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REVIEW

There is a Role in Detection of SARS-CoV-2 in Conjunctiva and Tears: a comprehensive review

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SUMMARY

Data on the involvement of the ocular surface and its relationship with Coronavirus disease 2019 (COVID-19) are still minimal and not univocal.
The respiratory tract is the structure most affected by COVID-19, and the serious form of the disease is characterized by severe pneumonia, acute respiratory distress syndrome and hypercoagulation. However, accumulating evidence shows that other organs could be reached by the virus, thus causing further comorbidities. To date, the exact route/routes of transmission of COVID-19 are still unclear. The respiratory tract is probably not the only route of transmission for this viral infection and some authors have also speculated that COVID-19 droplets, or infected hands, could contaminate the conjunctiva, which could therefore represent the initial site of an infection spread.
Theoretically, the role of the ocular surface, a biological site still relatively unexplored, appears scientifically relevant in understanding the Severe Acute Respiratory Syndrome - Coronavirus - 2 (SARS-CoV-2) infection. The purpose of this paper is to summarize the current literature in order to elucidate the potential role of tear and conjunctival sampling to detect SARS-CoV-2 for the diagnosis of COVID-19 and to monitor patients during follow-up.

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INTRODUCTION

Severe Acute Respiratory Syndrome-Corona-Virus-2 is a novel lineage in the phylogenetic tree of beta-coronaviruses. It displays 89% nucleotide identity with bat SARS-like CoVZXC21 and 82% with that of human SARS-CoV (Chan *et al.*, 2020). The spike proteins of SARS-CoV-2 associated with the host receptor angiotensin-converting enzyme 2 (ACE2) in target cells of sensitive tissues can result in infection. The mammalian serine protease Transmembrane Serine Protease 2 (TMPRSS2) or the protease Furin (also known as Paired basic Amino acid Cleaving Enzyme) appear to trigger the spike protein for interaction with ACE2 (Hoffmann *et al.*, 2020, Zhou *et al.*, 2020).
ACE2 is also the receptor for SARS-CoV; both types of virus have high human-to-human transmissibility and can cause severe acute respiratory disease (Benvenuto *et al.*, 2020).
Viral diagnosis plays an essential role in the control of any communicable disease, and this has been unequivocally shown in the recent global pandemic caused by SARS-CoV-2, also known as Coronavirus disease 2019 (COVID-19) (Li & Ma, 2020). Real-time reverse transcriptase polymerase chain reaction-based assays (RT-PCR) for detecting SARS-CoV-2 performed on respiratory specimens are the current reference standard for COVID-19 diagnosis. To limit the spread of infection, these assays are also used to track affected but asymptomatic patients, according to proposed guidelines that also consider sustainability of the flow.
Biological samples other than those from the respiratory tract, such as feces (Chen *et al.*, 2020c), saliva (Lm *et al.*, 2020, Williams *et al.*, 2020), urine (Peng *et al.*, 2020), and semen (Paoli *et al.*, 2020) have been investigated.
On the other hand, data published on the involvement of the ocular surface, in particular the conjunctival mucosa and the corneal epithelium, are still minimal and inconsistent.
Interestingly, SARS-CoV-2 has also been identified in tears, as was SARS-CoV in 2003 (Karimi *et al.*, 2020, Loon *et al.*, 2004, Xia *et al.*, 2020). The expression of ACE2 and TMPRSS2 has been identified in several tissues in the human body (such as lung alveolar mucosa, oral mucosa, gastrointestinal duct, kidney), including the cornea and conjunctiva (Lange *et al.*, 2020, Ma *et al.*, 2020), where they are both co-expressed (Sungnak *et al.*, 2020). These findings suggest that these tissues could be possible sites of original infection and potential reservoirs for diffusion (Sun *et al.*, 2020).
Although the expression level of ACE2 on the conjunctiva appears to be lower than that in the lung, at least in mice, (Zhang *et al.*, 2020) it could be a potential infection route of SARS-CoV-2 via the ocular surface. A comparison of SARS-CoV-2, SARS-CoV and MERS (Middle East respiratory syndrome) - CoV receptors on the ocular surface and in the lung is given in a recent review work by Willcox and collaborators (Willcox *et al.*, 2020). The diagnostic

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role of the conjunctival swab in investigating the local involvement of the ocular surface by SARS-CoV-2 has been reported in few studies and on a very limited number of patients, often occasional observations on a single patient (Chen *et al*., 2020b, Colavita *et al*., 2020, Xia *et al.*, 2020, Zhang *et al*., 2020). In particular, the conjunctival swab test was found positive for SARS-CoV-2 in only about 2.5-5% of patients, according to studies conducted in the Wuhan region (Wu *et al*., 2020, Zhou *et al.*, 2020). It is worth noting that the positivity of eye swabs persists over time compared with nasopharyngeal swabs. In particular, conjunctival swab positivity can be detected for over two weeks after the negativity of the nose pharyngeal swab (Colavita *et al*., 2020, Hu *et al.*, 2020).

THE OCULAR SURFACE AS A DEFENSIVE BARRIER TO PATHOGENS

The ocular surface system (Gipson 2007) includes the epithelia of the cornea, the conjunctiva, the corneo-scleral limbus with stem cells, lacrimal, accessory, and meibomian glands, tears, the eyelashes with their associated glands, and the nasolacrimal duct. All the various components of this system are integrated by the nervous, endocrine, vascular, and immune systems to provide a refractive smooth surface of the cornea and a primary defensive wall to environmental irritants, allergens and pathogens.

All the epithelia of the ocular surface are continuous, from the lacrimal glandular epithelium to the ductal epithelium of the nasolacrimal system, and share functional properties with the mucus membranes of the respiratory tracts (Knop & Knop, 2007, Paulsen, 2008). However, the need to maintain optical properties is crucial at the ocular surface and make it a unique mucosal immune lining. In fact, to effectively counteract microbial diseases without leading to impaired corneal transparency, the immune response must be precisely modulated through the recruitment of competent immune cells (Akpek & Gottsch, 2003, Foulsham *et al*., 2018, Galletti, *et al*., 2017).

The corneal and conjunctival epithelial cells are sealed by adherent junctions that act as a physical barrier against the external environment, and lie above a connective tissue, looser in conjunctiva and highly organized and compact in the cornea. Both epithelium and stromal tissue present immunocompetent resident cells belonging to the immune defense system, including CD8+ T and CD4+ T cells, macrophages (CD68+), Langerhans cells expressing HLA-DR (Pflugfelder & de Paiva, 2017). The first line of defense of the ocular surface is the presence of a healthy tear film that protects it from the external environment and provides an effective clearance and self-cleaning system under normal conditions.

Tears play a fundamental role in the innate defense against microorganisms (Garreis, *et al.*, 2010, Maarouf *et al.*, 2018, McDermott, 2013). Their antimicrobial function is exerted through three mechanisms:

1) blinking and reflex tearing of the tear film, which results in flushing and washout of invading microorganisms;
2) inhibition and/or killing of microorganisms by several antimicrobial proteins (including lysozyme, lactoferrin, lipocalin, and secretory immunoglobulins A (IgA));
3) entrapment of microorganisms by high-molecular weight glycoproteins (mucins, MUC), MUC2 and gel-forming MUC5Ac.

The lacrimal duct operates the passage of the virus from ocular to respiratory tract mucosa as it opens at the inferior meatus located underneath the inferior nasal turbinate. Transport may occur as a simple fluid exchange between sites through the conduit without onsite viral replication (Olofsson *et al.*, 2005). However, the possibility of virus inoculation in nasolacrimal duct epithelial cells exists, as these cells both secrete and reabsorb tear fluid (Paulsen, 2003).

THE OCULAR SURFACE AS A PORTAL OF INFECTION

In addition to the respiratory tract mucosa, the ocular surface certainly represents a site in the human body which is similarly exposed to contaminated and infectious droplets (Konjevoda *et al.*, 2020, Paulsen, 2008). Given the anatomical connection and immunological interdependence between the eye and the nose, the ocular surface may also be considered a portal to respiratory system infection. The ocular and respiratory tract mucosa share host cell receptors for several respiratory viruses, such as epithelial cell lectin-binding sites and, in particular, glycoproteins bearing terminal sialic acids (Kumlin *et al.*, 2008).

Human respiratory viruses display a variable ocular tropism, which can cause ocular infection and further respiratory tract infection following the first contact with the eye (*Figure 1*) (Belser *et al*., 2013). In fact, the virus is

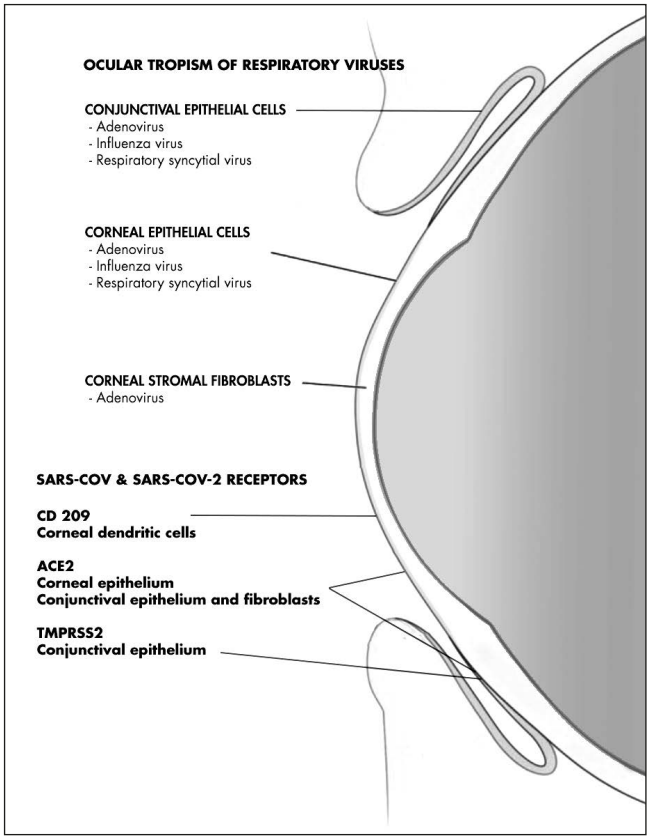

Figure 1 - *Ocular tropism of respiratory viruses and SARS-COV and SARS-COV-2 receptors.* Ocular tissues exhibiting tropism for respiratory viruses are shown along with the location of the receptors that mediate the entry of SARS-COV and SARS-COV-2.

capable of either replicating in the ocular surface epithelial cells or simply draining with tears into the nasolacrimal duct and further infecting the respiratory tract if the innate immune system does not neutralize it earlier. The adenovirus and influenza virus can frequently cause epidemic, highly contagious, severe ocular diseases such as keratoconjunctivitis or conjunctivitis (Belser *et al*., 2013, Creager *et al*., 2018), and tropism in conjunctival cells has been demonstrated in *in vitro* models (Chan *et al*., 2010).

Previous studies have indicated that coronavirus infections in humans rarely associate with ocular complications, but suggest that ocular exposure may be a portal of entry for these viruses and that contact with infected eyes could be one route of transmission. For instance, HCoVNH (New Haven coronavirus) positivity has been found in children affected by Kawasaki disease associated with bilateral conjunctivitis (Esper *et al*., 2005) but the etiology of the disease is still unclear. Kawasaki disease is an acute febrile systemic childhood vasculitis, which is suspected to be triggered by respiratory viral infections. Interestingly, peaks of severe Kawasaki-like disease have been described in association with the SARS-CoV-2 epidemic, suggesting that this could be a trigger for the disease and indicating the potential timing of an increase in incidence of the disease in COVID-19 epidemics (Ouldali *et al*., 2020).

In the SARS-associated coronavirus pneumonia identified in 2003, the virus was found to transmit via contact with mucous membranes, including those of the eyes (Peiris *et al*., 2003), as suggested by increased SARS transmission from infected patients to health care workers without eye protection (Raboud *et al.,* 2010). Although the World Health Organization in 2003 included tears in the body fluids potentially containing SARS-CoV, the clinical impact is not yet clear ('WHO | Update 27 - One month into the global SARS outbreak' n.d.).

SARS-associated coronavirus was detected by Real Time Polymerase Chain Reaction (RT-PCR) in conjunctival samples from suspected patients without ocular symptoms (Loon *et al*., 2004), whereas conjunctival and tear samples from confirmed SARS patients, similarly without ocular symptoms or signs, tested negative (Chan *et al*., 2004, Yuen *et al.*, 2004, Zhou *et al*., 2020).

The tropism of SARS-CoV2 to ocular surface cells remains controversial. In *in vitro* studies, SARS-CoV-2 replication was greater than SARS-CoV replication in conjunctival cells, but the release of pro-inflammatory cytokines was lower than in MERS-CoV and influenza virus H5N1 (Hui *et al*., 2020). A meta-analysis on six studies recently reported a very low pooled sensitivity of ocular tissue/fluid in detecting SARS-CoV-2 compared to samples from nasopharyngeal swabs (Ulhaq & Soraya, 2020). The prevalence of viral detection in infected patients is provided in the following section of this paper, where the literature was reviewed as of June 2, 2020.

OCULAR SURFACE AND COVID-19 INFECTION: REVIEW OF LITERATURE

An initial literature search was performed in several databases, including Web of Science, Ovid, Cochrane Database, and Scopus for original articles published up to August 2020. Keywords were used in combination, including: "COVID-19," "SARS-CoV-2," "coronavirus," "eye," "ocular," "conjunctival," "ocular surface," "ophthalmic," and "conjunctivitis." A second literature search was conducted by identifying relevant references of initially included articles. The searches were conducted by two independent investigators (FB, PV). Any discrepancies were resolved by discussion or by input from the third reviewer (MCR).

Study Selection. After removing duplicate publications, 2 reviewers (FB, PV) independently screened the titles and abstracts of all identified citations. The full text of citations judged as potentially eligible were obtained and independently screened for eligibility, and any disagreement was resolved by discussion with the authors.

Eligibility Criteria. The articles were considered eligible if the studies met the following inclusion criteria:

1) study type: case series or reports;
2) population: patients with COVID-19 who described ocular manifestations that were tested for SARS-COV-2 in ocular secretions via various sampling or detection methods. We excluded studies that described ocular manifestations of COVID-19 but did not document testing for SARS-COV-2 in ocular secretions.

A rapidly increasing number of studies has investigated ocular signs and symptoms in patients infected with SARS-CoV-2. (Chen *et al*., 2020a, Fang *et al*., 2020, Guan *et al.*, 2020, Hong *et al*., 2020, Karimi *et al*., 2020, Liang & Wu, 2020, Mungmungpuntipantip & Wiwanitkit, 2020, Seah *et al*., 2020, Wu *et al.*, 2020, Xia et al., 2020, Xie *et al*., 2020, Zhang *et al*., 2020, Zhou *et al*., 2020, Zhou *et al*., 2020). A recent meta-analysis that included 1167 COVID-19 patients showed that conjunctivitis may be a sign of COVID-19 infection associated to a more severe form of disease, suggesting the use of protective equipment for all persons potentially exposed to infected subjects (Loffredo *et al.*, 2020). Specifically, several studies reported conjunctival injection, chemosis and epiphora as the most common signs of ocular involvement in patients affected by COVID-19. In addition, the clinical picture could lead to a broad spectrum of symptoms, in particular foreign body sensation, dry eye and blurred vision (Xia *et al*., 2020; Wu *et al*., 2020, Chen *et al.,* 2020b). In addition, it cannot be excluded that SARS-CoV-2 could infect both the eye and the surrounding structures; the role of the ocular surface as a potential access or exit route is under debate (Napoli *et al*., 2020, Aiello *et al*., 2020, Sun *et al.*, 2020).

Ocular symptoms were common in a large series of COVID patients (Hong *et al.,* 2020). Hong and collaborators investigated ocular symptoms through the Ocular Surface Disease Index and Salisbury Eye Evaluation Questionnaire in 56 patients, and found that fifteen (27%) had aggravated ocular symptoms, of which 6 (11%) had prodromal ocular symptoms before disease onset (Hong *et al.*, 2020). The authors speculated that the micro-environment of the ocular surface and the stability of tear film could be affected by various factors, such as a systemic immune system reaction to the SARS-CoV-2 infection, a secondary infection by opportunistic ocular pathogens, and infection of ocular tissues by the SARS-CoV-2 virus.

However, other studies have shown a lower prevalence of ocular surface involvement. In a large series of 535 COVID-19 patients, Chen and collaborators found that 27 patients (5.0%) presented with conjunctival congestion. The study also showed that dry eye, blurred vision and foreign

body sensation were the most common ocular symptoms in all patients (20.9%, 12.7% and 11.8%, respectively) (Chen *et al.*, 2020a). In another large sample of 1099 patients with COVID-19, 0.9% showed signs of ocular inflammation (Guan *et al.*, 2020). Finally, Mungmungpuntipantip *et al.*, in a brief letter to the editor, reported that none of the 48 patients affected by COVID-19 presented any signs of ocular inflammation (Mungmungpuntipantip & Wiwanitkit, 2020).

The presence of SARS-CoV-2 RNA at the level of the ocular surface has been investigated by means of RT-PCR on conjunctival swab samples. In particular, Seah and collaborators prospectively evaluated 17 patients with COVID-19, investigating tear samples and ocular findings during 2 weeks of active infection (Seah *et al.*, 2020). All samples showed negative results for SARS-CoV-2 on viral isolation and RT-PCR, but 1 patient developed signs of ocular inflammation during hospitalization (Seah *et al.*, 2020). Moreover, Zhou *et al.* tested 121 COVID-19 patients, disclosing three (0.8%) positive RT-PCR from conjunctival swabs (Zhou *et al.*, 2020). Specifically, one patient showed both symptoms and positive conjunctival swab results and was classified as a critical case, and 2 patients showed no symptoms but revealed positive swab results, with one classified as a severe or critical case and another classified as moderate case (Zhou *et al.*, 2020). Zhang *et al.* reported that only two out of 72 (2.8%) COVID-19 patients presented with conjunctivitis and that only one (1.4%) of them showed SARS-CoV-2 RNA fragments found in ocular discharges. The authors proposed that the incidence of SARS-CoV-2 infection through the ocular surface should be extremely low, whereas nosocomial infection of SARS-CoV-2 through the eyes after occupational exposure is a potential route (Zhang *et al.*, 2020). Liang and coauthors evaluated 37 conjunctival swabs from confirmed SARS-CoV-2-infected patients. Three out of 37 patients (8.1%) had conjunctivitis. However, only one patient in serious condition, not suffering from conjunctivitis, presented a positive RT-PCR assay (Liang & Wu, 2020). Based on this finding, the authors suggested that conjunctival viral load could be directly proportional to severity of the disease (Liang & Wu, 2020).

Xia and coauthors prospectively evaluated the presence of SARS–CoV-2 in 30 affected patients (Xia *et al.*, 2020) and disclosed a positive RT-PCR assay in two consecutive swabs from the only patient with conjunctivitis symptoms (Xia *et al.*, 2020). On the contrary, Güemes-Villahoz showed that COVID-19 patients with and without conjunctivitis showed the same rate of positive samples from ocular fluids (5.5%), suggesting that the detection of SARSCoV-2 is not conditioned by the presence of conjunctivitis (Güemes-Villahoz *et al.*, 2020a) (Güemes-Villahoz *et al.*, 2020b). Wu *et al.* investigated ocular involvement and viral prevalence in the conjunctiva of 38 patients with COVID-19, and found that 12 patients (31.58%) presented with signs suggestive of conjunctivitis and that 5.26% of conjunctival specimens yielded positive findings for SARS-CoV-2 at RT-PCR. It should be considered that patients with positive conjunctival swabs presented ocular symptoms (Wu *et al.*, 2020). Karimi and coauthors disclosed only one patient with conjunctivitis among 43 affected by COVID-19. However, three patients (7%), including the one with conjunctivitis, had tear samples positive for SARS-CoV-2 (Karimi *et al.*, 2020). These results suggest that, although ocular manifestation seems to be rare in COVID-19 patients, the possibility of ocular transmission should be considered even in the absence of ocular manifestations. Fang *et al.* found that five (15.63%) out of 32 patients with COVID-19 showed positive SARS-CoV-2 RNA via RT-PCR tear swabs. However, they did not investigate the potential ocular involvement of affected patients (Fang *et al.*, 2020). Xie *et al.* performed ocular surface swabs on 33 consecutive COVID-19 patients without any ocular manifestation and found that SARS-CoV-2 tested highly positive in both eyes from 2 patients (Xie *et al.*, 2020). These findings demonstrated that SARS-CoV-2 RNA could be detected from the normal ocular surface of COVID-19 patients.

Table 1 - *Characteristics and main findings of studies evaluating ocular surface involvement in patients with COVID-19 infection.*

Author	*Design*	*Patients (n)*	*Ocular Involvement (no. patients) %*	*Positive Ocular Surface Swabs*	*Ocular Swabs Timing*
Chen *et al.*, 2020a	Retrospective	535	(27) 5%	NP	
Fang *et al.*, 2020	Retrospective	32	NP	15.6%	NA
Guan *et al.*, 2020	Retrospective	1099	(10) 0.9%	NP	
Güemes-Villahoz *et al.*, 2020	Cross-sectional	36 (18 with ocular involvement + 18 without ocular involvement)	NA	5.5% in each group	NA
Hong *et al.*, 2020	Retrospective	56	(15) 27%	NP	
Karimi *et al.*, 2020	Prospective	43	(1) 2.3%	7 %	1–7 days
Liang & Wu, 2020	Retrospective	37	(3) 8.1%	1%	NA
Mungmungpuntipantip &Wiwanitkit 2020	Retrospective	48	0%	NP	
Seah *et al.*, 2020	Prospective	17	(1) 5.8%	0%	3-20 days
Wu *et al.*, 2020	Retrospective	38	(12) 31.5%	5.2%	NA
Xia *et al.*, 2020	Prospective	30	(1) 3.3%	3.3%	3-16 day
Xie *et al.*, 2020	Retrospective	33	0%	6.1%	1-7 days
Zhang *et al.*, 2020	Cross Sectional	72	(2) 2.8%	1.4%	6-46 days
Zhou *et al.*, 2020	Retrospective	121	(7) 6.6%	0.8%	1-38

NP: Not performed, NA: Not applicable.

Table 1 summarizes the characteristics and main findings of studies (published as of June 2, 2020) that evaluated ocular surface involvement in patients with COVID-19 infection. In the thirteen studies taken into consideration, only 70 patients (3.2%) out of 2176 COVID-19 patients were diagnosed as suffering from ocular surface involvement.

It should be considered that most of these studies suffer from a retrospective design. In addition, because this is a potentially lethal disease and because hospitalization often requires careful systemic monitoring, the presence of signs of ocular inflammation may have been clinically underestimated. Furthermore, sampling time during the course of infection was found highly variable across studies, whenever indicated. The diagnostic window for molecular-based techniques (and serological testing) has been clarified only recently (Younes *et al*., 2020), and it is also highly feasible that this fundamental parameter may have been missed in the ophthalmological studies performed in the early, very troubling period of the SARS-CoV-2 pandemic. In addition, and closely related to the ocular surface site, late sampling may have activated the immune system with significant increases in tears containing immunoglobulins exudated from plasma and antimicrobial compounds which can inhibit viral binding to ACE2 (Lang *et al*., 2011, Orr-Burks *et al.*, 2014).

These observations suggest that the ocular surface as a potential portal of entry for this virus should not be underestimated, as the possibility that the conjunctiva might be a gate for the virus is consistent with biological findings so far. In addition, the possibility of disease transmission through the ocular surface should be considered even in the absence of ocular manifestations.

OCULAR SURFACE AND LABORATORY DIAGNOSIS OF COVID-19: CURRENT ISSUES AND CHALLENGES

Ineffcient diagnostic methods have been accused of being responsible for the variability of coronavirus findings on the ocular surface (Zhang *et al.*, 2020). A detailed discussion of the several technical issues that could be involved is beyond the scope of this paper. An exhaustive discussion of this issue has been conducted by previous studies (Deeks *et al.*, 2020, Sethuraman, Jeremiah & Ryo, 2020, Tang *et al.*, 2020). In addition, data on technical aspects are almost totally lacking in the papers published to date dealing with tear or conjunctival samples in SARS-CoV2 detection.

We would like here to briefly mention some arguments that deserve further study:

– Viral load in tears. Current RT-PCR protocols may have low sensitivity in detecting SARS-CoV-2 in conjunctival and tear samples. This could be related to small amounts of viral RNA in these secretions, taking into account that it reflects a disease course-dependent balance between viral replication and immune system response, as was previously shown for MERS-CoV (Memish *et al.*, 2014). Furthermore, MERS-CoV concentration and genome fraction was shown to be dissimilar in different sites of the respiratory tract (Memish *et al*., 2014). When distribution of SARS-CoV-2 was investigated in bronchoalveolar fluid, pharyngeal swabs, blood, sputum, feces, nasal samples, and urine of patients clinically diagnosed as COVID-19 positive, virus was detected all tissues except urine, with the highest viral load in nasal swabs [mean cycle threshold value of 24.3 (1.4×10^6 copies/mL) as compared to more than 30 ($<2.6\times10^4$ copies/mL) in all the other tissues] (Wang *et al*., 2020). Interestingly, a recent meta-analysis showed that the sensitivity of saliva samples is slightly lower than that of nasopharyngeal samples (91% compared to 98%), suggesting that it may be an effective method for the detection of SARS-CoV-2, with less heterogeneity among studies (Czumbel *et al.*, 2020). On the whole, data on the accuracy of RT-PCR testing suggest that test sensitivity may vary by type of specimen, but these data did not include tears.
– Sampling time. Some authors reported that conjunctival samples collected in the late phase of the disease are CoV RNA-negative (Chan *et al*., 2004). The contribution of antimicrobial agents increasing post-infection, as reported above, may contribute to support CoV RNA as present only in the early phase of the disease. MERS-CoV RNA in the conjunctiva had been detected within only 6 days post-infection in an animal model (de Wit *et al.*, 2016). A high percentage of positivity was found in tears or conjunctiva sampled from the 4^{th} to 9^{th} day (median value being 5^{th}) from the onset of symptoms (Arora *et al*., 2020), with a significant decrease in positivity from the second week and beyond (Seah *et al*., 2020, Zhang *et al*., 2020). Other authors have noted that the positivity of eye swabs to SARS-CoV-2 was prolonged over time (Colavita *et al.*, 2020), remaining for over two weeks after nose and pharyngeal swabs had become negative (Hu *et al.*, 2020).

Taken as a whole, studies with a large sample size and repeated collection of conjunctival/tears in each patient during the course of the disease are needed.

– Collection technique may be appropriate or standardized: most of the studies have reported conjunctival swabs for tear collection (Arora *et al.*, 2020, Karimi *et al*., 2020, Wu *et al*., 2020, Xia *et al.*, 2020, Zhou *et al*., 2020), except one where the Schirmer test was used (Seah *et al*., 2020). For specimen sampling, the World Health Organization recommends (CDC 2020) the use of only synthetic fiber swabs rather than calcium alginate swabs which may contain substances interfering with PCR testing. Similarly, topical anesthesia is not recommended for tear and conjunctival sample collection, as it might negatively influence viral viability (CDC 2020).
– Primer design: hundreds of TaqMan RT-PCR assays for the detection of SARS-CoV-2 have been proposed and dozens are under development for both manual and automated assay (https://www.finddx.org/covid-19/pipeline). Furthermore, scientific validation of their diagnostic accuracy, tailored to the specimen sampled, including tears and conjunctiva, should be performed in real-life prospective trials.

In summary, there is strong evidence that SARS-CoV-2 can affect the ocular surface. Because of its anatomical and functional structure, the ocular surface could represent a potential infection route and allow both the entry and exit of the virus. To date, studies on the involvement of the ocular surface have given conflicting results, and there is still no consensus on the methodological techniques to be applied to effectively detect SARS-CoV-2 at the ocular level. Testing of specimens from multiple sites, including the ocular surface, and the standardization and validation of a method for the detection of SARS-CoV-2 may improve sensitivity and reduce false-negative test results, with the aim of implementing management of the disease at all stages (Ho *et al.*, 2020).

References

Akpek E.K., Gottsch J.D. (2003). Immune defense at the ocular surface. *Eye* (Lond). **17**, 949-956.

Arora R., Goel R., Kumar S., Chhabra M., Saxena S., Manchanda V., Pumma P. (2020). Evaluation of SARS-CoV-2 in tears of moderate to severe COVID-19 patients. *Ophthalmology*.

Belser J.A., Rota P.A., Tumpey T.M. (2013). Ocular tropism of respiratory viruses. *Microbiol Mol Biol Rev.* **77**, 144-156.

Benvenuto D., Giovanetti M., Ciccozzi A., Spoto S., Angeletti S., Ciccozzi M. (2020). The 2019-new coronavirus epidemic: Evidence for virus evolution. *Journal of Medical Virology.* **92**, 455-459.

CDC. (2020). Information for Laboratories about Coronavirus (COVID-19). Centers for Disease Control and Prevention.

Chan J.F.-W., Kok K.-H., Zhu Z., et al. (2020). Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. *Emerging Microbes & Infections*. **9**, 221-236.

Chan M.C.W., Chan R.W.Y., Yu W.C.L., et al. (2010). Tropism and innate host responses of the 2009 pandemic H1N1 influenza virus in *ex vivo* and *in vitro* cultures of human conjunctiva and respiratory tract. *Am J Pathol.* **176**, 1828-1840.

Chan W.M., Yuen K.S.C., Fan D.S.P., et al. (2004). Tears and conjunctival scrapings for coronavirus in patients with SARS. *Br J Ophthalmol.* **88**, 968-969.

Chen L., Deng C., Chen X., et al. (2020). Ocular manifestations and clinical characteristics of 535 cases of COVID-19 in Wuhan, China: a cross-sectional study. *Acta Ophthalmol.*

Chen L., Liu M., Zhang Z., et al. (2020). Ocular manifestations of a hospitalised patient with confirmed 2019 novel coronavirus disease. *Br J Ophthalmol.* **104**, 748-751.

Chen Y., Chen L., Deng Q., et al. (2020). The presence of SARS-CoV-2 RNA in the feces of COVID-19 patients. *J Med Virol.*

Colavita F., Lapa D., Carletti F., et al. (2020). SARS-CoV-2 Isolation From Ocular Secretions of a Patient With COVID-19 in Italy With Prolonged Viral RNA Detection. *Ann Intern Med.*

Creager H.M., Kumar A., Zeng H., et al. (2018). Infection and Replication of Influenza Virus at the Ocular Surface. *J Virol.* **92**.

de Wit E., van Doremalen N., Falzarano D., Munster V.J. (2016): SARS and MERS: recent insights into emerging coronaviruses. *Nature Reviews Microbiology.* **14**, 523-534.

Deeks J.J,. Dinnes J., Takwoingi Y., et al. (2020). Diagnosis of SARS-CoV-2 infection and COVID-19: accuracy of signs and symptoms; molecular, antigen, and antibody tests; and routine laboratory markers. *Cochrane Database of Systematic*. Reviews.

Esper F., Shapiro Z.D., Weibel C., Ferguson D., et al. (2005). Association between a novel human coronavirus and Kawasaki disease. *J Infect Dis*. **191**, 499-502.

Fang Z., Zhang Y,. Hang C., Ai J., et al. (2020). Comparisons of viral shedding time of SARS-CoV-2 of different samples in ICU and non-ICU patients. *J Infect.*

Foulsham W., Coco G., Amouzegar A., et al. (2018): When Clarity Is Crucial: Regulating Ocular Surface Immunity. *Trends Immunol.* **39**, 288-301.

Galletti J.G., Guzmán M., Giordano M.N. (2017). Mucosal immune tolerance at the ocular surface in health and disease. *Immunology.* **150**, 397-407.

Garreis F., Gottschalt M., Paulsen F.P. (2010). Antimicrobial peptides as a major part of the innate immune defense at the ocular surface. *Dev Ophthalmol.* **45**, 16-22.

Gipson I.K. (2007). The Ocular Surface: The Challenge to Enable and Protect Vision: The Friedenwald Lecture. *Invest Ophthalmol Vis Sci.* **48**, 4391-4398.

Guan W., Ni Z., Hu Y., et al. (2020). Clinical Characteristics of Coronavirus Disease 2019 in China. *New England Journal of Medicine.* **382**, 1708-1720.

Güemes-Villahoz N., Burgos-Blasco B., Arribi-Vilela A., et al. (2020). Detecting SARS-CoV-2 RNA in conjunctival secretions: Is it a valuable diagnostic method of COVID-19? *J Med Virol.* [Online ahead of print] 10.1002/jmv.26219.

Güemes-Villahoz N., Burgos-Blasco B., Vidal-Villegas B., et al. (2020). Novel Insights into the Transmission of SARS-CoV-2 Through the Ocular Surface and its Detection in Tears and Conjunctival Secretions: A Review. *Adv Ther.* **37**, 4086-4095.

Ho D., Low R., Tong L., et al. (2020). COVID-19 and the Ocular Surface: A Review of Transmission and Manifestations. *Ocular Immunology and Inflammation*. **28**, 726-734.

Hoffmann M., Kleine-Weber H., Schroeder S., et al. (2020). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell.* **181**, 271-280.e8.

Hong N., Yu W., Xia J, et al. (2020). Evaluation of ocular symptoms and tropism of SARS-CoV-2 in patients confirmed with COVID-19. A*cta Ophthalmol.* [Online ahead of print] 10.1111/aos.14445.

Hu Y., Chen T., Liu M., et al. (2020). Positive detection of SARS-CoV-2 combined HSV1 and HHV6B virus nucleic acid in tear and conjunctival secretions of a non-conjunctivitis COVID-19 patient with obstruction of common lacrimal duct. *Acta Ophthalmol.* [Epub ahead of print] 10.1111/aos.14456.

Hui K.P.Y., Cheung M.-C., Perera R.A.P.M., et al. (2020). Tropism, replication competence, and innate immune responses of the coronavirus SARS-CoV-2 in human respiratory tract and conjunctiva: an analysis in ex-vivo and in-vitro cultures. *Lancet Respir Med.* **8**, 689-695.

Karimi S., Arabi A., Shahraki T., Safi S. (2020). Detection of severe acute respiratory syndrome Coronavirus-2 in the tears of patients with Coronavirus disease 2019. *Eye* (Lond). **34**, 1220-1223.

Knop E., Knop N. (2007). Anatomy and immunology of the ocular surface. *Chem Immunol Allergy.* **92**, 36-49.

Konjevoda S., Canovic S., Pastar Z., et al. (n.d.). Ophthalmic manifestations of novel coronaviruses: precautionary measures and diagnostic possibilities. *J Glob Health.* **10**, 010430.

Kumlin U., Olofsson S., Dimock K., Arnberg N. (2008). Sialic acid tissue distribution and influenza virus tropism. *Influenza Other Respir Viruses*. **2**, 147-154.

Lang J., Yang N., Deng J., et al. (2011). Inhibition of SARS Pseudovirus Cell Entry by Lactoferrin Binding to Heparan Sulfate Proteoglycans. *Plos One.* **6**, e23710.

Lange C., Wolf J., Auw-Haedrich C., et al. (2020). Expression of the COVID-19 receptor ACE2 in the human conjunctiva. J Med Virol [Online ahead of print] 10.1002/jmv.25981

Li X., Ma X. (2020). Acute respiratory failure in COVID-19: is it "typical" ARDS? *Critical Care*. **24**, 198.

Liang L., Wu P. (2020). There may be virus in conjunctival secretion of patients with COVID-19. *Acta Ophthalmol.* **98,** 223.

Czumbel L.M., Kiss S., Farkas N., et al. (2020). Saliva as a Candidate for COVID-19 Diagnostic Testing: A Meta-Analysis. *Frontiers in medicine.* **7**, 465.

Loffredo L., Pacella F., Pacella E., et al. (2020). Conjunctivitis and COVID-19: A meta-analysis. J Med Virol [Online ahead of print] 10.1002/jmv.25938.

Loon S.-C., Teoh S.C.B., Oon L.L.E., et al. (2004). The severe acute respiratory syndrome coronavirus in tears. *Br J Ophthalmol.* **88**, 861-863.

Ma D., Chen C.-B., Jhanji V., et al. (2020). Expression of SARS-CoV-2 receptor ACE2 and TMPRSS2 in human primary conjunctival and pterygium cell lines and in mouse cornea. *Eye* (Lond) **34**, 1212-1219.

Maarouf M., Rai K.R., Goraya M.U., Chen J.-L. (2018). Immune Ecosystem of Virus-Infected Host Tissues. *Int J Mol Sci.* **19**, 1379.

McDermott A.M. (2013). Antimicrobial compounds in tears. *Exp Eye Res.* **117**, 53-61.

Memish Z.A., Al-Tawfiq J.A., Makhdoom H.Q., et al. (2014). Respiratory Tract Samples, Viral Load, and Genome Fraction Yield in Patients With Middle East Respiratory Syndrome. *J Infect Dis*. **210**, 1590-1594.

Mungmungpuntipantip R., Wiwanitkit V. (2020). Ocular manifestation, eye protection, and COVID-19. *Graefes Arch Clin Exp Ophthalmol.* **258**, 1339.

Younes N., Al-Sadeq D.W., Al-Jighefee H., et al. (2020). Challenges in Laboratory Diagnosis of the Novel Coronavirus SARS-CoV-2. *Viruses*. **12**, 582.

Napoli P.E., Nioi M., d'Aloja E., Fossarello M. (2020). The Ocular Surface and the Coronavirus Disease 2019: Does a Dual 'Ocular Route' Exist? *J Clin Med.* **9**, 1269.

Olofsson S., Kumlin U., Dimock K., Arnberg N. (2005). Avian influenza and sialic acid receptors: more than meets the eye? *The Lancet Infectious Diseases*. **5**, 184-188.

Orr-Burks N., Gulley S.L., Gallardo R.A., Toro H., Ginkel F.W. van (2014). Immunoglobulin A as an Early Humoral Responder After Mucosal Avian Coronavirus Vaccination. *avdi.* **58**, 279-286.

Ouldali N., Pouletty M., Mariani P., et al. (2020). Emergence of Kawasaki disease related to SARS-CoV-2 infection in an epicentre of the French COVID-19 epidemic: a time-series analysis. The Lancet Child & Adolescent Health **4**, 662-668.

Paoli D., Pallotti F., Colangelo S., et al. (2020). Study of SARS-CoV-2 in semen and urine samples of a volunteer with positive naso-pharyngeal swab. *J Endocrinol Invest*. [Online ahead of print] 10.1002/jmv.25936.

Paulsen F. (2003). The human nasolacrimal ducts. *Adv Anat Embryol Cell* Biol. **170**, 1-106.

Paulsen F. (2008). Functional anatomy and immunological interactions of ocular surface and adnexa. *Dev Ophthalmol.* **41**, 21-35.

Peiris J.S.M., Yuen K.Y., Osterhaus A.D.M.E., Stöhr K. (2003). The severe acute respiratory syndrome. *N Engl J Med.* **349**, 2431-2441.

Peng L., Liu J., Xu W., et al. (2020). SARS-CoV-2 can be detected in urine, blood, anal swabs, and oropharyngeal swabs specimens. J Med Virol [Online ahead of print.] 10.1002/jmv.25936.

Pflugfelder S.C., de Paiva C.S. (2017). The Pathophysiology of Dry Eye Disease: What We Know and Future Directions for Research. *Ophthalmology.* **124**, S4-S13.

Raboud J., Shigayeva A., McGeer A., et al. (2010). Risk Factors for SARS Transmission from Patients Requiring Intubation: A Multicentre Investigation in Toronto, Canada. *Plos One.* **5**, e10717.

Seah I.Y.J., Anderson D.E., Kang A.E.Z., et al. (2020): Assessing Viral Shed-

ding and Infectivity of Tears in Coronavirus Disease 2019 (COVID-19) Patients. *Ophthalmology.* **127**, 977-979.
Sethuraman N., Jeremiah S.S., Ryo A. (2020). Interpreting Diagnostic Tests for SARS-CoV-2. *JAMA.* **323**, 2249-2251.
Sun C.-B., Wang Y.-Y., Liu G.-H., Liu Z. (2020). Role of the Eye in Transmitting Human Coronavirus: What We Know and What We Do Not Know. *Front Public Health.* **8**, 155.
Sungnak W., Huang N., Bécavin C., et al. (2020). SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nat Med.* **26**, 681-687.
Tang Y.-W., Schmitz J.E., Persing D.H., Stratton C.W. (2020). Laboratory Diagnosis of COVID-19: Current Issues and Challenges. *Journal of Clinical Microbiology.* **58**, e00512-20.
Ulhaq Z.S., Soraya G.V. (2020). The prevalence of ophthalmic manifestations in COVID-19 and the diagnostic value of ocular tissue/fluid. *Graefes Arch Clin Exp Ophthalmol.* **258**, 1351-1352.
Wang W., Xu Y., Gao R., et al. (2020). Detection of SARS-CoV-2 in Different Types of Clinical Specimens. *JAMA.* **323**, 1843.
WHO | Update 27 - One month into the global SARS outbreak: Status of the outbreak and lessons for the immediate future (2020). WHO. World Health Organization.
Willcox M.D., Walsh K., Nichols J.J., et al. (2020). The ocular surface, coronaviruses and COVID-19. *Clin Exp Optom.* **103**, 418-424.
Williams E., Bond K., Zhang B., et al. (2020). Saliva as a non-invasive specimen for detection of SARS-CoV-2. *Journal of Clinical Microbiology.* **58**, e00776-20.
Wu P., Duan F., Luo C., et al. (2020). Characteristics of Ocular Findings of Patients With Coronavirus Disease 2019 (COVID-19) in Hubei Province, China. *JAMA Ophthalmol.* **138**, 575-578.
Xia J., Tong J., Liu M., et al. (2020). Evaluation of coronavirus in tears and conjunctival secretions of patients with SARS-CoV-2 infection. *J Med Virol.* **92**, 589-594.
Xia J., Tong J., Liu M., et al. (2020). Evaluation of coronavirus in tears and conjunctival secretions of patients with SARS-CoV-2 infection. *Journal of Medical Virology.* 92, 589-594.
Xie H.-T., Jiang S.-Y., Xu K.-K., et al. (2020). SARS-CoV-2 in the ocular surface of COVID-19 patients. *Eye Vis* (Lond) **7**, 23.
Xu L., Zhang X., Song W., et al. (2020). Conjunctival polymerase chain reaction-tests of 2019 novel coronavirus in patients in Shenyang, China. medRxiv 2020.02.23.20024935.
Yuen K.S.C., Chan W.-M., Fan D.S.P., et al. (2004). Ocular screening in severe acute respiratory syndrome. *Am J Ophthalmol.* **137**, 773-774.
Zhang B.N., Wang Q., Liu T., et al. (2020). [Expression analysis of 2019-nCoV related ACE2 and TMPRSS2 in eye tissues]. Zhonghua Yan Ke Za Zhi. **56**, E011.
Zhang X., Chen X., Chen L., et al. (2020). The evidence of SARS-CoV-2 infection on ocular surface. Ocul Surf **18**, 360-362.
Zhou L., Xu Z., Castiglione G.M., Soiberman U.S. Eberhart C.G., Duh E.J. (2020). ACE2 and TMPRSS2 are expressed on the human ocular surface, suggesting susceptibility to SARS-CoV-2 infection. *Ocul Surf.* **18**, 537-544.
Zhou Y., Duan C., Zeng Y., et al. (2020). Ocular Findings and Proportion with Conjunctival SARS-COV-2 in COVID-19 Patients. *Ophthalmology.* **127**, 982-983.