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ACE2 & TMPRSS2 Expressions in Head & Neck Tissues: A Systematic Review.

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Abstract:

Objective: To review the data about the angiotensin converting enzyme-2 (ACE2) and transmembrane protease serine-2 (TMPRSS2) expressions in head and neck tissues.

Methods: Scopus, Cochrane Library and PubMed/MEDLINE were searched by four independent investigators for studies investigating ACE2 or TMPRSS2 expressions in head and neck tissues. The following outcomes were considered: sample origin (animal *versus* human); detection method; anatomical location and cell types. PRISMA checklist and modified population, intervention, comparison, outcome, timing and setting (PICOTS) framework were used to perform the review.

Results: Of the 24 identified studies, 17 met our inclusion criteria. Thirteen studies were conducted during the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic. ACE2 and TMPRSS2 were expressed in oral, pharyngeal, sinusal and nasal human mucosa. The following cell types expressed ACE2: basal, apical, goblet, minor salivary, and endothelial cells. TMPRSS2 was found in goblet and apical respiratory cells. ACE2 and TMPRSS2 were found in olfactory region, especially in sustentacular non-neural and neural stem cells. Animal studies suggested that ACE2 expression may vary regarding age. There was an important heterogeneity between studies about the methods used to detect ACE2 and TMPRSS2, leading to a potential detection bias.

Conclusion: The SARS-CoV-2 receptors, ACE2 and TMPRSS2, are both expressed in many head and neck tissues, enabling the virus entry in the host organism.

Key words: COVID-19; coronavirus; ACE2; TMPRSS2; receptor; virus; entry; head; neck.

Introduction

The renin angiotensin aldosterone system is one of the most important systems regulating the homeostasis of cardiovascular and pulmonary functions, which involves many molecules as the angiotensin converting enzyme-2 (ACE2).¹ ACE2 is also known to be the functional receptor of some coronavirus species as firstly found in 2003 during the epidemic of severe acute respiratory syndrome coronavirus (SARS-CoV).² The current pandemic of coronavirus disease 2019 (COVID-19) has brought to light the importance of ACE2 regarding the host contamination, the virus spread and the development of the clinical COVID-19.³ In the same time, another SARS-CoV2 receptor has been identified: the transmembrane protease serine -2 (TMPRSS2).⁴

ACE2 and TMPRSS2 tissue expressions are particularly important to identify the virus entry pathways and to better understand the organ-related clinical expression of the disease.^{3,4} According to the recent data reporting otolaryngological clinical pictures of the disease,⁵⁻⁷ the study of ACE2 and TMPRSS2 expressions in ear, nose and throat tissues makes particularly sense.

The aim of this systematic review is to summarize the current data about the ACE2 and TMPRSS2 expressions in head & neck tissues.

Methods

The review was conducted regarding the Preferred Reporting Items for a Systematic Review and Meta-analysis (PRISMA) checklist.⁸ A modified population, intervention, comparison, outcome, timing and setting (PICOTS) framework was used to structure the review process.⁹ Precisely, the PICOTS structure was kept but adapted to experimental/basic research studies on human and animal tissues.

Studies: Animal and human experimental published studies in English-language peer-reviewed journals were considered. Preprint studies were also considered regarding the current pandemic and the need to rapidly get substantial data. Investigators needed to assess ACE2 or TMPRSS2 expressions in head & neck tissues through immunochemistry (IHC), *in situ* hybridization, Western Blot, RNA sequencing (RNA-seq), or reverse transcription polymerase chain reaction (RT-PCR).

Participants and inclusion criteria: The papers had to include human, monkey or mouse subjects to analyze ACE2 or TMPRSS2 tissue expressions. The authors extracted substantial information about the sample characteristics (species) and ACE2 and TMPRSS2 identification method.

Outcomes: The main studied outcome was the tissue expression of ACE2 and TMPRSS2. The anatomical location; the types of cells that expressed both receptors were studied. A particular attention has been done on the method used to detect ACE2-TMPRSS2 in tissues. Additional useful information, such as virus impact on the functioning of the tissue/cell that expressed receptor or inter-individual differences, were collected.

Intervention and comparison: Because the aim of the study was to investigate the tissue ACE2 expression, we did not consider potential intervention on patient or animal models.

Timing:

Timing and Setting: In case of receptor analysis in infected subjects, any timing in the disease process was considered.

Search strategy

The PubMed/MEDLINE, Scopus and Cochrane search was conducted by 4 independent authors (JRL, TR, CCH, GD) to identify papers published between January 1990 and April 2020. The authors screened publications with database abstracts and available full texts referring to the condition. The following keywords were used for the search strategy: ‘ACE2’; ‘TMPRSS2’; ‘COVID-19’; ‘coronavirus’; ‘Receptor’; ‘Head’; ‘Neck’; ‘Nasal’; ‘ear, nose throat (ENT)’; ‘Tissue’ and ‘Cell’. The authors investigated papers for number of samples/individuals, study type & design, inclusion criteria and ACE2/TMPRSS2 detection outcomes.

Results:

The electronic search identified 24 papers, of which 17 met our inclusion criteria (Table 1).¹⁰⁻²⁶ A total of 16 studies investigated the expression of ACE2 and TMPRSS2 on human head and neck tissues, while 5 and 2 researches focused on mouse and monkey samples (Table 1). The chart flow of the study is available in Figure 1. One study focused on ACE2 genetic analysis, without anatomical expression report.¹⁷ Three studies were preprint.^{15,18,20}

Tissue expression in Human

ACE2 expression

ACE2 was studied in 16 studies.¹¹⁻²⁶ The expression of ACE2 was found in all mucosa of the respiratory upper tract, including trachea,^{12,13,20,22,25} sinus and nasal cavities.^{11,16,20} Among the respiratory mucosa, ACE2 was expressed in several types of cells, including epithelial, goblet,

and endothelial cells.^{11,12,26} One study reported that ACE2 was expressed on ciliated epithelial cells and not on non-ciliated (goblet) cells.²⁰ Butowt *et al.* compared the intensity of expression of both ACE2 in human respiratory tract regarding the cell localization (upper *versus* lower airway).¹⁶ They found that nasal epithelial cells had lower levels of ACE2 expression compared with epithelial cells of the lower respiratory tract.¹⁶ Among the nasal region, two studies investigated the ACE2 expression in the mucosa of the olfactory region, including olfactory bulb.^{15,16} ACE2 receptor was identified in sustentacular/non-neuronal cells of the olfactory tissues. Moreover, ACE2 was found in a low proportion of neuronal stem cells of the olfactory bulb.^{15,16} The expression of ACE2 in olfactory neurons (non-stem cells) remains uncertain because Butowt *et al.* and Brann *et al.* observed that ACE2 has only non-neuronal expression pattern in olfactory epithelium.^{15,16}

Five studies investigated ACE2 expression in oral and pharyngeal regions, including oral, hypo- oro- and nasopharyngeal spaces.^{11,19,23-25} The study that explored ACE2 expression in human nasopharynx,¹⁹ did not exhibit significant ACE2 immunostaining in nasopharyngeal cells.¹⁹ ACE2 receptor was identified in oral endothelial,¹¹ epithelial,^{11,23,25} and salivary²⁴ cells. Xu *et al.* found that ACE2 was also expressed in T and B cells as well as fibroblasts of the oral cavity.²³ Moreover, ACE2 was expressed in major salivary gland tissues¹⁸ and thyroid tissue.²¹ In many publications, authors reported the type of cells (goblet *versus* epithelial *versus* stem cells) that expressed ACE2 or TMPRSS2 (Table 2).

The genetic analysis of Cao *et al.* reported that there are 15 unique expression quantitative trait loci variants in East Asian population, supporting a gene polymorphism and tissue-related differences between individuals.¹⁷

TMPRSS2 expression

TMPRSS2 expression was investigated in 5 studies.^{10,15,16,22,26} Similarly to ACE2, TMPRSS2 was identified in nasal^{16,22,26} and respiratory mucosa cells,²² including both epithelial and goblet cells, with higher expression in lower airway compared with upper airway.²² Moreover, TMPRSS2 receptor was identified in sustentacular and neuronal olfactory cells^{15,16} but not in olfactory neurons.¹⁵ TMPRSS2 was also identified in salivary major gland tissue.¹⁰

ACE2 & TMPRSS2 tissue expression in Mouse and Monkey

Six studies used animal models to assess ACE2 or TMPRSS2 expressions in head and neck tissues.^{10,13-16,26} The mouse studies of Butowt *et al.* and Bilinska *et al.* revealed that that elderly mice had higher expression of both ACE2 and TMPRSS2 in nasal mucosa

compared with young specimen.^{14,15} In olfactory tissue, ACE2 was identified in sustentacular/non-neuronal and neural stem cells of mice.¹⁴⁻¹⁶ Liu *et al.* analyzed ACE2 expression in monkey,¹³ reporting a higher ACE2 expression in tracheal, naso-, oro- and hypopharyngeal tissues as well as in the salivary ducts of the pharyngeal gland, and, therefore, in saliva. In this study, the cell expression was mainly localized in lamina propria. In the same vein, Vaarala *et al.* reported TMPRSS2 expression in mouse salivary tissues.¹⁰

Cell Detection methods

The following methods have been used for detecting ACE2 and TMPRSS2 in cells of human and animal tissues: RNAseq (N=12); IHC (N=5); RT-PCR (N=1); in situ hybridization (N=1) and WB (N=1). Different detection approaches were used in 2 studies.^{12,14} One study reported specific genetic analyzes.¹⁷ There were significant differences about XX, XX, and XX regarding the following methods: XX, XX, XX.(Geraldine part)

Discussion:

The COVID-19 infection may present several clinical forms from 'benign' isolated anosmia to severe multiple organ failure. The mechanisms underlying the COVID-19 polymorphism are still unknown. To infect tissues, SARS-CoV2 needs to entry into the cells, which is allowed through ACE2 and TMPRSS2 receptors.⁴ The identification of virus receptor expression in the tissues makes particularly sense to better understand the clinical expression of the disease. This systematic review sheds light many points.

First, ACE2 and TMPRSS2 receptors are overall expressed in epithelial and non-epithelial cells of head and neck tissues. The head and neck expression may support the otolaryngological clinical picture of the disease, which was recently found in European and U.S. COVID-19 patients.^{6,7,27,28} The virus would entry into the epithelial cells of the upper aerodigestive tract mucosa, leading to an inflammatory reaction and the development of the otolaryngological symptoms. The nasal entrance of the virus through high ACE2 expression was supported in the study of Wu *et al.* who found a higher virus concentration in nasal swabs compared with throat swabs.²⁵

The olfactory cleft is a nasal region that drawn attention of many researchers over the past few weeks. Indeed, recent data supported that more than 70% of COVID-19 patients developed olfactory dysfunction, especially when patients suffered from mild-to-moderate forms of the disease.^{5,7,28,29} Because ACE2 and TMPRSS2 are both expressed in the nasal mucosa of the olfactory cleft, the entry way of the virus into the olfactory bulb seems obvious.

Once in the bulb, according to some human studies,^{15,16} the virus could infect cells that express ACE2 or TMPRSS2, namely glial and neuronal stem cells. The raise of glial cells, which are important in the neuron support, could overall impair the functioning of the olfactory way, leading to transient olfactory dysfunction.^(Julien: ok ? References?) Because the duration of glial cell recovery is shorter than that of neurons, the duration to clinically recover the smell sense would be shorter. In clinical studies, the recovery time of olfaction ranged from 4 to 14 days in more than 40% of patients,^{5,7,28,30} which supports this pathophysiological hypothesis. An additional pathophysiological mechanism that could explain the early recovery of some patients is the anosmia related to edema of the olfactory cleft mucosa. As observed in a recent imaging study, some patients had bilateral olfactory cleft edema, which was associated with olfactory dysfunction.³¹ However, the study also support that the majority of patients did not exhibit such findings over the clinical course of the disease, strengthening the ‘neurological’ anosmia. Interestingly, in another study, we reported magnetic resonance imaging abnormalities in the olfactory bulb of anosmic COVID-19 patients.³² These additional findings also support the neurological hypothesis of olfactory dysfunction.

In this systematic review, we found that three studies reported high ACE2 expression in major or minor salivary gland human tissues.^{10,18,24} These data corroborate the literature findings that reported a salivary pattern of SARS-CoV-2 and related parotitis.^{33,34} Moreover, the virus spread into the salivary gland tissues allows to better understand the mechanisms underlying the saliva contamination. Interestingly, in 2011, Liu *et al.* observed that Monkeys infected by SARS-CoV had a salivary virus spread, which was associated with a salivary virion excretion.¹³ These data support the need to conduct future studies investigating the presence of SARS-CoV-2 in the saliva of infected human and to corroborate the saliva findings with the ACE2 salivary gland expression.

The ENT expression of ACE2 and TMPRSS2 and the related ENT clinical pattern seems obvious but could vary across individuals and populations. Furthermore, Lee *et al.* observed that ACE2 expression is influenced by patient demographics, clinical characteristics, comorbidities and medication.²⁰ As reported in the genetic analysis of Cao *et al.*, there would be different susceptibilities or responses to SARS-CoV2 in different populations.¹⁷ The polymorphism of ACE2 and TMPRSS2 expression could explain the clinical differences between individuals. Indeed, many physicians reported in clinical and epidemiological studies different clinical presentation of the disease,^{28,30} which could be associated with virus

mutations.^{35,36} The virus mutations and the related impact on receptor binding and infectivity is another point that has to be considered in future studies.

The present study has several limitations. First, the heterogeneity between studies about the detection method may lead to detection bias because some approaches are more sensitive than others. (Geraldine + une ou deux phrases sur la qualité de marquage et les étapes nécessaires pour obtenir un bon marquage? Ont ils été trop vite? Quid de la qualité de leur marquage)

Second, the majority of studies that were conducted during the SARS-CoV-2 pandemic did not consider many demographic and clinical factors such as the age of patients from who the tissues were extracted, the use of ACE inhibitor drugs and its potential impact on ACE2 expression, etc. Third, some otolaryngological region remains non-investigated such as vocal folds. The investigation of these remaining regions makes sense regarding recent study reporting some unusual clinical expression of the disease such as severe dysphonia.³⁷

Conclusion:

ACE2 and TMPRSS2 are both expressed in head and neck tissues, which may explain the otolaryngological clinical pattern of the disease and the entry of SARS-CoV-2 into the host organism. Future studies considering demographical and clinical characteristics of patients from who the tissues are extracted are needed to better understand the cell entry mechanisms of SARS-CoV-2.

References:

1. Crackower MA, Sarao R, Oudit GY, et al. Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature*. 2002; 417(6891):822-8.
2. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, Choe H, Farzan M. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature*. 2003; 426(6965):450-4.
3. Li Y, Zhou W, Yang L, You R. Physiological and pathological regulation of ACE2, the SARS-CoV-2 receptor. *Pharmacol Res*. 2020; 157:104833. doi: 10.1016/j.phrs.2020.104833.
4. Shang J, Wan Y, Luo C, Ye G, Geng Q, Auerbach A, Li F. Cell entry mechanisms of SARS-CoV-2. *Proc Natl Acad Sci U S A*. 2020. pii: 202003138. doi: 10.1073/pnas.2003138117.
5. Vaira LA, Hopkins C, Salzano G, et al. Olfactory and gustatory function impairment in COVID-19 patients: An Italian objective multicenter-study. *Head Neck*. Doi: 10.1002/hed.26269.
6. Lechien JR, Chiesa-Estomba CM, Place S, Van Laethem Y, Cabaraux P, Mat Q, Huet K, Plzak J, Horoi M, Hans S, Barillari MR, Cammaroto G, Fakhry N, Martiny D, Ayad T, Jouffe L, Hopkins C, Saussez S; COVID-19 Task Force of YO-IFOS. Clinical and Epidemiological Characteristics of 1,420 European Patients with mild-to-moderate Coronavirus Disease 2019. *J Intern Med*. 2020. doi: 10.1111/joim.13089.
7. Lechien JR, Chiesa-Estomba CM, De Siaty DR, et al. Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): a multicenter European study. *Eur Arch Otorhinolaryngol*. 2020. doi: 10.1007/s00405-020-05965-1.
8. McInnes MDF, Moher D, Thombs BD, et al. Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies: The PRISMA-DTA Statement. *JAMA*. 2018;319(4):388-396. doi:10.1001/jama.2017.19163
9. Thompson M, Tiwari A, Fu R, Moe E, Buckley DI. *A Framework To Facilitate the Use of Systematic Reviews and Meta-Analyses in the Design of Primary Research Studies*. Rockville (MD): Agency for Healthcare Research and Quality (US); 2012. <http://www.ncbi.nlm.nih.gov/books/NBK83621/>. Accessed February 22, 2020.
10. Vaarala MH, Porvari KS, Kellokumpu S, Kyllönen AP, Vihko PT. Expression of transmembrane serine protease TMPRSS2 in mouse and human tissues. *J Pathol*. 2001; 193(1):134-40. doi: 10.1002/1096-9896(2000)9999:9999
11. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol*. 2004; 203(2):631-7. doi: 10.1002/path.1570.
12. Jia HP, Look DC, Shi L, et al. ACE2 receptor expression and severe acute respiratory syndrome coronavirus infection depend on differentiation of human airway epithelia. *J Virol*. 2005; 79(23):14614-21. doi: 10.1128/JVI.79.23.14614-14621.2005.
13. Liu L, Wei Q, Alvarez X, et al. Epithelial cells lining salivary gland ducts are early target cells of severe acute respiratory syndrome coronavirus infection in the upper respiratory tracts of rhesus macaques. *J Virol*. 2011; 85(8):4025-30. doi: 10.1128/JVI.02292-10.
14. Bilinska K, Jakubowska P, Von Bartheld CS, Butowt R. Expression of the SARS-CoV-2 Entry Proteins, ACE2 and TMPRSS2, in Cells of the Olfactory Epithelium: Identification of Cell Types and Trends with Age. *ACS Chem Neurosci*. 2020. doi: 10.1021/acchemneuro.0c00210.
15. Brann DH, Tsukahara T, Weinreb C et al. Non-neuronal expression of SARS-CoV-2 entry genes in the olfactory system suggests mechanisms underlying COVID-19-associated anosmia. Preprint. <https://doi.org/10.1101/2020.03.25.009084>.

16. Butowt R, Bilinska K. SARS-CoV-2: Olfaction, Brain Infection, and the Urgent Need for Clinical Samples Allowing Earlier Virus Detection. *ACS Chem Neurosci*. 2020; 11(9):1200-1203. doi: 10.1021/acchemneuro.0c00172.
17. Cao Y, Li L, Feng Z, Wan S, Huang P, Sun X, Wen F, Huang X, Ning G, Wang W. Comparative genetic analysis of the novel coronavirus (2019-nCoV/SARS-CoV-2) receptor ACE2 in different populations. *Cell Discov*. 2020; 6:11. doi: 10.1038/s41421-020-0147-1.
18. Chen R, Wang K, Yu J et al. The spatial and cell-type distribution of SARS-CoV-2 receptor ACE2 in human and mouse brain. Preprint. <https://doi.org/10.1101/2020.04.07.030650>.
19. Hikmet
20. Lee IT, Nakayama T, Wu CT, et al. Robust ACE2 protein expression localizes to the motile cilia of the respiratory tract epithelia and is not increased by ACE inhibitors or angiotensin receptor blockers. Preprint. <https://doi.org/10.1101/2020.05.08.20092866>.
21. Li MY, Li L, Zhang Y, Wang XS. Expression of the SARS-CoV-2 Cell Receptor Gene ACE2 in a Wide Variety of Human Tissues. *Infect Dis Poverty*, 2020; 9(1):45. doi: 10.1186/s40249-020-00662-x.
22. Sungnak W, Huang N, Bécavin C, et al. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nat Med*. 2020; 26(5):681-687. doi: 10.1038/s41591-020-0868-6.
23. Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X, Li T, Chen Q. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. *Int J Oral Sci*. 2020; 12(1):8. doi: 10.1038/s41368-020-0074-x.
24. Xu J, Li Y, Gan F, Du Y, Yao Y. Salivary Glands: Potential Reservoirs for COVID-19 Asymptomatic Infection. *J Dent Res*. 2020: 22034520918518. doi: 10.1177/0022034520918518.
25. Wu
26. Ziegler CGK, Allon SJ, Nyquist SK, et al. SARS-CoV-2 Receptor ACE2 Is an Interferon-Stimulated Gene in Human Airway Epithelial Cells and Is Detected in Specific Cell Subsets across Tissues. *Cell*. 2020: S0092-8674(20)30500-6. doi: 10.1016/j.cell.2020.04.035.
27. Kaye R, Chang CWD, Kazahaya K, Brereton J, Denny JC 3rd. COVID-19 Anosmia Reporting Tool: Initial Findings. *Otolaryngol Head Neck Surg*. 2020: 194599820922992. doi: 10.1177/0194599820922992.
28. Lechien JR, Chiesa-Estomba CM, Hans S, Barillari MR, Jouffe L, Saussez S. Loss of Smell and Taste in 2,013 European Mild-to-Moderation COVID-19 Patients. Accepted for publication in *Ann Int Med*. 2020.
29. Vaira LA, Salzano G, Deiana G, De Riu G. Anosmia and Ageusia: Common Findings in COVID-19 Patients. *Laryngoscope*. 2020: 10.1002/lary.28692. doi: 10.1002/lary.28692.
30. Spinato G, Fabbris C, Polesel J, Cazzador D, Borsetto D, Hopkins C, Boscolo-Rizzo P. Alterations in Smell or Taste in Mildly Symptomatic Outpatients With SARS-CoV-2 Infection. *JAMA*. 2020: e206771. doi: 10.1001/jama.2020.6771.
31. Lechien JR, Michel J, Radulesco T, et al. Clinical and Radiological Evaluations of COVID-19 Patients with Anosmia: Preliminary Report. Submitted. 2020
32. Chetrit A, Lechien JR, Chekkoury-Idrissi Y, et al. Magnetic Resonance Imaging of Olfactory Structures in COVID-19 Patients: Preliminary Report. Submitted for publication.
33. Lechien JR, Chetrit A, Chekkoury-Idrissi Y et al. Parotitis-like Symptoms associated with COVID-19 disease, France, March-April, 2020. *Emerg Infect Disease*. 2020.
34. Acute Parotitis: A Possible Precocious Clinical Manifestation of SARS-CoV-2 Infection? *Otolaryngol Head Neck Surg*. 2020:194599820926992. doi: 10.1177/0194599820926992.

35. Coppee F, Lechien JR, Decleves AE, Tafforeau L, Saussez S. SARS-CoV-2: virus mutations in specific European populations. *New Micr New Infec.* 2020. <https://doi.org/10.1016/j.nmni.2020.100696>
36. Saha P, Banerjee AK, Tripathi PP, Srivastava AK, Ray U. A virus that has gone viral: amino acid mutation in S protein of Indian isolate of Coronavirus COVID-19 might impact receptor binding, and thus, infectivity. *Biosci Rep.* 2020; 40(5):BSR20201312. doi: 10.1042/BSR20201312.PMID: 32378705
37. Lechien JR, Chiesa-Estomba CM, Cabaraux P, et al. Features of Mild-to-Moderate COVID-19 Patients with Dysphonia. *J Voice*, 2020.

Table 1: Studies Reporting ACE2 or TMPRSS2 Head and Neck Expression.

Table 1 footnotes: Abbreviations: ACE2=Angiotensin Converting Enzyme-2; Immunohistochemistry=IHC; RT-PCR=reverse transcription polymerase chain reaction; SARS-CoV-2= severe acute respiratory syndrome coronavirus-2; TMPRSS2: transmembrane protease serine-2; WB=Western Blotting.

Table 2: Summary of Cell Expression of ACE2 and TMPRSS2.

Table 2 footnotes: Abbreviations: ACE2=Angiotensin Converting Enzyme-2; NA=not available; TMPRSS2: transmembrane protease serine-2.

Figure 1: Chart flow.

Figure 1 footnotes: Abbreviations: ACE2=Angiotensin Converting Enzyme-2; TMPRSS2: transmembrane protease serine-2.