



Absence of Severe Acute Respiratory Syndrome-Coronavirus-2 RNA in ocular tissues

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ABSTRACT

Purpose: To evaluate the status of ocular donor tissues of a COVID-19 postmortem donor.

Methods: SARS-CoV-2 was detected via a pharyngeal swab and broncho-alveolar lavage in the COVID-19 suspect. Postmortem tissue procurement and preparation were performed with personal protective equipment (PPE) and the necessary protective measures. qRT-PCR-testing was performed for the following ocular tissues and fluids: conjunctival fluid swabs, bulbar conjunctiva, corneal epithelium, corneal stroma, corneal endothelium, anterior chamber fluid, lens, iris, vitreous, retina, uvea, sclera, and optic nerve. Informed consent and Institutional Review Board approval was obtained prior to this study (196/2020BO2; Date of approval: 03/26/2020; Ethics Committee of the University of Tuebingen).

Results: In all ocular tissue and fluid samples no SARS-CoV-2 RNA was detected via qRT-PCR of the confirmed COVID-19 postmortem donor.

Conclusions and Importance: Late-stage COVID-19 patients might not harbor an ocular reservoir of SARS-CoV-2. The risk of transmitting SARS-CoV-2 via ocular tissues and fluids might be low. This may bear future implications for patient management in ophthalmological practice, surgery and transplantation.

1. Introduction

Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) first detected in December 2019 in Wuhan, China, features a high rate of transmission and an estimated death rate of 4.8% among confirmed cases (March 31st 2020; <http://coronavirus.jhu.edu/map.html>).^{1,2} One of the first healthcare professionals to raise the alarm was the Chinese ophthalmologist Li Wenliang, who died from coronavirus disease 2019 (COVID-19) on February 7, 2020, at the age of 33, after he contracted the virus from an infected patient.³ Recently, it was suggested that an unprotected exposure of the eyes to SARS-CoV-2 serves as viral entry portal.⁴

2. Materials and methods

2.1. Informed consent, approval of independent institutional review board

Informed consent, adherence to the Declaration of Helsinki and

approval of an independent Ethics Committee (institutional review board) was obtained prior to commencing with the study (196/2020BO2).

Characteristics of the COVID-19 donor: The patient was a 76-year old male who developed Acute Respiratory Distress Syndrome (ARDS), septic shock and multi-organ failure. The patient had no ocular symptoms or findings during his severe clinical course of disease. Coinfection by HSV, CMV, RSV, parainfluenza, and influenza were excluded through qRT-PCR. The lymphopenic patient was intubated for 9 days and received continuous venovenous hemofiltration needing extracorporeal membrane oxygenation (ECMO) for 5 days in our intensive care unit. Pleural effusion and atrial fibrillation developed during the acute course of the disease. Known previous medical illnesses were limited to a status post trans-urethral resection of a benign tumor of bladder and prostate and controlled arterial hypertension.

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2.2. Detailed medical history and timeline

At the beginning our patient suffered from an infection of the upper respiratory tract with an initially dry cough (day 0), fever up to 104 °F (40.0 °C) and progressive shortness of breath. Eleven days after the onset of symptoms, he was admitted to an external hospital with a feverish infection and severe dyspnea. On chest X-ray diffuse bilateral infiltrates were seen (day 11). Any history of exposure to or contact with COVID-19 affected patients could not be traced back. Due to profound respiratory insufficiency, the patient was isolated, transferred to the intensive care unit of the external hospital and subsequently intubated. The patient was tested positive for SARS-CoV-2 (via pharyngeal swab) and negative for influenza. Further microbiological diagnostics using tracheal secretion and blood cultures showed no findings. After a 3-day intensive care stay in the external hospital, the patient was transferred to our intensive care unit for further ARDS therapy. A second qRT-PCR test confirmed SARS-CoV-2 using pharyngeal swab and bronchoalveolar lavage (day 14). On day 16 anemia developed requiring transfusion, on day 18 critical hypoxemia occurred forcing commencement of ECMO and on day 19 anuria ensued demanding hemofiltration. On day 20 spontaneous oozing hemorrhages developed at ECMO lines and the intubation tubing. The patient passed away 23 days after the onset of symptoms. The number of days between the postmortem tests, the initial symptoms and the last positive SARS-CoV-2 test was 23 days and 9 days, respectively. The postmortem tests were performed 13 h after death.

2.3. Guidelines for tissue procurement and personal protective equipment

Guidelines according to the local supervisory authorities for the enucleation team is outlined in the following: To be checked prior to the enucleation of a COVID-19 postmortem donor is the place of enucleation which is defined as an area and/or room needing permission to access. The time spent at the location has to be documented. In addition, the place of enucleation is not allowed to be used by another person at the time of tissue extraction. Any kind of aerosol and/or turbulence has to be prevented by the performing person. The necessary equipment has to be discarded after usage and/or disinfected depending on the specific utensils used. To preclude any kind of self-harm personal protective equipment (PPE) has to be used appropriately. This includes gowns (overalls and apron), double gloves (as indicator system), hood and face mask FFP-3 level with 0.6 µm/99% filtration. The infectious wastes have to be disposed of in a one-time lockable container and any sharp utensils in a suitable, second container.

2.4. Enucleation and preparation protocols

A routine tissue procurement protocol for bio-banking was employed for the left globe of the donor with the application of iodine. The respective right globe was kept naïve during the enucleation and preparation steps. The enucleation was performed at the designated COVID-19 autopsy room of the Institute for Pathology and Neuro-pathology of the University Hospital of Tuebingen. Direct preparation and further testing of donor tissues was performed at a BSL2 laboratory (under a sterile workbench) of the Institute for Medical Virology of the University of Tuebingen. A detailed description of the protocols is given as an appendix.

2.5. RNA extraction and quantitative Reverse Transcription-Polymerase Chain Reaction

The RNA extraction and qRT-PCR was performed using RealStar SARS-CoV-2 RT-PCR Kit 1.0 (altona Diagnostics GmbH, Hamburg, Germany) and LightMix® Modular SARS-CoV (COVID19) kit (TIB Molbiol Syntheselabor GmbH, Berlin, Germany). The quality approved protocols included controls. DNase digest was done with RNase-Free DNase Set (#79254) and purification with RNeasy Mini Kit (Qiagen

#74106).

3. Results

We report here the absence of SARS-CoV-2 RNA in ocular tissues obtained from a COVID-19 postmortem donor using qRT-PCR on the following tissues: conjunctival fluid swabs, bulbar conjunctiva, corneal epithelium, corneal stroma, corneal endothelium, anterior chamber fluid, lens, iris, vitreous, retina, uvea, sclera, and optic nerve. All tissue samples tested negative for SARS-CoV-2 viral RNA coding for its S and E genes, indicating absence of viral infection in the eye.

4. Discussion

This early-stage pilot study provides valuable insight into an important clinical issue and may bear future implications for patient management in ophthalmological practice, surgery and transplantation. The risk of transmitting SARS-CoV-2 via ocular tissues and fluids is assumed to take place given most recent reports.⁵ In this study we analyzed via qRT-PCR the status of SARS-CoV-2 RNA in essentially all ocular tissues and fluids of a COVID-19 postmortem donor. We identified no significant levels of SARS-CoV-2 RNA in any sample of tissue or fluid. This appears similar to Ebola virus (EBOV) infections, where ocular fluids and tissues were negative for EBOV RNA by qRT-PCR in aqueous humor/vitreous fluid and conjunctiva at a median of 19 months and an expected incidence of EBOV in ocular fluids of less than or equal to 5% (confidence interval of Ebola Virus Persistence in Ocular Tissues and Fluids (EVICT) study: 0.95–1.00; N = 50).⁶ Relevant original articles on this topic are currently scarce. Some authors even consider the majority of available data on SARS-CoV-2 colonization of ocular and periorbital tissues and secretions as controversial.⁷ Adequate protection of ophthalmologists is advised as long as an increased risk for infection may exist.⁸ This study is considered early stage pilot and was used to test the design. In order to confirm or refute the preliminary data a full-scale study needs to be implemented.

5. Conclusions

Although limited to a single patient, our findings suggest that *late-stage* COVID-19 patients do not carry an ocular reservoir of SARS-CoV-2. This bears implications for the management of COVID-19 patients in ophthalmological emergency and general surgery and, in particular, for tissue procurement and processing of potential COVID-19 donors as well as corneal transplantation surgery. Nevertheless, until more extensive studies are available, protective measures need to be maintained.

Consent

Written consent was obtained prior to the study.

Disclosures

None.

Declaration of competing interest

Tarek Bayyoud, MD: Conflicts of interest: none.
 Angelika Iftner: Conflicts of interest: none.
 Thomas Iftner, PhD: Conflicts of interest: none.
 Karl Ulrich Bartz-Schmidt, MD: Conflicts of interest: none.
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 Sebastian Thaler, MD: Conflicts of interest: none.

CRedit authorship contribution statement

Tarek Bayyoud: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing. **Angelika Iftner:** Validation, Formal analysis, Investigation, Writing - review & editing. **Thomas Iftner:** Conceptualization, Methodology, Validation, Resources, Writing - review & editing, Supervision, Project administration. **Karl Ulrich Bartz-Schmidt:** Conceptualization, Methodology, Validation, Resources, Writing - review & editing, Supervision, Project administration. **Marius Ueffing:** Conceptualization, Methodology, Validation, Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration. **Michael Schindler:** Conceptualization, Methodology, Validation, Resources, Writing - review & editing, Supervision, Project administration. **Sebastian Thaler:** Conceptualization, Methodology, Validation, Resources, Writing - review & editing, Supervision, Project administration.

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None.

Appendix

Enucleation and preparation protocols

The enucleation team had to check the set of surgical instruments, double check the identity of the postmortem donor and the consent form and confirm the cause of death (COVID-19). According to the standard operating procedures in place donor side (right/left), place of enucleation and time of enucleation were documented. Proper usage of PPE including a fitting test of the FFP-3/N-95 mask was obligatory. This was followed by the inspection of body bag and corpus, preparation of transport media and vessel (left globe: sterile gauze, 10 mL NaCl, 10 mL gentamicinsulfate (5 mg/mL); right globe: sterile gauze, 10 mL NaCl; each vessel marked: COVID-19 donor tissues R or L). Flushing of the superior and inferior fornix of the left globe (Betasisodona®; 1:10 diluted in sterile NaCl equivalent to 1% of free iodine, flushing with sterile NaCl after 5 min) ensued with prior periocular wiping with Betasisodona®. The right globe was kept naïve. The provided drape, vessel and PPE was used appropriately to prevent any kind of self-harm. The process of enucleation was done with *single-use* surgical set (eyelid blocker, forceps, scissors, hooks) to obtain an intact globe with conjunctivae (5–10 mm) and prevent any potential contaminations. Prosthesis selection,

insertion and closure of palpebral fissures ensued. The transfer of each globe into the pre-defined transport vessel and a marked re-lockable container followed (marked: “COVID-19 donor tissues”). Final disposal of the used PPE and potentially infectious materials was performed to current standards of hygiene and occupational safety. The transport took place via a re-lockable, marked container (“COVID-19 donor tissues”) during which temperature was recorded and kept between 33.8 °F and 50 °F (+1 to +10 °C) using cooling packs and a cooling box thereby avoiding direct contact to ice (Liberio T1, Elpro, Switzerland). Appropriate use of PPE including fitting test for FFP-3/N-95 mask, disinfection of the sterile workbench (Descosept-AF, desiccation of 15 min) and disinfection of globe (left) in diluted iodine solution (5 min in Betasisodona® (7.5%, Braun, #3864154)/NaCl (250 mL, Fresenius Kabi, PZN-00809049) 1:20) and thorough rinse (in 50 mL NaCl) was performed. The right globe was kept naïve. The preparation of the donor tissues and fluids with a *single-use* surgical set (surgical forceps, 15 mm trephine, 30G-cannulas, Kolibri-forceps, Vannas-scissors, Westcott-scissors, hockey knife and vacuum holder) and extraction of tissue/fluid samples for quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) testing on SARS-CoV-2 RNA was done to the highest standards of bio-banking to obtain bulbar conjunctiva, corneal epithelium, corneal stroma, corneal endothelium, anterior chamber fluid, lens, iris, vitreous, retina, uvea, sclera, and optic nerve samples.

References

1. Wang C, Horby PW, Hayden FG, et al. A novel coronavirus outbreak of global health concern. *Lancet*. 2020 Feb 15;395(10223):470–473. [https://doi.org/10.1016/S0140-6736\(20\)30185-9](https://doi.org/10.1016/S0140-6736(20)30185-9). Epub 2020 Jan 24.
2. Sommer A. Humans, viruses, and the eye—an early report from the COVID-19 front line. *JAMA Ophthalmol*. 2020 Mar 31 <https://doi.org/10.1001/jamaophthalmol.2020.1294> [Epub ahead of print].
3. https://en.wikipedia.org/wiki/Li_Wenliang.
4. Lu CW, Liu XF, Jia ZF. 2019-nCoV transmission through the ocular surface must not be ignored. *Lancet*. 2020 Feb 22;395(10224):e39. [https://doi.org/10.1016/S0140-6736\(20\)30313-5](https://doi.org/10.1016/S0140-6736(20)30313-5). Epub 2020 Feb 6.
5. Navel V, Chiambaretta F, Dutheil F. Haemorrhagic conjunctivitis with pseudomembranous related to SARS-CoV-2. *Am. J. Ophthalmol. Case Rep.* 2020 May 6:100735 <https://doi.org/10.1016/j.ajoc.2020.100735> [Epub ahead of print].
6. Shantha JG, Mattia JG, Goba A, et al. Ebola virus persistence in ocular tissues and fluids (EVICT) study: Reverse transcription-polymerase Chain reaction and cataract surgery outcomes of Ebola survivors in Sierra Leone. *EBioMedicine*. 2018 Apr;30: 217–224.
7. Aiello Francesco, Afflitto Gabriele Gallo, Mancino Raffaele, et al. Coronavirus disease 2019 (SARS-CoV-2) and colonization of ocular tissues and secretions: a systematic review. *Eye*. 2020 May 18:1–6. <https://doi.org/10.1038/s41433-020-0926-9>. Online ahead of print.
8. Kuo Irene C. A rashomon moment? Ocular involvement and COVID-19. *Ophthalmology*. 2020 Apr 21. <https://doi.org/10.1016/j.ophtha.2020.04.027>. Online ahead of print.