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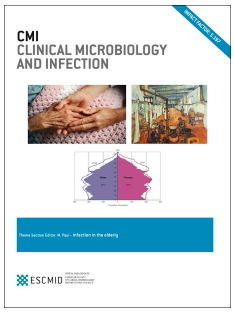


In search of viable SARS-CoV-2 in the tear film: a prospective clinical study in hospitalized symptomatic patients.

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# In search of viable SARS-CoV-2 in the tear film: a prospective clinical study in hospitalized symptomatic patients.

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To the Editor,

SARS-CoV-2 RNA in tears has been described in patients with and without conjunctivitis (1), implying that this disease might be transmitted via this body fluid. Moreover the corneal epithelium contains angiotensin 2 converting enzyme as well as transmembrane Serine Protease 2 protein, both essential for the binding and entrance of the SARS-Cov-2 spike protein (2).

We conducted a prospective study in 30 patients admitted to the non-ICU COVID unit of the University Hospitals Leuven, Belgium. This study was approved by the Ethics Committee Research UZ / KU Leuven, Belgium in accordance with the principles of the Declaration of Helsinki. This project was registered on ClinicalTrials.gov (NCT04799704).

First, we wanted to investigate the presence of SARS-CoV-2 in the tear film by reverse transcription (RT) - quantitative (q)PCR (RT- qPCR). This is the most common used test to detect SARS-CoV-2 in clinical laboratories (3). However, molecular tests such as RT-qPCR cannot distinguish between non-infectious residual viral RNA and replicating virus. To detect viable and replicating virus, subgenomic (sg) RNA testing and viral culture on Vero E6 cells was performed on SARS-CoV-2 positive conjunctival samples at the Laboratory of Clinical and Epidemiological Virology (Rega institute), Katholieke Universiteit Leuven. Viral culture is a well-known technique to prove active viral shedding. The main limitations of this technique are the low sensitivity, special infrastructure and expertise needed, the timely effort and the required biosafety level 3 conditions. SgRNA, on the other hand, is an intermediate product produced during the process of active replication of the SARS-CoV-2 virus and a potential marker for active infection and viral replication (3,4).

Eighty percent of the included patients were over 50 years old (n=24, mean 65 ± 16) and 37% (n=11) of all included patients were female. There was no significant difference between patients with SARS-CoV-2 positive and SARS-CoV-2 negative conjunctival swabs considering age and gender. A questionnaire was completed by 27 patients, four (14.8%) reported ocular symptoms (red eye,

irritation, pain and/or epiphora), all in combination with nasal congestion; none of them had anosmia or ageusia. All but one patient developed ocular symptoms within the first week of the presenting COVID-19 symptoms. These ocular symptoms occurred 1 to 4 weeks before hospitalization; none of these four patients required ICU admission.

All patients underwent bilateral conjunctival swabbing at least once. A total of 176 swabs were collected; 3 patients did not proceed with the serial swabbing due to discomfort. In total, in 13 swabs (7%) of 7 patients (23%) SARS-CoV-2 was detected by RT-qPCR (Table 1). Three were found to be strongly positive (5-7 log copies/ml) and 10 were weakly positive (<3 log copies per ml). Only 2 patients had consecutive positive conjunctival swabs, with the first one being strongly positive and the consecutive ones only weakly positive. Interestingly, none of the patients with a positive conjunctival swab reported symptoms of conjunctivitis. Six of the 13 positive samples (46%) were also positive for sgRNA and 2 of those 6 samples showed growth on viral cultures, confirming viability.

To summarize, we not only demonstrated the presence of SARS-CoV-2 in tears by RT-qPCR, but 6 samples (46%) also tested positive for the presence of sgRNA and 2 of those samples showed growth after inoculation on viral culture (Table 1). The low sensitivity of viral culture may explain why some samples are positive for sgRNA and negative on viral culture. Of note, only samples strongly positive on RT-qPCR showed viral growth.

Four previously published papers described viral culture of conjunctival swabs of SARS-CoV-2 positive patients (5–8), only one case report noticed a cytopathic effect on Vero E6 cells (8). Casagrande et al. found sgRNA in corneal discs of deceased patients with COVID-19, but they failed to isolate the virus (9).

The added value of our research project is the demonstration of the replication and shedding of the virus in the tear film by sgRNA assays and viral culture. This makes tears a potential route of viral transmission, especially in procedures such as pneumotonometry and excimer refractive laser

surgery, both transforming tears to small droplets by either the use of a jet of air or a laser beam (10,11). Since none of the patients with positive conjunctival swabs reported signs of conjunctivitis, the presence and shedding of SARS-CoV-2 in the tear film should be considered in both patients with and without conjunctivitis.

The limitations of our study are the small sample size and the dependence on self-reported symptoms by using questionnaires. Furthermore, we only included patients with a nasopharyngeal swab positive for SARS-CoV-2 on PCR test. We cannot provide information on the presence of SARS-CoV-2 in the tears in case of a negative nasopharyngeal swab. The strength of this study lies in the repetitive and bilateral sampling approach and the exploration of the presence of SARS-CoV-2 through both sgRNA-testing and viral cultures.

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We have no conflict of interest to declare.

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74 **Authors' contributions**

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| Heleen Delbeke MD | Conceptualization, Methodology, Investigation, Writing – Original Draft, Formal Analysis, Funding acquisition, Supervision, Role assignment |
| Stefanie Desmet ParmD | Writing – Review & Editing, Formal Analysis |
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Table 1: Overview of patients with a SARS-CoV-2 positive conjunctival swab

| | Total n of swab samples | Positive swab | RE/LE | PCR | Viral culture | sgRNA | 1st COVID Symptoms reported |
|---|---|---|---|---|---|---|---|
| **Patient 1** | 2 | First | LE | Weak | Neg | Neg | 2 Weeks |
| **Patient 2** | 8 | First | RE | Weak | Neg | Neg | 2 Weeks |
| **Patient 3** | 3 | First | RE | Strong | Pos | Pos | 1 Week |
| | | First | LE | Strong | Pos | Pos | 1 Week |
| | | Second | LE | Weak | Neg | Neg | 1 Week |
| **Patient 4** | 5 | Second | RE | Weak | Neg | Neg | 1 Week |
| | | Second | LE | Weak | Neg | Neg | 1 Week |
| **Patient 5** | 3 | First | LE | Strong | Neg | Neg | 3 Weeks |
| | | Second | RE | Weak | Neg | Neg | 3 Weeks |
| | | Second | LE | Weak | Neg | Pos | 3 Weeks |
| **Patient 6** | 1 | First | RE | Weak | Neg | Pos | 1 Week |
| | | First | LE | Weak | Neg | Pos | 1 Week |
| **Patient 7** | 9 | First | LE | Weak | Neg | Pos | 1 Week |

n: number; LE: left eye, RE: right eye, Neg: negative, Pos: positive
Strong positive: 5-7 log copies/ml; weak positive <3 log copies per ml.