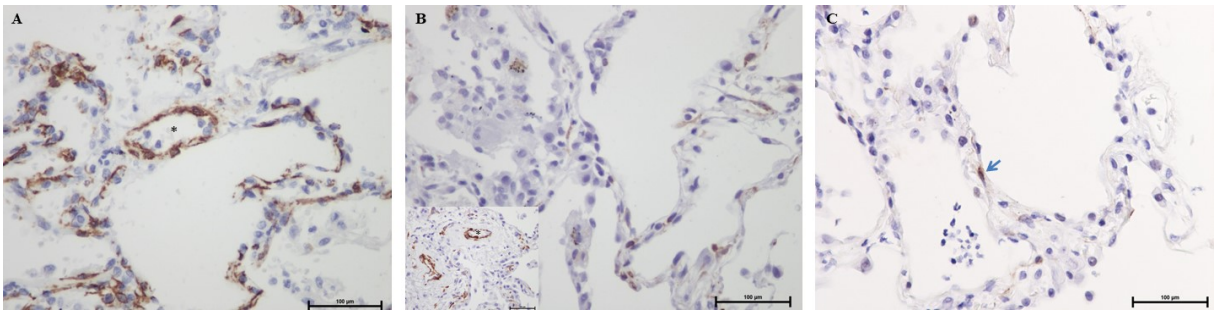
References

1. Helms J, Kremer S, Merdji H, Clere-Jehl R, Schenck M, Kummerlen C, Collange O, Boulay C, Fafi-Kremer S, Ohana M, Anheim M, Meziani F, (2020) Neurologic Features in Severe SARS-CoV-2 Infection. The New England journal of medicine
2. Lang M, Som A, Mendoza DP, Flores EJ, Reid N, Carey D, Li MD, Witkin A, Rodriguez-Lopez JM, Shepard JO, Little BP, (2020) Hypoxaemia related to COVID-19: vascular and perfusion abnormalities on dual-energy CT. Lancet Infect Dis
3. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, Vanstapel A, Werlein C, Stark H, Tzankov A, Li WW, Li VW, Mentzer SJ, Jonigk D, (2020) Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in COVID-19. The New England journal of medicine
4. Chen L, Li X, Chen M, Feng Y, Xiong C, (2020) The ACE2 expression in human heart indicates new potential mechanism of heart injury among patients infected with SARS-CoV-2. Cardiovasc Res
5. Ziegler CGK, Allon SJ, Nyquist SK, Mbano IM, Miao VN, Tzouanas CN, Cao Y, Yousif AS, Bals J, Hauser BM, Feldman J, Muus C, Wadsworth MH, Kazer SW, Hughes TK, Doran B, Gatter GJ, Vukovic M, Taliaferro F, Mead BE, Guo Z, Wang JP, Gras D, Plaisant M, Ansari M, Angelidis I, Adler H, Sucre JMS, Taylor CJ, Lin B, Waghray A, Mitsialis V, Dwyer DF, Buchheit KM, Boyce JA, Barrett NA, Laidlaw TM, Carroll SL, Colonna L, Tkachev V, Peterson CW, Yu A, Zheng HB, Gideon HP, Winchell CG, Lin PL, Bingle CD, Snapper SB, Kropski JA, Theis FJ, Schiller HB, Zaragosi L-E, Barbry P, Leslie A, Kiem H-P, Flynn JL, Fortune SM, Berger B, Finberg RW, Kean LS, Garber M, Schmidt AG, Lingwood D, Shalek AK, Ordovas-Montanes J, (2020) SARS-CoV-2 receptor ACE2

is an interferon-stimulated gene in human airway epithelial cells and is detected in specific cell subsets across tissues. Cell

Figure 1. Pericyte loss in the lung of COVID infected patient.

Pericytes, highlighted by α SMA immunostaining (x 400), were dramatically decreased in alveolar capillaries in COVID + lung (B) while they were abundant in normal parenchyma (A) (asterisk shows venules lumen). Pericytes seemed preserved in venular walls in COVID+ (B insert). Cleaved caspase 3 staining revealed apoptosis of a pericyte in inter-alveolar septum of COVID+ lung (C).



Supplementary methods

Detection of SARS-CoV-2 RNA

Total nucleic acids present within 200 µL of samples were extracted with automated system *NucliSENS® easyMAG®* (bioMérieux, Marcy-l'Étoile, France) after proteinase K pretreatment and offboard lysis. Presence of SARS-CoV-2 specific RNA was assessed by reverse transcription-PCR based on the protocol recommended by the French National Reference Center for Respiratory Viruses (Institut Pasteur, Paris, France) [(ECDC), 2020 #81900;Pasteur, 2020 #81901]. Primers and probes used in this protocol were designed to amplify two different targets (IP2 and IP4) in the RNA-dependent RNA polymerase (RdRp) SARS-CoV-2 specific gene (Table S2) [Pasteur, 2020 #81901]. Reverse transcription-PCR assays were realized by *StepOnePlus Real-Time PCR system* (Applied Biosystems, Foster City, CA, USA). The threshold cycle values (Ct) were collected from PCR assays as an indicator of viral load for positive samples.

Histology

Histological and immununohistochemical (IHC) analyses were performed on 2µm thick-sections of formaldehyde-fixed paraffin-embedded biopsies. Sections were stained with Hematoxylin/Eosin-stained (HE) for standard histological examination.

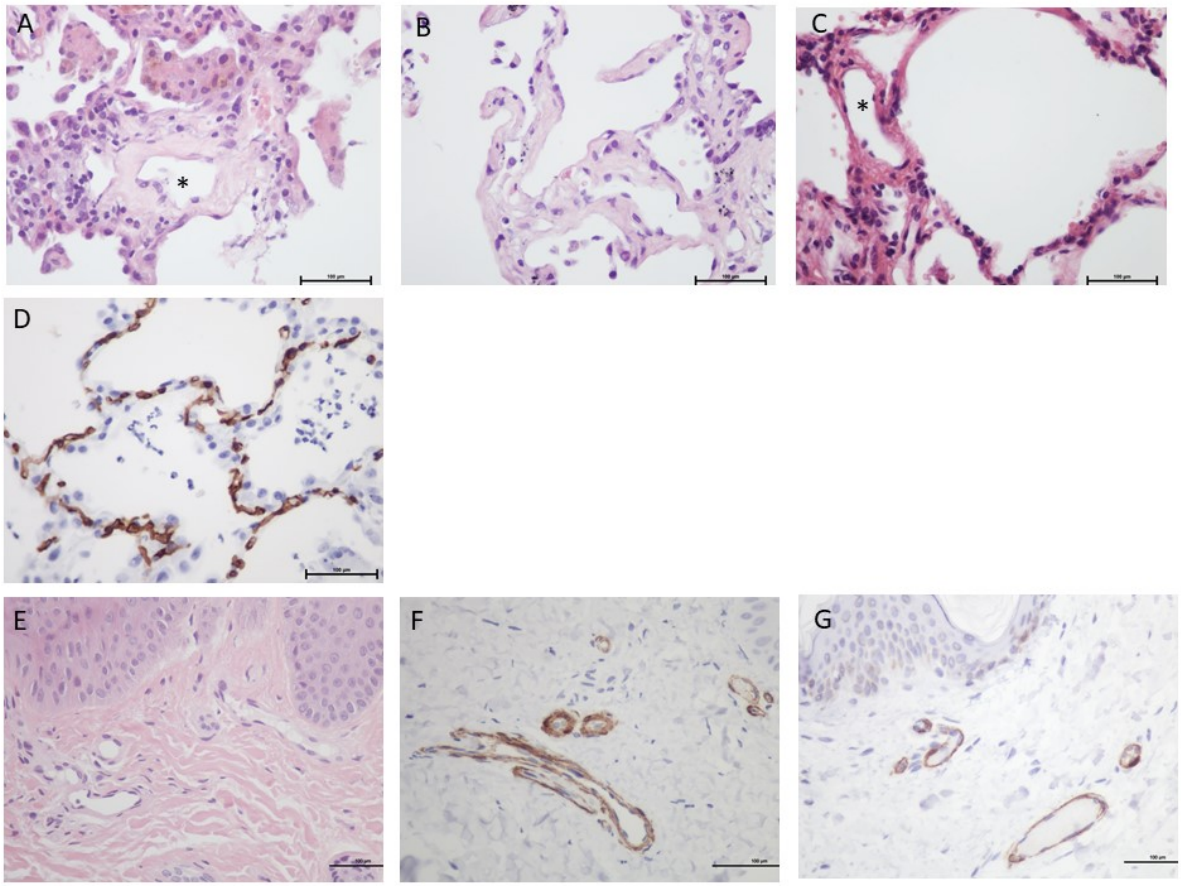
Immunohistochemistry

IHC was performed with a Dako Autostainer Link (Glostrup, Denmark) using the cleaved caspase-3 (Asp175) rabbit polyclonal antibody (#9661, Cell Signaling, 1:200) and the following prediluted antibodies: alpha smooth muscle actin (SMA) (CLONE 1A4, Mouse, DAKO), Desmin (clone D 33, Mouse, DAKO), CD31 (clone JC70A, Mouse, DAKO), CD34 (clone Qbend 10, DAKO). Deparaffinization, rehydration and antigen retrieval were performed using the pretreatment module PTlink (Dako) at low pH for SMA, CD31 and high pH for Desmin, CD34 and cleaved caspase 3. Primary antibodies were incubated for 20 min. Revelation was performed using the Envision Flex Kit (Dako), with 3–3′ diaminobenzidine (DAB) as a chromogen. Sections were counterstained with Hematoxylin.

Supplementary Figure

Histological analysis of lung and skin micro-vascularization of normal and COVID+ patients

In COVID+ lung, walls of venule (A) and alveolar capillaries (B) were thickened as compared to normal pulmonary vessels (C) (asterisk shows venules lumen). CD34 staining showed no alteration of endothelial cells in the same territory (D). Mild thickening of small sized vessel wall in superficial dermis without inflammation on skin biopsy of hospitalized patient for SARS-CoV-2 infection in intensive care unit (HE staining x400) (E). Immunostaining with αSMA shows mild pericyte hyperplasia in hospitalized COVID+ patient skin (F) compared to healthy skin control (G) (HE, 400X).



Cardot-Leccia N. et al. **Pericyte alteration sheds light on micro-vasculopathy in COVID-19 infection.** ***Intensive Care Medicine*** **(2020);** DOI: 10.1007/s00134-020-06147-7

Intensive Care Medicine

LETTER TO THE EDITOR Un-edited accepted proof

Cardot-Leccia N. et al. **Pericyte alteration sheds light on micro-vasculopathy in COVID-19 infection.** ***Intensive Care Medicine*** **(2020);** DOI: 10.1007/s00134-020-06147-7