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Detection of Coronavirus in Tear Samples of Hospitalized Patients With Confirmed SARS-CoV-2 From Oropharyngeal Swabs

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Purpose: This study was designed to detect CoV-RNA in the tears of polymerase chain reaction (PCR)-confirmed SARS-CoV-2 positive patients.

Methods: We performed a prospective case series study of hospitalized patients who have been confirmed SARS-CoV-2 positive by oropharyngeal swab within the previous 5 days. Tear samples obtained with a laboratory capillary and oropharyngeal swabs were analyzed by real-time PCR using the Altona SARS-CoV-2 Assay or the Roche SARS-CoV-2 LightMix PCR, depending on the availability. Patient history was documented, and ophthalmoscopy was used to assess for ocular surface disease.

Results: Of all 18 patients recruited in April 2020, 5 suffered from respiratory failure and were submitted to an intensive care unit. None of our patients had signs of viral conjunctivitis although all patients in intensive care showed chemosis and conjunctival hyperemia because of third-spacing or fluid overload. The presence of coronavirus RNA was confirmed by PCR in 5 of 18 patients (28%) in tears and 72% for oropharyngeal swabs.

Conclusions: Using a tear fluid sampling technique similar to oropharyngeal lavage presents a higher percentage of SARS-CoV-2 positive tears in contrast to earlier reports that used a conjunctival swab. This does not automatically indicate viral shedding in ocular tissue or contagiousness of tear fluid.

Key Words: coronavirus, SARS-CoV-2, conjunctivitis, case series, PCR

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SARS-CoV-2 is an enveloped single-stranded RNA virus from the Coronaviridae family of viruses that are known to cause respiratory and gastrointestinal symptoms.[1,2] According to the World Health Organization, SARS-CoV-2 most commonly causes fever, dry cough, and tiredness, and less commonly pains, sore throat, diarrhea, headache, loss of taste or smell, and conjunctivitis. In recent weeks, vasculitis in children and thrombotic events in the elderly have been reported to be a growing concern.[3] The mean incubation period is around 5 days with a range of up to 14 days.[4,5] SARS-CoV-2 is typically confirmed by nasopharyngeal swab polymerase chain reaction (PCR) although the sensitivity of the different tests is no more than 56% to 83%.[6] Therefore, clinicians and patients are advised to stay in quarantine if COVID-19 is suspected, even if they had initially tested negative. The binding and entry of SARS-CoV-2 is mediated by the angiotensin-converting enzyme 2 receptor, which is expressed highly in the respiratory and cardiovascular system, explaining the dominance of respiratory symptoms.[7] Nevertheless, angiotensin-converting enzyme 2 is also expressed by conjunctival epithelial cells and by the retina.[8,9]

Bilateral follicular conjunctivitis has been described in a limited number of cases in patients with active SARS-CoV-2 infection.[10] This common presentation of viral conjunctivitis is nonspecific for COVID-19, and therefore, some theoretical risk exists for ophthalmologists who unknowingly come into contact with SARS-CoV-2. But how many infected patients carry the virus in their tears? To clarify this, we tested the tear fluid of confirmed hospitalized SARS-CoV-2 patients by PCR using a method not previously used for the collection of tear samples.

MATERIALS AND METHODS

Clinical Samples

Written consent was given by patients in accordance with the European data protection law. All but 2 of the patients who participated in the study provided consent themselves for conjunctival swab samples to be taken. The remaining 2 patients' written consent was given by a first-degree relative, who had the patient's permission to do so for medical purposes. The study was approved by the ethics committee of Heinrich Heine University, Düsseldorf. Ophthalmic symptoms and medical and ocular histories were reviewed for each patient. Oropharyngeal and tear samples were collected from patients hospitalized at Kliniken der Stadt Köln GmbH (Cologne, Germany) and from both Luisenhospital and Marienhospital, Aachen, Germany, in April 2020. All patients who tested positive for SARS-CoV-2 within 5 days of our sampling were included in our study. To stimulate tear production and collect

AQ:3

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AQ:2

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cellular debris, the eyes of each patient were gently massaged wearing sterilized gloves. Directly after, 1 drop of saline solution was applied as a lavage to flush out maximum protein. In some cases, an initial tear ran down the cheek after the patient blinked. Then, 2× 15 μL of the tear solution was taken from the medial canthus and lower fornix using a laboratory capillary. The capillaries were stored in RNA-later at −20°C for up to 1 week.

For collecting oropharyngeal swabs, a polyester swab with a plastic shaft is used to swab the bilateral tonsils and posterior pharyngeal wall while depressing the tongue. The swab is then placed in a test tube containing RNA-later viral transport medium before being sent directly to the in-house hospital laboratory.

Real-Time PCR

The usage of different extraction methods and PCR protocols was due to delivery chains being broken early on in the pandemic, meaning that not all assays were available all the time. All methods were validated internally against round robin trial samples obtained from INSTAND or against a positive control sample obtained from the Institute of Virology, Charité, Berlin. For RNA extraction, the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) was used according to the manufacturer's protocol. Alternatively, RNA was extracted using the Maxwell Viral RNA kit (Promega, Darmstadt, Germany) for the initial disease confirmation of oropharyngeal swabs in the Kliniken der Stadt Köln laboratory. Total RNA was extracted using the Promega Total Viral Nucleic Acid kit according to the manufacturer's instructions. Before extraction, an internal extraction control (Altona Diagnostics, Hamburg, Germany) was added to the sample. This extraction control was detected in a third target channel. Fragmentation of the RNA during the PCR would lead to invalid results also of this internal control. Only those samples in which no fragmented RNA was present and which led to valid results were included in this study. PCR was performed using the Real-Time PCR Cycler (LightCycler 480 II; Roche, Switzerland) in combination with the LightMix SarbecoV E-gene/EAV control 530/660 (TIB MOLBIOL Syntheselabor GmbH, Berlin, Germany). A 25 μL reaction contained 5 μL of RNA, 12.5 μL of 2× reaction buffer provided by the Superscript III one-step RT-PCR system with Platinum Taq Polymerase (Invitrogen, Darmstadt, Germany; containing 0.4 mM of each deoxyribont triphosphates and 3.2 mM magnesium sulfate), 1 μL of reverse transcriptase/Taq mixture from the kit, 0.4 μL of a 50 mM magnesium sulfate solution (Invitrogen), and 1 μg of non-acetylated bovine serum albumin (Roche). As primers, the LightMix SarbecoV E-gene and EAV control 530/660 (TIB MOLBIOL Syntheselabor GmbH, Berlin, Germany) were used. All oligonucleotides were synthesized and provided by TIB MOLBIOL (Berlin, Germany). Thermal cycling was performed at 55°C for 10 minutes for reverse transcription, followed by 95°C for 3 minutes and then 45 cycles of 95°C for 15 seconds and 58°C for 30 seconds. The laboratory used the Roche Light Cycler 480II (Roche).

AQ:4

Long-term monitoring of the patients was performed on the RotorGeneQ (Qiagen), in combination with the RealStar SARS-CoV-2 RT-PCR (Altona Diagnostics), in accordance with the manufacturer's protocol.

RESULTS

Patients' History

Eighteen patients were included: 9 were male and 9 were female. The mean age was 66.3 years old, ranging from 42 to 90 years old. All 18 patients were hospitalized: 16 because of acute respiratory insufficiency, 1 because of cachexia, and 1 because of aphasia with hyperkalemia.

Anterior Segment Examination

None of the patients showed typical signs of viral conjunctivitis such as follicular conjunctivitis. All nonintubated patients declared no novel ocular symptoms within the past month. Examinations of the anterior segment revealed no ocular surface diseases except for dry eye syndrome. By contrast, 5 patients intubated because of respiratory failure presented chemosis and conjunctival hyperemia.

Real-Time PCR

PCR confirmed coronavirus RNA in the tears of 5 of 18 patients (28%) and from oropharyngeal swabs in 13 of 18 patients (72%). For tear samples, the volume obtained differed substantially because of the difference in tear volume of each patient. A spill over the lid margin of the saline/tear mixture reduced the comparability of virus load within tears as well. Therefore, the virus load was not symmetrically quantified and compared. Nevertheless, a mean ct value of 30.8 (SD ±4.8) was comparable with the ct value of oropharyngeal swabs of 32.3 (SD ±3.8). One patient tested positive in tears but tested negative in the oropharyngeal swab, whereas the other 4 patients tested positive in both types of samples.

DISCUSSION

In this study, we could confirm SARS-CoV-2 RNA positive tear samples by PCR in as many as 28% of determined SARS-CoV-2 patients by oropharyngeal swabs. Only 72% of oropharyngeal swabs tested positive again within 5 days of the initial positive test. This could primarily be explained by the dynamics of viral shedding that peak either on or before symptom onset and decrease thereafter.[11] This number is clearly higher than in previous reports. Xia et al[12] obtained 52 conjunctival swabs of Schirmer stripes of 30 COVID-19 patients, and only 1 test came back positive for a patient suffering from conjunctivitis. In another study of 38 clinically confirmed COVID-19 patients, only 2 (5.2%) had a positive tear swab, whereas 73% had a positive oropharyngeal swab.[13] In a more recent cross-sectional study, only 1 (1.38%) conjunctival swab of 72 confirmed SARS-CoV-2 cases was tested positive.[14] Test reliability is probably the cause of this discrepancy. The amount of protein obtained from tear swabs is small, even our earliest attempt to gain tear fluid simply by capillary forces without adding saline revealed only single-to-double digit microliter volumes. We suspect that, by adding saline, a wash-out effect could have brought about more material and therefore a higher rate of CoV-RNA positive tears.

Clinically, in the study by Wu et al[13] as mentioned above, 31% of COVID-19 patients had ocular manifestations, such as chemosis, secretion, and epiphora, classified as conjunctivitis.

AQ:5 Considering our 5 patients presented with chemosis and conjunctival hyperemia, we could not draw the conclusion that these are cases of SARS-CoV-19-related conjunctivitis. Although previous reports of SARS-CoV-2 follicular conjunctivitis have been reported in the literature,[10,15] we could not confirm that in any of our patients who presented with ocular signs. The chemosis and conjunctival hyperemia seen in our 5 acutely ill patients are more likely to represent fluid overload or third-spacing rather than SARS-CoV-2 viral conjunctivitis.

To answer the question on how infectious tear fluid in such cases is, Zhou et al[16] performed a clinical analysis on 4 patients with positive conjunctival swab samples. Although these 4 patients infected further individuals, none of these new infections presented positive conjunctival swabs. This gives hope that the ocular route of transmitting SARS-CoV-19 is unlikely although the low sample size and the detection method of conjunctival swabs do not give certainty. In addition, up until now, there is no AQ:6 evidence of active virions in tear fluid. Seah et al[17] detected no cytopathic effects or the presence of viral shedding in SARS-CoV-2 RNA positive tears if incubated with Vero-E6 cells, in comparison with oropharyngeal samples. However, lacking a BSL3 facility, no cell culture attempts were performed in this study. This gives hope that the ocular route of transmitting SARS-CoV-19 is unlikely although the low sample size and the detection method of conjunctival swabs do not give certainty.

In our study, despite high levels of viral RNA in tears, we were unable to confirm the presence of viral conjunctivitis AQ:7 in patients with proven SARS-CoV infection. This, although we describe a comparably high rate of CoV-RNA positive tear samples.

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