Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Persistence of SARS-CoV-2 RNA in lung tissue after mild COVID-19

On Dec 1, 2020, we reported a successful case of double-lung transplantation from a SARS-CoV-2 seropositive donor 105 days after the onset of mild COVID-19.[1] Although repeated quantitative (q)RT-PCR analyses of donor nasopharyngeal swabs were negative, this technique detected RNA of the SARS-CoV-2 *N* gene (delta Ct 35) from a biopsy of the right lung taken during organ procurement. Viral culture of this biopsy was negative and donor-to-recipient transmission did not occur. Complementary orthogonal methods were needed to corroborate and interpret the qRT-PCR results.

Therefore, we did ultrasensitive single-molecule fluorescence RNA in-situ hybridisation with RNAscope technology on formalin-fixed paraffin-embedded sections of the same lung biopsy (appendix p 1), and compared the results with those of a lung biopsy from a deceased patient with acute COVID-19 (figure A and B; appendix p 2). We stained 14 slides of the donor lung biopsy, each containing one 5 µm section, as follows: five slides with a probe for the *N* gene; five slides with a probe for the *S* gene; and four slides with probes for *N* and *S*. A probe for the *basigin* gene, which has been proposed to encode an alternative host recipient for SARS-CoV-2, served as a positive control on the ten slides stained for *N* or *S* only.[2] We identified characteristic RNAscope puncta in three out of nine slides for the *N* probe, and in six out of nine slides for the *S* probe (figure C and D). These puncta appeared to be located in clumps of sloughed-off material, and no cells or cell nuclei could be discerned in this debris-like tissue.

To our knowledge, this is the first report of long-term (>100 days) persistence of SARS-CoV-2 RNA in lung tissue of an immunocompetent patient after convalescing from COVID-19. The debris-like tissue that contained SARS-CoV-2 RNA might be composed of degenerated endothelial cells that had detached from vessel walls, dysmorphic syncytial elements of pneumocytes, or dead neutrophilic plugs in the interstitium.[3,4] We speculate that this debris-like tissue might shield SARS-CoV-2 RNA from degradation.

Data on sputum, nasopharyngeal swabs, and bronchoalveolar lavage fluid indicate that prolonged detection of SARS-CoV-2 RNA is rare and limited to a few weeks.[5] By contrast, SARS-CoV-2 RNA persisted in the lung parenchyma for 105 days after the onset of a mild course of COVID-19. Nonetheless, at the time of writing, 11 months after transplantation, the recipient is in good health. Our data show that the persistence of SARS-CoV-2 RNA in this donor lung tissue has been inconsequential.

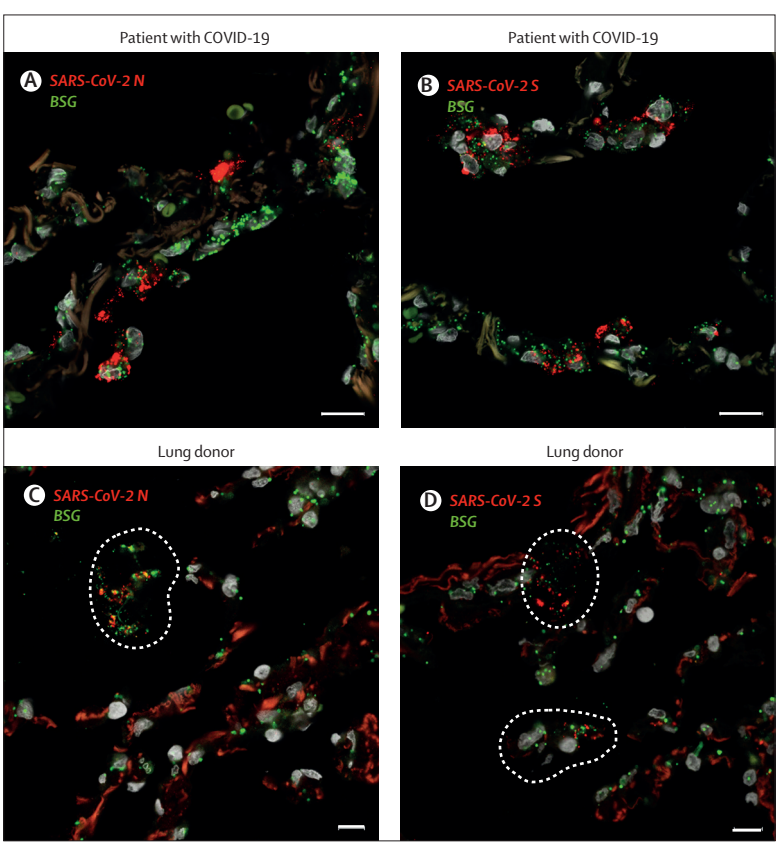

Figure: **Ultrasensitive single-molecule fluorescence RNA in-situ hybridisation with RNAscope**
Formalin-fixed paraffin-embedded sections of a lung biopsy from a patient with COVID-19 who died 5 days after a positive quantitative RT-PCR result from a nasopharyngeal swab (A, B). Red puncta show RNA for the SARS-CoV-2 *N* gene (A) or the SARS-CoV-2 *S* gene (B), and green puncta show RNA for *BSG*. The nuclear stain DAPI is shown in grey. Scale bar is 20 µm. Formalin-fixed paraffin-embedded sections of the right lung biopsy from the lung donor (C, D). Red puncta show RNA for the SARS-CoV-2 *N* gene (C) or the SARS-CoV-2 *S* gene (D), and green puncta show RNA for *BSG*. The nuclear stain DAPI is shown in grey. The dotted traces denote areas with debris-like tissue that contains puncta for SARS-CoV-2 *N* or *S* and for *BSG*. Scale bar is 10 µm. *BSG=basigin*.

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See **Online** for appendix

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*Laurens J Ceulemans, Mona Khan, Seung-Jun Yoo, Bolek Zapiec, Laura Van Gerven, Jan Van Slambrouck, Arno Vanstapel, Dirk Van Raemdonck, Robin Vos, Els Wauters, Joost Wauters, Peter Carmeliet, Peter Mombaerts
laurens.ceulemans@uzleuven.be

Department of Thoracic Surgery and Lung Transplantation (LJC, JVS, DVR), Department of Otorhinolaryngology (LVG), Department of Respiratory Diseases (RV, EW), and Department of Internal Medicine (JW), University Hospitals Leuven, Leuven, Belgium; Department of Chronic Diseases and Metabolism, Laboratory of Respiratory Diseases and Thoracic Surgery (LJC, AV, DVR, RV, EW), Department of Neurosciences, Research unit Experimental Otorhinolaryngology (LVG), Department of Microbiology, Immunology, and Transplantation, Research unit Allergy and Clinical Immunology (LVG), Department of Imaging and Pathology (AV), Department of Microbiology, Immunology, and Transplantation, Laboratory for Clinical Infectious and Inflammatory Disorders (JW), and Laboratory of Angiogenesis and Vascular Metabolism, Centre for Cancer Biology, VIB, Department of Oncology, Leuven Cancer Institute (PC), Katholieke Universiteit Leuven, Leuven, Belgium; Max Planck Research Unit for Neurogenetics, Frankfurt, Germany (MK, S-JY, BZ, PM); Department of Biomedicine, Aarhus University, Aarhus, Denmark (PC); and State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Centre, Sun Yat-Sen University, Guangzhou, Guangdong, China (PC)

1 Ceulemans LJ, Van Slambrouck J, De Leyn P, et al. Successful double-lung transplantation from a donor previously infected with SARS-CoV-2. *Lancet Respir Med* 2021; **9:** 315–18.
2 Wang K, Chen W, Zhang Z, et al. CD147-spike protein is a novel route for SARS-CoV-2 infection to host cells. *Signal Transduct Target Ther* 2020; **5:** 283.
3 Bussani R, Schneider E, Zentilin L, et al. Persistence of viral RNA, pneumocyte syncytia and thrombosis are hallmarks of advanced COVID-19 pathology. *EBioMed* 2020; **61:** 103104.
4 Schurink B, Roos E, Radonic T, et al. Viral presence and immunopathology in patients with lethal COVID-19: a prospective autopsy cohort study. *Lancet Microbe* 2020; **1:** e290–99.
5 Wölfel VM, Corman W, Guggemos M, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* 2020; **581:** 465–69.