ACE2 downregulation in olfactory mucosa: Eosinophilic rhinosinusitis as COVID-19 protective factor?

To the Editor,

Loss of smell (LoS) in COVID-19 patients is one of the most early and common symptoms, has a sudden onset and variability in severity ranging from hyposmia to anosmia, being present in a 77% of COVID-19 patients. Via entry angiotensin-converting enzyme-2 (ACE2) and transmembrane protease serine-2 (TMPRSS2) in sustentacular cells, SARS-CoV-2 may induce inflammation in the olfactory neuroepithelium (ONE), leading to olfactory neurons damage, and this being one possible via employed by the virus to enter into the central nervous system.

Identification of risk and protective factors for COVID-19 is critical to better understand, as well as to direct the development of new treatments and possible prevention strategies. In this line, high ACE2 expression has been shown to increase cell susceptibility to SARS-CoV-2. In addition, understanding ACE2 expression and regulation under different pathological conditions may help to predict patient’s susceptibility to COVID-19 and clinical outcomes.

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a respiratory disease characterized by persistent polypoid inflammation of sinonasal mucosa, and symptoms such as nasal obstruction, nasal discharge, facial pain/pressure, and decreased/LoS for ≥12 weeks, with an estimated prevalence of 2–5% in the general population. CRSwNP predominantly displays a type 2 immune signature involving significant increase in key cytokines and eosinophils.

It has been suggested that CRSwNP patients might have a decreased risk for COVID-19. In this line, a reduction in ACE2 and TMPRSS2 expressions has been observed in airway epithelial cells including nasal polyps, suggesting that ACE2 expression in airway pathways may not increase the risk for developing COVID-19. However, ACE2/TMPRSS2 expressions in the ONE from CRSwNP patients, that may be a risk factor for SARS-CoV-2 infection of the olfactory bulbs and brain entry, remain to be investigated. The study objective was to investigate the expression of SARS-CoV-2 entry proteins in the ONE from eosinophilic CRSwNP compared to healthy controls.

We studied the ACE2 and TMPRSS2 expressions in the ONE from eosinophilic CRSwNP patients (>10 eosinophils/high power field (HPF) (n = 13) and healthy controls (n = 11)). Samples from ONE were obtained from superior turbinate and the upper part of the nasal septum, being histologically characterized by hematoxylin-eosin staining. Immunohistological studies were performed to mark eosinophil infiltration (BMK13+), whereas immunofluorescence was used to identify olfactory neurons (OMP+), sustentacular cells (Hsp25+), and ACE2 protein expression. ACE2 protein expression was confirmed by Western blotting. Real-time PCR studies were performed to quantify ACE2 and TMPRSS2 mRNA expressions. This study was approved by the ethics committee of the Hospital Clinic, Barcelona. All study participants signed a written informed consent.

No demographical differences existed between CRSwNP patients and controls regarding age and gender, but being different for asthma and non-steroidal anti-inflammatory drug-exacerbated respiratory disease (N-ERD) comorbidities associated with CRSwNP (Table S1). The expression of ACE2 protein (p < 0.01) (Figure 1A–D), sustentacular cells (p < 0.05) (Hsp25+, Figure 1A,E), and olfactory neurons (p < 0.01) (OMP+, Figure 1B,F) was significantly decreased in the ONE from CRSwNP. ACE2 and TMPRSS2 mRNAs were significantly decreased in the olfactory mucosa (p < 0.05) (Figure 1G). In addition, CRSwNP patients showed a significant increase in the number of eosinophils in the olfactory mucosa compared to controls (p < 0.05) (Figure 2, Table S2). A positive correlation was found between sustentacular cells (Hsp25+) and ACE2 protein expression (r = 0.920, p < 0.001). A mild-to-moderate negative correlation was found between eosinophil cells (BMK13+) and ACE2 protein (r = −0.42) and with sustentacular cells (r = −0.53) (Figure S1).

These findings suggest that ACE2/TMPRSS2 downregulation in the ONE of CRSwNP patients might be related to tissue predominantly type 2 inflammation, potentially leading to a decreased risk for further ONE damage and SARS-CoV-2 entry to the olfactory bulbs and brain.

KEYWORDS
ACE2, chronic rhinosinusitis, COVID-19, eosinophil, olfactory neuroepithelium, SARS-CoV-2, TMPRSS2

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FIGURE 1 Expression of SARS-CoV-2 entry proteins (ACE2 and TMPRSS2) and OMP in olfactory mucosa in CRSwNP patients vs. healthy controls. Controls consisted in patients undergoing nasal corrective surgery due to non-inflammatory obstructive nasal pathology or undergoing pituitary surgery for benign diseases or olfactory dysfunction. (A) Representative cross sections through the olfactory neuroepithelium (ONE) probed with the following specific human antibodies: goat polyclonals anti-ACE2 (R&D Systems #AF933, red channel) and anti-Hsp25 (Proteintech #18284-1-AP, green channel) in a control case and in a CRSwNP patient. Nuclei were stained with DAPI. A higher magnification of the outlined area is shown as an inset. Merging confocal images (63×) from DAPI, ACE2, and Hsp25 reveal that cell bodies of the sustentacular cells contain most of the ACE2 protein, being decreased in CRSwNP patient, scale bar, 50 μm. (B) Representative cross sections through the ONE probed with antibodies against ACE2 (red channel) and against OMP (Wako Fujifilm #544-10001, green channel) in a control case and in a CRSwNP patient. Nuclei were stained with DAPI. A higher magnification of the outlined area is shown as an inset. Merging images (20×) from ACE2 and OMP reveal that ORNs contain OMP but not ACE2, being decreased in CRSwNP patient, scale bar, 100 μm. (C) Quantification of thickness and OD of ACE2 immunoreactivity in the ONE from CRSwNP patients and healthy controls showing a significant decrease in CRSwNP patients. (D) Representative Western blotting images confirming a decreased ACE2 protein expression in olfactory mucosa tissue homogenate of CRSwNP patients. GAPDH was used as a housekeeping protein. (E and F) Quantification of thickness and OD of Hsp25 and OMP immunoreactivity, respectively, in the ONE from CRSwNP patients and in control cases showing a significant decrease in CRSwNP patients. (G) mRNA expression levels, as revealed by RT-PCR, of genes encoding for SARS-CoV-2 entry protein (ACE2 and TMPRSS2) in olfactory mucosa from CRSwNP patients and control cases. Relative expression levels were normalized to controls expression (100%) using GADPH and β-actin as housekeeping genes. Statistical analysis of RT-PCR results for group comparisons (CRSwNP vs controls) was performed using the non-parametric Mann-Whitney U test. Non-paired Student’s t test was applied for comparisons in the immunohistochemistry studies. Data are expressed as mean ± SEM; ‘p < 0.05, **p < 0.01 vs control cases. ACE2, angiotensin-converting enzyme 2; CRSwNP, chronic rhinosinusitis with nasal polyps; DAPI, 4′,6-diamidino-2-phenylindole; GAPDH, gliceraldehyde-3-phosphate dehydrogenase; Hsp25, heat shock protein-25; OMP, olfactory marker protein; OD, integrated optical density; ORN, olfactory receptor neuron; SEM, standard error of the mean; TMPRSS2, transmembrane protease serine-2
LETTER TO THE EDITOR

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CONFLICT OF INTEREST

Dr. Alobid has consulted for Sanofi, Novartis, GlaxoSmithKline, Menarini, and Mylan. Dr. Mullol has participated in advisory boards for, or participated in speakers’ bureaus for AstraZeneca, Genentech, GlaxoSmithKline, Glenmark, Menarini, Mitsubishi-TanabePharma, MSD, Viatrius (Mylan-Meda Pharmaceuticals), Novartis, Procter & Gamble, Regeneron Pharmaceuticals, Inc., Sanofi-Genzyme, UCB Pharma, and UriachGroup. Dr. Marin, Dr. Tubita, Dr. Langdon, M. Fuentes, M.J. Rojas-Lechuga, and Dr. Valero declare that they have no relevant conflict of interest.

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FIGURE 2. Infiltration of eosinophils in the olfactory mucosa from CRSwNP patients vs. healthy controls. (A) Quantification of the number of eosinophils (BMK13+ cells, mouse monoclonal anti-BMK13, Milipore #CBL419). A significant increase in the number of eosinophils was observed in CRSwNP patients when compared to controls. Non-paired Student’s t test was applied for comparisons. Data are expressed as mean ± SEM; *p < 0.05 vs. control cases. (B) Representative cross sections from olfactory mucosa probed with anti-BMK13 antibody, scale bar 100 μm. CRSwNP, chronic rhinosinusitis with nasal polyps; BMK13, anti-eosinophil major basic protein antibody, clone BMK13; SEM, standard error of the mean

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.