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LETTER TO THE EDITOR

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A missing link between SARS-CoV-2 and the eye?: ACE2 expression on the ocular surface

Dear Editor,

We applaud Lange et al[1] for their extensive efforts to analyze entry factors for severe acute respiratory syndrome coronavirus (SARS-CoV-2) into conjunctival epithelial cells covering the ocular surface, which is an important albeit controversially discussed issue.[1,2] In this journal, Lange et al[1] using high-throughput RNA sequencing and immunohistochemistry could not find any significant expression of angiotensin converting enzyme (ACE2) in the conjunctiva. In contrast, Sungnak et al[2] recently demonstrated expression of ACE2 in the epithelium but not in the stroma of human conjunctiva using single-cell RNA-sequencing. This is a clinically highly relevant and urgent issue, since conjunctivitis has been reported as an ocular manifestation of coronavirus disease 2019 (COVID-19)[3-5] and tears have the potential to spread the infection by draining into the nasopharynx through the naso-lacrimal system.

Here, using the same anti ACE2-antibody as Lange et al[1] under modified experimental conditions (basic instead of acidic antigen retrieval buffer, different dilution and incubation period of primary antibody) we could clearly demonstrate ACE2 expression in the conjunctiva by immunohistochemistry, as determined by Sungnak et al[2] on a RNA level (Figure 1). After obtaining written informed consent, ACE2 was, for the first time to our knowledge, detected in formalin

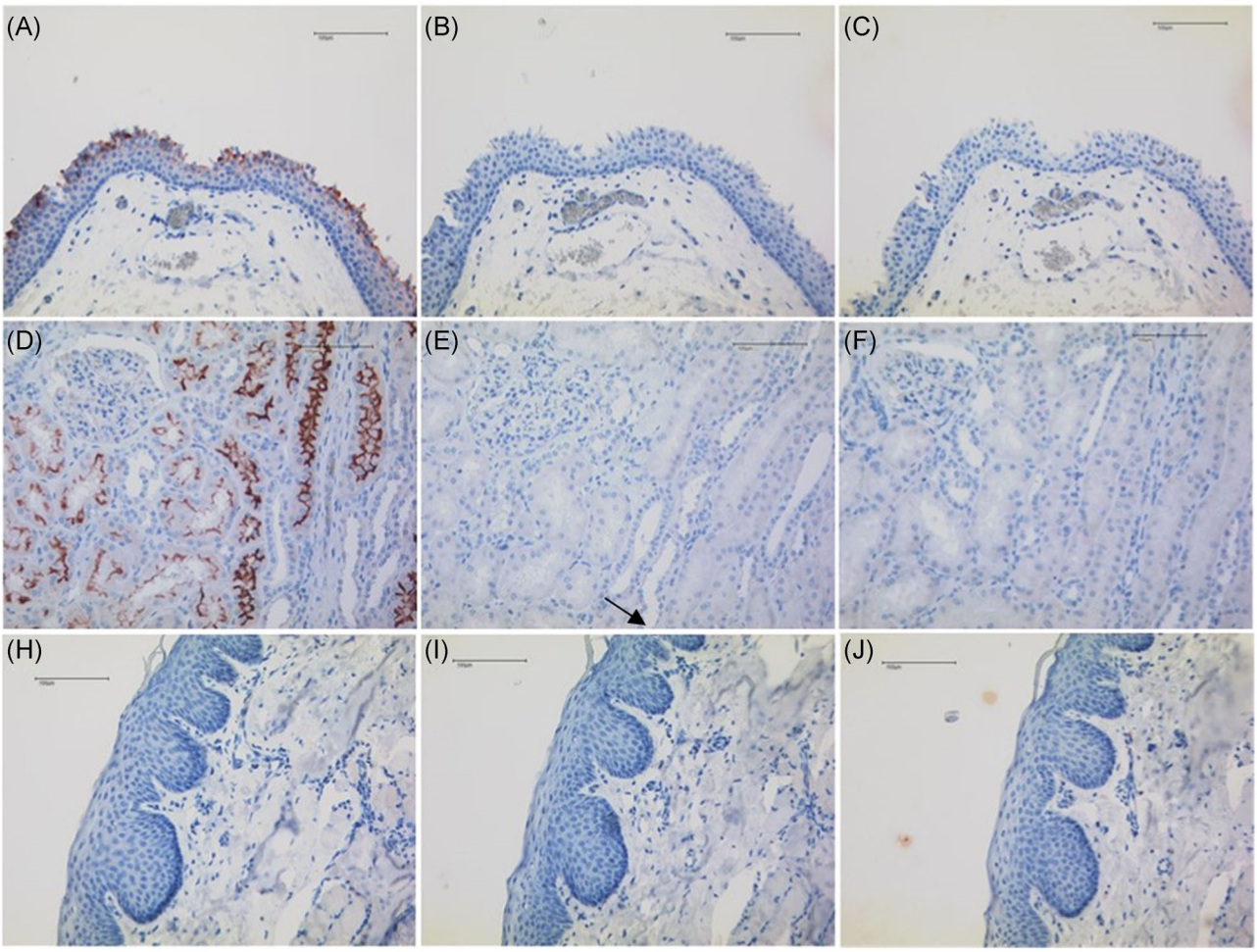

FIGURE 1 Angiotensin converting enzyme (ACE2) is expressed in healthy human conjunctiva

fixed paraffin-embedded sections of healthy human conjunctiva using mouse anti-human ACE2 monoclonal antibody (catalog #MAB933, monoclonal mouse IgG2a anti-human ACE2, clone #171606; dilution 1:100; R&D Systems) at 5 μg/mL for 60 minutes at room temperature. Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Target Retrieval Solution pH 9 (catalog #S2367; Dako). Tissue was stained using DCS DetectionLine, Polylink and Peroxidase Label Horseradish Peroxidase(catalog #PD000RP; DCS) and as the substrate chromogen AEC + High Sensitivity (catalog #K3461; DakoCytomation). The counterstain was hematoxylin (blue). Staining revealed immunopositivity specifically confined to the conjunctival epithelium (Figure 1A). The conjunctival stroma was devoid of ACE2 immunopositivity (Figure 1A). Healthy human conjunctiva sections of seven other patients revealed ACE2 immunopositivity of the epithelium in all samples. Three of these eight patients (age range 8 weeks to 78 years, median age 56 years) were healthy, including the patient in Figure 1A-C (age 43 years), four had arterial hypertension that was treated with ACE inhibitors or ACE-II-receptor antagonists. Although we did not see a relation of ACE2 expression to these and other clinical parameters, the sample size is too small for definitive conclusions.

Staining both without the primary antibody (Figure 1B) as well as an isotype control (Figure 1C; monoclonal mouse immunoglobulin 2a [IgG2a] antibody, clone #20102; dilution 1:100; R&D Systems) demonstrated no staining. Human kidney tissue served as a positive control and showed a specific staining of epithelial cells in convoluted tubules (Figure 1D; NO primary antibody: E, isotype control: F). Human skin served as a negative control (Figure 1H-J, respectively).

The location of ACE2 expression at the ocular surface enables direct contact of SARS-CoV-2 with conjunctival cells, raising the question how often this occurs and if protective mechanisms of tears and conjunctiva might prevent conjunctivitis to happen more frequently. For example, it has to be taken into account that people are breathing in many litres of air potentially containing many viral particles that can be distributed on the huge mucosal surface of the airways and the lung. In contrast, the much smaller mucosal surface of the conjunctiva might only accidentally be hit by single particles, except when contagious aerosols are being blown or coughed directly into the face. Also, tears might contain antiviral substances such as interferons.

Furthermore, it is not clear, whether isolated conjunctivitis may occur without concurrent organ manifestation such as pneumonia, as suggested by current studies,[5] since patients without other symptoms than conjunctivitis are not routinely tested for SARS-CoV-2. Ophthalmologists and eye-care personnel have been described at risk for transmission of COVID-19 due to close contact to patients during examination.[6]

In summary, our results provide an important addition to the results of Lange et al[1] and other works by clearly demonstrating specific ACE2 expression in conjunctival epithelial cells, providing the receptor for direct entry of SARS-CoV-2. Together, these findings emphasize the urgent need for further research regarding the eye as a possible alternative route for transmission of SARS-CoV-2.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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