

# Olfactory dysfunction in COVID-19 patients: observational cohort study and systematic review

Tom Wai-Hin Chung, MRCP(UK) <sup>a\*</sup>; Siddharth Sridhar, FRCPATH <sup>a,b,c\*</sup>; Anna Jinxia Zhang, PhD <sup>a\*</sup>; Kwok-Hung Chan, PhD <sup>a\*</sup>; Hang-Long Li, BSc <sup>d</sup>; Fergus Kai-Chuen Wong, FRCSEd(ORL) <sup>e</sup>; Ming-Yen Ng, MD <sup>f</sup>; Raymond King-Yin Tsang, MS <sup>g</sup>; Andrew Chak-Yiu Lee, PhD <sup>a</sup>; Zhimeng Fan, MSc <sup>a</sup>; Ronnie Siu-Lun Ho, FHKCPATH <sup>h</sup>; Shiobhon Yiu Luk, FRCR <sup>i</sup>; Wai-Kuen Kan, MBChB <sup>i</sup>; Sonia Hiu-Yin Lam, FRCR <sup>j</sup>; Alan Ka-Lun Wu, FRCPATH <sup>k</sup>; Sau-Man Leung, MSc <sup>a</sup>; Wai-Ming Chan, MD <sup>l</sup>; Pauline Yeung Ng, MBBS <sup>l,m</sup>; Kelvin Kai-Wang To, MD <sup>a,b,c</sup>; Vincent Chi-Chung Cheng, MD <sup>a</sup>; Kwok-Cheung Lung, FRCP(Edin) <sup>n</sup>; Professor Ivan Fan-Ngai Hung, MD <sup>b,m</sup>; Professor Kwok-Yung Yuen, MD <sup>a,b,c,o</sup>

<sup>a</sup> Department of Microbiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China; <sup>b</sup> State Key Laboratory of Emerging Infectious Diseases, The University of Hong Kong, Hong Kong, China; <sup>c</sup> Carol Yu Centre for Infection, The University of Hong Kong, Hong Kong, China; <sup>d</sup> Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China; <sup>e</sup> Department of Ear, Nose and Throat Surgery, Pamela Youde Nethersole Eastern Hospital, Hong Kong, China; <sup>f</sup> Department of Diagnostic Radiology, The University of Hong Kong, Hong Kong, China; <sup>g</sup> Division of Otolaryngology, Department of Surgery, The University of Hong Kong, Hong Kong, China; <sup>h</sup> Department of Pathology, Queen Mary Hospital, Hong Kong, China; <sup>i</sup> Department of Diagnostic Radiology, Pamela Youde Nethersole Eastern Hospital, Hong Kong, China; <sup>j</sup> Department of Diagnostic Radiology, Queen Mary Hospital, Hong Kong, China; <sup>k</sup> Department of Clinical Pathology, Pamela Youde Nethersole Eastern Hospital, Hong Kong,

© The Author(s) 2020. Published by Oxford University Press on behalf of Infectious Diseases Society of America.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact [journals.permissions@oup.com](mailto:journals.permissions@oup.com)

China; <sup>l</sup> Department of Adult Intensive Care, Queen Mary Hospital, The University of Hong Kong, Hong Kong, China; <sup>m</sup> Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China; <sup>n</sup> Department of Medicine, Pamela Youde Nethersole Eastern Hospital, Hong Kong, China; <sup>o</sup> The Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The University of Hong Kong, Hong Kong, China

\*contributed equally

Accepted Manuscript

## Correspondence:

(1) Corresponding author: Kwok-Yung Yuen, Carol Yu Centre for Infection, State Key Laboratory of Emerging Infectious Diseases, Department of Microbiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Queen Mary Hospital, Pokfulam, Hong Kong. Phone: (852) 22552378. Fax: (852) 28724555. E-mail: [kyyuen@hku.hku.hk](mailto:kyyuen@hku.hku.hk)

(2) Alternate corresponding author: Tom Wai-Hin Chung, Department of Microbiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Queen Mary Hospital, Pokfulam, Hong Kong. Phone: (852) 22552378. Fax: (852) 28724555. E-mail: [tomchungwaihin@gmail.com](mailto:tomchungwaihin@gmail.com)

Accepted Manuscript

## Summary:

COVID-19-related olfactory dysfunction is common (67%). Recovery can be slow (>3 weeks). Sinusitis and olfactory cleft obstruction were absent in most patients. Immunohistochemical analysis of nasal biopsy revealed the presence of infiltrative CD68+ macrophages expressing SARS-CoV-2 antigen in the stroma.

Accepted Manuscript

## **Abstract**

### *Background*

Olfactory dysfunction (OD) has been reported in coronavirus disease 2019 (COVID-19). However, there are knowledge gaps about the severity, prevalence, etiology, and duration of OD in COVID-19 patients.

### *Methods*

Olfactory function was assessed in all participants using questionnaires and the butanol threshold test (BTT). COVID-19 patients with abnormal olfaction were further evaluated using the smell identification test (SIT), sinus imaging and nasoendoscopy. Selected patients received nasal biopsies. Systematic review was performed according to PRISMA guidelines. PubMed items from 1<sup>st</sup> January 2020 to 23<sup>rd</sup> April 2020 were searched. Studies which reported clinical data on olfactory disturbances in COVID-19 patients were analyzed.

### *Results*

We included 18 COVID-19 patients and 18 controls. Among COVID-19 patients, 12/18 (67%) reported olfactory symptoms and OD was confirmed in 6 patients by BTT and SIT. OD was the only symptom in two patients. Mean BTT score of patients was worse than controls (p-value = 0.004, difference in means = 1.8; 95% CI: 0.6 – 2.9). Sinusitis and olfactory cleft obstruction were absent in most patients. Immunohistochemical analysis of nasal biopsy revealed the presence of infiltrative CD68+ macrophages harboring SARS-CoV-2 antigen in the stroma. OD persisted in two patients despite clinical recovery. Systematic review showed that the prevalence of olfactory disturbances in COVID-19 ranged from 5% to 98%. Most studies did not assess olfaction quantitatively.

## *Conclusion*

OD is common in COVID-19 and may be the only symptom. COVID-19-related OD can be severe and prolonged. Mucosal infiltration by CD68+ macrophages expressing SARS-CoV-2 viral antigen may contribute to COVID-19-related OD.

**Keywords:** COVID-19; SARS-CoV-2; anosmia; smell impairment; olfactory dysfunction

Accepted Manuscript

## Introduction

Coronavirus disease 2019 (COVID-19) was declared a global pandemic by the World Health Organization on 11<sup>th</sup> March 2020. COVID-19 is caused by a novel betacoronavirus: severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 is highly adapted to humans and is capable of efficient person-to-person transmission.<sup>1</sup> As of 11<sup>th</sup> May 2020, COVID-19 has already infected 4 million people worldwide, with over 270,000 associated deaths.<sup>2</sup> Due to inadequacies in testing programs and large proportions of mild infections, the exact community burden of COVID-19 is unknown and will require population-wide seroepidemiological studies for definitive elucidation.

The common symptoms of COVID-19 include fever, cough, dyspnea, malaise, myalgia, and gastrointestinal disturbances.<sup>3</sup> In addition, olfactory dysfunction (OD) or anosmia has been reported in COVID-19 patients.<sup>4-6</sup> The majority of studies investigating OD in COVID-19 have utilized retrospective questionnaires or self-reported symptoms by patients.<sup>7-9</sup> These methods of assessment are prone to reporting bias and might fail to identify subtle OD. Therefore, objective measurements of olfactory function in COVID-19 patients are required. Furthermore, olfactory function in COVID-19 patients has not been compared to healthy controls, which is necessary to prove that olfaction in COVID-19 patients is compromised. A systematic assessment of the severity, prevalence, etiology, and duration of OD in COVID-19 patients is also lacking.

In this study, we quantitated olfactory function in COVID-19 patients and compared them to healthy controls. We also evaluated the severity, prevalence, etiology, and duration of OD in COVID-19 patients. In addition, a systematic review of the existing literature on olfactory disturbances in COVID-19 patients was performed.

## Methods

### *Study setting*

The study was conducted in Queen Mary Hospital and Pamela Youde Nethersole Eastern Hospital in Hong Kong. Both institutions are designated referral centers providing acute care to COVID-19 patients. Ethics approval for this study was obtained from the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (UW 20-310).

### *Patients & controls*

Patients between 18 and 60 years of age with mild COVID-19 infection (defined as not requiring supplemental oxygen therapy) who were consecutively admitted to either institutions between 6<sup>th</sup> April to 9<sup>th</sup> April 2020 were eligible for inclusion. All patients received standard care during hospitalization. Their demographic characteristics, clinical features and laboratory results were retrieved from the electronic patient records. COVID-19 patients were compared to a control group in a 1:1 ratio. The control group comprised of healthy volunteers who were students or healthcare workers who reported no recent symptoms of upper respiratory tract infection, and were age-matched to the patient group  $\pm$  5 years to control for the potential effect of age on olfaction. COVID-19 patients and controls were excluded if they had an underlying history of allergic rhinitis or nasal polyps, which might confound the results of olfactory assessment. All participants completed a detailed questionnaire relating to olfaction (see Supplementary Material) as previously described with modifications.<sup>10</sup> This was followed by functional olfactory assessment.

### *Quantitative assessment of olfactory function*

The butanol threshold test (BTT) was used as the screening method for quantitation of olfactory function in all participants. BTT was performed as previously described with modifications.<sup>11</sup> Briefly, 99.9% 1-butanol AnalaR NORMAPUR® (VWR International limited, Briare, France) was diluted in deionized water to produce a range of 10 serial dilutions (see Supplementary Table 1), from 0.00002% (step 10) to 4% (step 1). Identical bottles containing deionized water were used as controls. All solutions were colorless. During the assessment, the test subjects were blindfolded. Each nostril was tested independently, starting from the lowest concentration (0.00002% 1-butanol, step 10). Each concentration was tested along with a control in a forced-choice paradigm, in which the participant was required to differentiate between the test solution and control. The concentration step at which the participant was able to correctly differentiate between the test solutions and controls over four consecutive trials was the BTT score. The averaged scores of both nostrils was the participant's final BTT score. If the participant failed to identify the highest concentration (4% 1-butanol, step 1), a BTT score of zero was given. Mean BTT scores of COVID-19 patient and control groups were compared.

For COVID-19 patients who reported impaired olfaction at the time of study entry and received a BTT score of less than four, the Smell Identification Test™ (SIT; Sensonics International, New Jersey, USA) was performed as a confirmatory and discriminatory test of olfactory function.<sup>12</sup> The SIT consisted of 40 test odorants. The test score was the total of all correct answers. The SIT results were compared against an age-matched and gender-matched normative SIT score, which were further categorized according to severity.

Patients were defined as having OD if they met the following criteria: (a) self-reported ongoing olfactory impairment, (b) a BTT score of less than four, and (c) an abnormal SIT result.

#### *Virologic assessment and diagnosis*

COVID-19 was diagnosed by real-time reverse transcriptase–polymerase chain reaction (RT-PCR) of pooled nasopharyngeal and throat swab specimens targeting the E-gene (TIB Molbiol, Berlin, Germany) of SARS-CoV-2. Serial cycle threshold (Ct) values of SARS-CoV-2 RT-PCR were assessed from diagnosis to discharge. Other respiratory viral infections including influenza A, influenza B, respiratory syncytial virus, adenovirus, and rhinovirus/enterovirus were excluded by commercial multiplex and in-house PCR assays.

#### *Computer tomography (CT) scans*

COVID-19 patients fulfilling the definition of OD received plain CT scans of the sinuses and thorax. All scans were reviewed by a radiologist. The degree of rhinosinusitis was evaluated using the Lund-Mackay (LM) score, which is a three-point scoring system used to describe the degree of sinus opacification (no opacification = 0, partial opacification = 1, or total opacification = 2).<sup>13</sup> A combined LM score of 0 (no sinus opacification) to 24 (total opacification of all sinuses) is possible.

#### *Nasoendoscopy and nasal biopsy*

Nasoendoscopy was performed for COVID-19 patients with OD. The examination was performed at a seated and upright position at bedside. Local anaesthetic, Co-phenylcaine Forte (Mayne Pharma, North Carolina, USA) was applied to both nostrils by spray. The nasal anatomy was assessed using a 4 mm diameter 30° rigid nasal endoscope (Karl Storz SE & Co., Tuttlingen, Germany) with tele-system (TELE PACK; Karl Storz SE & Co., Tuttlingen,

Germany). Nasal biopsy was obtained at the discretion of the operating surgeon. A cotton pledget soaked with Co-phenylcaine Forte was inserted under endoscopic guidance for vasoconstriction and regional anaesthesia. Nasal biopsies were obtained at the high anterior septum, just superior to the level of the axilla of the middle turbinate, using a 2.7 mm diameter biopsy forceps (Type 8150.00; Richard Wolf GmbH, Knittlingen Germany).

#### *Histological assessment and immunofluorescence staining*

Nasal biopsy specimens were fixed in 10% formalin and embedded in paraffin blocks. 4 µm tissue sections were stained with standard haematoxylin and eosin (H&E). The presence of macrophages was confirmed by immunohistofluorescence staining with mouse anti-human CD68 (clone KP-1) (Lifespan Biosciences, WA, USA) and FITC conjugated donkey anti-mouse IgG (Jackson ImmunoResearch, PA, USA). SARS-CoV-2 nucleoprotein (NP) was detected by immunofluorescence staining using rabbit anti-SARS-CoV NP antibody or mouse anti-SARS-2 NP protein antibody as previously described.<sup>14,15</sup>

#### *Statistical analysis*

Means of BTT scores between case and control groups were compared using Student's *t* test. BTT follow-up results for COVID-19 patients with OD were compared using paired *t* test. Categorical variables were compared using the Fisher's exact test. Correlation between BTT scores and Ct values were assessed using Spearman's Rank test. Statistical analysis was performed using XLSTAT (Addinsoft, New York, USA) and Prism (GraphPad, San Diego, USA).

## *Systematic review*

Systematic review was carried out according to Preferred Reporting Items for Systematic reviews and Meta-analysis (PRISMA) guidelines.<sup>16</sup> PubMed items from 1<sup>st</sup> January 2020 to 23<sup>rd</sup> April 2020 were searched using the keywords: COVID-19, SARS-CoV-2, 2019-nCoV, or novel coronavirus. Duplicates were removed. Records were screened by the title and abstract. Two authors, TWHC and HLL, carried out screening and determined the eligibility of studies independently. Disagreements were resolved by another author (SS). The inclusion criteria included (1) study population: adult patients diagnosed with COVID-19; (2) study design: (randomized) controlled trials, case-control studies, prospective or retrospective cohort studies, case series, and case reports; (3) publications written in English. The exclusion criteria included (1) records that did not report anosmia or hyposmia or smell disturbances as symptoms; and (2) correspondences, editorials, review articles which did not contain new clinical data. For included articles, we collected basic information (authors, country, sample size, and primary outcomes), patient demographics (age and sex), the method(s) used to assess olfaction, the prevalence of abnormal olfaction reported in the article, relevant imaging and endoscopic findings, and proposed etiology of impaired olfaction.

## Results

### *Patient characteristics*

A total of 20 cases and 21 controls were assessed for study inclusion. Two COVID-19 patients and three controls were excluded due to known history of nasal polyps and allergic rhinitis, respectively. Overall, 18 cases and 18 controls were included in the study. COVID-19 patients and controls did not differ significantly in terms of age, sex, smoking status, drinking history and medical co-morbidities (table 1). One (1/18, 6%) COVID-19 patient was diagnosed with adenovirus co-infection. Other common respiratory viruses were excluded. The blood sodium levels were normal in COVID-19 patients on admission.

### *Self-reported olfactory symptoms*

Olfactory symptoms were reported by 12/18 (67%) COVID-19 patients compared to 0/18 controls ( $p < 0.001$ ). Of the 12 patients with olfactory symptoms, six reported recovered olfaction at the time of study inclusion, including the patient who was diagnosed with adenovirus co-infection. The remaining six patients had never experienced olfactory symptoms. Age, gender distribution and initial presenting symptoms of COVID-19 patients with ( $n = 12$ ) and without ( $n = 6$ ) olfactory symptoms were not significantly different. Of the twelve patients reporting olfactory symptoms, the median time from symptom onset to olfactory symptoms was 0.5 days (interquartile range; IQR: 0 to 4.75 days), while the time from onset of olfactory symptoms to study recruitment was 14 days (IQR: 10.5 to 16.25 days). Only 2/12 (17%) patients with olfactory symptoms reported concomitant rhinorrhea or nasal congestion, while 9/12 (75%) reported dysgeusia. Two patients were asymptomatic apart from impaired sense of smell.

### *Butanol threshold test (BTT)*

Quantitative olfactory function was screened for all 36 COVID-19 patients and controls by BTT. Mean BTT of COVID-19 patients was 4.1 (SD = 2.2) while that of controls was 5.9 (SD = 1.1). The mean BTT scores were significantly higher for healthy controls compared to COVID-19 patients, indicating impaired olfactory function in the latter group (figure 1A,  $p$ -value = 0.004, difference in means = 1.8 with 95% CI: 0.6 – 2.9). All six patients who reported ongoing olfactory disturbances at study entry had a BTT score < 4. One COVID-19 patient had a BTT score of 0. Young COVID-19 patients (age <22) performed worse in the BTT; their mean score was lower when compared to COVID-19 patients from other age groups ( $p$  = 0.002) as well as controls ( $p$  < 0.001). This may have been due to shorter mean duration between symptom onset and BTT in the younger age group less than 22 years of age ( $p$  = < 0.001, difference in means = 12.7 days with 95% CI: 7.5 – 17.9). Above age 22, the mean BTT score in COVID-19 patient group also tended to be lower than in control group but did not reach statistical significance (figure 1B). Admission SARS-CoV-2 RT-PCR Ct values and BTT scores were not correlated ( $p$  = 0.882).

### *Smell identification test (SIT)*

From the BTT score and history taking, six COVID-19 patients met the criteria for SIT confirmatory assessment, which showed that two patients (33%) were anosmic, two patients (33%) suffered from severe microsmia, and one patient each had moderate and mild microsmia. Their demographic, clinical and olfactory function profiles are described in table

2.

### *Radiographic assessment by CT scans*

CT sinus and thorax was performed for the six patients confirmed with OD. No radiological evidence of sinusitis was found, as represented by a LM score of  $\leq 2$ . Specifically, bilateral olfactory cleft obstructions were documented in two (33%) patients only, while partial obstruction was found in one patient. The remaining three patients (50%) had patent olfactory passages (figure 2). CT thorax revealed peripheral ground glass opacification (two patients), linear parenchymal bands (one patient), consolidative nodules (one patient), or no abnormalities (two patients).

### *Nasoendoscopy and biopsy*

Patients with OD were assessed by nasoendoscopy. No endoscopic evidence of olfactory cleft obstruction, nasal polyps, or active sinusitis were found. Nasal biopsies were performed in three patients, of which one specimen was positive for SARS-CoV-2 by RT-PCR. H&E staining of nasal biopsy samples showed minimal inflammatory changes, as represented by minor infiltrations of lymphocytes, plasma cells and occasional neutrophils in the stroma. In one of the samples, intraepithelial neutrophils were also observed (Figure 3A & B).

Immunofluorescence staining for CD68 established the presence of macrophages within the epithelium (Figure 3C) and the stroma (Figure 3D). Scattered cells positive for the SARS-CoV-2 NP protein were found in the stroma (Figure 3E). These cells were confirmed to be macrophages (Figure 3H to I).

### *Follow-up evaluation by BTT assessment*

For the six patients with OD, follow-up BTT assessments were arranged between 7 to 9 days after the initial examination when all other symptoms of COVID-19 had already subsided (figure 4). Four patients had measurable and significant improvements in their BTT scores in the second assessment ( $p = 0.005$ ). One patient had static olfactory function. Another patient's BTT score deteriorated further at follow-up. Her nasopharyngeal swab SARS-CoV-2 RT-PCR was already negative at this stage. This shows that OD can persist even after virologic clearance in a subset of patients.

### *Systematic review*

The PRISMA flow diagram is shown in figure 5. A total of 5,514 records from PubMed were screened. We identified 17 cohort studies and six case reports from: China (1 study), France (4 studies), Germany (1 study), Greece (1 study), Iran (3 studies), Italy (5 studies), Republic of Korea (1 study), Spain (3 studies), UK (1 study), USA (2 studies) and multinational (1 study).<sup>4-9,17-33</sup> The sample sizes of the cohort studies ranged from four to 4,707 patients. Self-reporting through questionnaires or clinical consultations were the most commonly adopted method for the assessment of olfaction. Only one study performed SIT on patients.<sup>21</sup> The reported prevalence of olfactory symptoms ranged from 5% to 98%. There was no difference in prevalence of anosmia based on sex. Clinical recovery of olfaction was documented in five reports (4 case cohorts and 1 case report), which showed that majority of patients recovered within 14 days from onset of olfactory disturbances. Details of individual reports are listed in tables 3 and 4.

## Discussion

In this study, we have demonstrated that OD is a characteristic symptom of COVID-19, which is reported by a significant proportion (67%) of patients. We have objectively proved that olfaction is compromised in COVID-19 patients when compared to healthy controls. Remarkably, we found that a subset of COVID-19 patients suffered from profound OD in the absence of typical respiratory and systemic symptoms. Although measurable improvements were documented on follow-up evaluation, OD persisted in a subset of patients even after virologic clearance and hospital discharge.

The etiology of OD in COVID-19 is enigmatic. One case report had proposed that inflammatory obstruction of the olfactory clefts may be a possible cause of anosmia in COVID-19, however half of the patients with OD in our study had patent olfactory passages. This argues against simple conductive impairment.<sup>17</sup> Alternatively, previous studies have suggested that the inflammatory cytokine milieu in nasal cavity affects olfactory neuronal function in patients with chronic rhinosinusitis.<sup>39</sup> However, only 17% of patients with OD in our study had rhinorrhea or nasal congestion, and minimal inflammatory infiltrates were found in nasal biopsy specimens, suggesting that local inflammation may not be the only contributing factor for OD in COVID-19 infection. Our previous study on SARS-CoV-2 replication in *ex vivo* human lung tissue also demonstrated that inflammation induced by SARS-CoV-2 is mild and less severe compared to 2003 SARS coronavirus.<sup>15</sup>

An alternate hypothesis is direct invasion of the olfactory neurons by SARS-CoV-2. OD has been replicated in mice infected with Sendai virus with detectable virus in both olfactory epithelium and bulbs.<sup>34</sup> Similarly, olfactory system infection has also been linked to mouse hepatitis virus and neuroinvasive H5N1 infection in animal models.<sup>35,36</sup> *In vitro*, we have recently demonstrated that SARS-CoV-2 is capable of growth in the neural cell line U251.<sup>37</sup>

However, olfactory sensory neurons do not appear to express *ACE2* and *TMPRSS2* genes.<sup>38</sup>

On the other hand, *ACE2* and *TMPRSS2* genes are found in the supporting cells of the human neuroepithelium, including sustentacular cells, olfactory horizontal cells, microvillar cells and Bowman's glands, which represent viable targets for SARS-CoV-2 infection.<sup>38</sup> While our histological analysis of nasal biopsy specimens showed that local inflammation may be minimal or absent, it was notable that SARS-CoV-2 retains the ability to cause infiltrative infection, as demonstrated by CD68+ macrophages carrying viral antigen in the stroma. These macrophages may be able to traffic SARS-CoV-2 to the olfactory neuroepithelium. Such cell-to-cell transfer is well described in HIV infection where macrophages can secondarily infect lymphocytes. Therefore, transmission of SARS-CoV-2 by CD68+ macrophages to the supporting cells of the olfactory neuroepithelium may disrupt the biochemical and electrophysiological homeostasis of the olfactory receptor neurons, leading to chemosensory dysfunction and impaired neuronal turnover.

In our systematic review, we found that OD was seldom reported in COVID-19 patient cohorts from mainland China. Prevalence of OD in Chinese COVID-19 patients also appeared lower than in Western cohorts of comparable size.<sup>8</sup> Although this might be due to under-reporting, we note that intranasal interferon- $\alpha$  is widely prescribed in China for COVID-19 infection in accordance with national treatment guidelines.<sup>40</sup> Intranasal interferon treatment is not widely adopted by the rest of the world including Hong Kong. We speculate that early intranasal interferon- $\alpha$  may abrogate local SARS-CoV-2 replication in the olfactory epithelium sufficiently to reduce OD. Prospective interventional trials for the treatment of OD in COVID-19 patients are needed to confirm this important observation.

Olfactory dysfunction and dysgeusia in COVID-19 should not be overlooked because of the associated psychosocial impact on affected patients. Our patients reported distress due to their inability to smell, which affected their appetite and desires for eating. Patients might

become socially withdrawn as they cannot share culinary experiences with their loved ones. Studies have shown that OD is linked to lower quality of life and depression.<sup>41</sup>

There were a few limitations in our study. First, the study sample size was limited by the number of confirmed COVID-19 patients in Hong Kong. However, quantitative olfactory assessments were performed for all participants, which allowed for objective comparison between patient cohorts and controls. Second, imaging and nasoendoscopy were not performed in all patients to limit exposure of patients without OD to invasive assessments. Third, nasal biopsy specimens were obtained from the middle turbinate, which may not contain olfactory neuroepithelium. However, biopsy of the olfactory neuroepithelium at the cribriform plate of the ethmoid bone is not possible in routine bedside endoscopic assessment. Fourth, the control group was not tested for SARS-CoV-2, due to limited number of test kits available, outside of clinical needs. However due to the low prevalence of COVID-19 in Hong Kong, it is unlikely that these healthy volunteers were infected by SARS-CoV-2.

In conclusion, this study proves that OD is common in COVID-19 patients using objective functional measurements. We also demonstrated that olfactory cleft obstruction, nasal congestion, and sinusitis were absent in most COVID-19 patients with OD. Notably, while overt inflammation may be absent, we have identified CD68+ macrophages carrying SARS-CoV-2 antigen in the stroma of nasal biopsy samples. This raises the possibility that COVID-19-related OD may be caused by infiltrative infection of the sustentacular cells and supporting cells of the olfactory neuroepithelium.

## Authors' contributions

TWHC and SS designed the experiments and wrote the manuscript draft. AJZ, ACYL and ZF performed immunohistochemical analysis of biopsy samples and critically reviewed the manuscript. HLL performed systematic review together with TWHC and SS. FKCW and RKYT performed nasoendoscopies and biopsies from patients. KHC and SML performed RT-PCR analyses. MYN, SYL, WKK, SHYL analyzed the computed tomography films. RHSL performed histological analysis of nasal biopsy samples. KCL, AKLW, WMC, PYN, VCCC, KKWT and IFNH were involved in patient recruitment, data analysis and critical review. KYY conceived the study and critically reviewed the manuscript. TWHC had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. TWHC, SS, and KYY were responsible for the decision to submit the manuscript.

## **Declaration of interests**

S.S has received speaker's honoraria from Sanofi-Aventis Hong Kong Limited and Abbott laboratories Limited. The other authors declare no conflicts of interest.

## **Funding**

This work was supported by the Shaw Foundation Hong Kong; Michael Seak-Kan Tong; Richard Yu and Carol Yu; May Tam Mak Mei Yin; Jessie & George Ho Charitable Foundation; Perfect Shape Medical Limited; Respiratory Viral Research Foundation; Hui Ming, Hui Hoy and Chow Sin Lan Charity Fund Limited; Sanming Project of Medicine in Shenzhen, China (SZSM201911014); High Level-Hospital Program, Health Commission of Guangdong Province, China; Consultancy Service for Enhancing Laboratory Surveillance of Emerging Infectious Diseases and Research Capability on Antimicrobial Resistance for Department of Health of the Hong Kong Special Administrative Region Government; Theme-Based Research Scheme (T11/707/15) of the Research Grants Council; Hong Kong Special Administrative Region. The funding sources played no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

## **Acknowledgment**

We thank Ms. Tracy Chui-Chi Kwok and her nursing team for their kind assistance and logistics coordination.

## References

1. Chan JF, Yuan S, Kok KH, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* 2020; **395**(10223): 514-23.
2. World Health Organization. Coronavirus disease 2019 (COVID-19) Situation Report - 112 2020. [https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200511-covid-19-sitrep-112.pdf?sfvrsn=813f2669\\_2](https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200511-covid-19-sitrep-112.pdf?sfvrsn=813f2669_2) (accessed 12/05/2020).
3. Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *The New England journal of medicine* 2020.
4. Yan CH, Faraji F, Prajapati DP, Boone CE, DeConde AS. Association of chemosensory dysfunction and Covid-19 in patients presenting with influenza-like symptoms. *Int Forum Allergy Rhinol* 2020.
5. Galougahi MK, Ghorbani J, Bakhshayeshkaram M, Naeini AS, Haseli S. Olfactory Bulb Magnetic Resonance Imaging in SARS-CoV-2-Induced Anosmia: The First Report. *Acad Radiol* 2020.
6. Gane SB, Kelly C, Hopkins C. Isolated sudden onset anosmia in COVID-19 infection. A novel syndrome? *Rhinology* 2020.
7. Lechien JR, Chiesa-Estomba CM, De Siati 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.
8. Mao L, Jin H, Wang M, et al. Neurologic Manifestations of Hospitalized Patients With Coronavirus Disease 2019 in Wuhan, China. *JAMA Neurol* 2020.
9. Characteristics of Health Care Personnel with COVID-19 - United States, February 12-April 9, 2020. *MMWR Morb Mortal Wkly Rep* 2020; **69**(15): 477-81.

10. Malaty J, Malaty IA. Smell and taste disorders in primary care. *American family physician* 2013; **88**(12): 852-9.
11. Cain WS, Gent JF, Goodspeed RB, Leonard G. Evaluation of olfactory dysfunction in the Connecticut Chemosensory Clinical Research Center. *The Laryngoscope* 1988; **98**(1): 83-8.
12. Doty RL, Shaman P, Dann M. Development of the University of Pennsylvania Smell Identification Test: a standardized microencapsulated test of olfactory function. *Physiol Behav* 1984; **32**(3): 489-502.
13. Hopkins C, Browne JP, Slack R, Lund V, Brown P. The Lund-Mackay staging system for chronic rhinosinusitis: how is it used and what does it predict? *Otolaryngol Head Neck Surg* 2007; **137**(4): 555-61.
14. Chan JF, Zhang AJ, Yuan S, et al. Simulation of the clinical and pathological manifestations of Coronavirus Disease 2019 (COVID-19) in golden Syrian hamster model: implications for disease pathogenesis and transmissibility. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2020.
15. Chu H, Chan JF, Wang Y, et al. Comparative replication and immune activation profiles of SARS-CoV-2 and SARS-CoV in human lungs: an ex vivo study with implications for the pathogenesis of COVID-19. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2020.
16. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med* 2009; **6**(7): e1000097.
17. Eliezer M, Hautefort C, Hamel AL, et al. Sudden and Complete Olfactory Loss Function as a Possible Symptom of COVID-19. *JAMA otolaryngology-- head & neck surgery* 2020.

18. Gutierrez-Ortiz C, Mendez A, Rodrigo-Rey S, et al. Miller Fisher Syndrome and polyneuritis cranialis in COVID-19. *Neurology* 2020.
19. Jang Y, Son HJ, Lee S, Lee EJ, Kim TH, Park SY. Olfactory and taste disorder: the first and only sign in a patient with SARS-CoV-2 pneumonia. *Infection control and hospital epidemiology* 2020: 1-4.
20. Klopfenstein T, Kadiane-Oussou NJ, Toko L, et al. Features of anosmia in COVID-19. *Med Mal Infect* 2020.
21. Moein ST, Hashemian SMR, Mansourafshar B, Khorram-Tousi A, Tabarsi P, Doty RL. Smell dysfunction: a biomarker for COVID-19. *Int Forum Allergy Rhinol* 2020.
22. Ollarves-Carrero MF, Rodriguez-Morales AG, Bonilla-Aldana DK, Rodriguez-Morales AJ. Anosmia in a healthcare worker with COVID-19 in Madrid, Spain. *Travel Med Infect Dis* 2020: 101666.
23. Benezit F, Le Turnier P, Declerck C, et al. Utility of hyposmia and hypogeusia for the diagnosis of COVID-19. *The Lancet Infectious diseases* 2020.
24. Marzano AV, Genovese G, Fabbrocini G, et al. Varicella-like exanthem as a specific COVID-19-associated skin manifestation: multicenter case series of 22 patients. *Journal of the American Academy of Dermatology* 2020.
25. Zayet S, N'Dri Juliette KO, Royer PY, Toko L, Gendrin V, Klopfenstein T. Coronavirus disease 2019: new things to know! *Journal of medical virology* 2020.
26. Monti S, Balduzzi S, Delvino P, Bellis E, Quadrelli VS, Montecucco C. Clinical course of COVID-19 in a series of patients with chronic arthritis treated with immunosuppressive targeted therapies. *Annals of the rheumatic diseases* 2020; **79**(5): 667-8.
27. Giacomelli A, Pezzati L, Conti F, et al. Self-reported olfactory and taste disorders in SARS-CoV-2 patients: a cross-sectional study. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2020.

28. Lytras T, Dellis G, Flountzi A, et al. High prevalence of SARS-CoV-2 infection in repatriation flights to Greece from three European countries. *Journal of travel medicine* 2020.
29. Toscano G, Palmerini F, Ravaglia S, et al. Guillain-Barre Syndrome Associated with SARS-CoV-2. *The New England journal of medicine* 2020.
30. Wolfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* 2020.
31. Beltran-Corbellini A, Chico-Garcia JL, Martinez-Poles J, et al. Acute-onset smell and taste disorders in the context of Covid-19: a pilot multicenter PCR-based case-control study. *European journal of neurology* 2020.
32. Spinato G, Fabbris C, Polesel J, et al. Alterations in Smell or Taste in Mildly Symptomatic Outpatients With SARS-CoV-2 Infection. *Jama* 2020.
33. Heidari F, Karimi E, Firouzifar M, et al. Anosmia as a prominent symptom of COVID-19 infection. *Rhinology* 2020.
34. Tian J, Pinto JM, Cui X, et al. Sendai Virus Induces Persistent Olfactory Dysfunction in a Murine Model of PVOD via Effects on Apoptosis, Cell Proliferation, and Response to Odorants. *PloS one* 2016; **11**(7): e0159033.
35. Plourde JR, Pyles JA, Layton RC, Vaughan SE, Tipper JL, Harrod KS. Neurovirulence of H5N1 infection in ferrets is mediated by multifocal replication in distinct permissive neuronal cell regions. *PloS one* 2012; **7**(10): e46605.
36. Perlman S, Jacobsen G, Afifi A. Spread of a neurotropic murine coronavirus into the CNS via the trigeminal and olfactory nerves. *Virology* 1989; **170**(2): 556-60.
37. Chu H, Chan JF-W, Yuen TT-T, et al. Comparative tropism, replication kinetics, and cell damage profiling of SARS-CoV-2 and SARS-CoV with implications for clinical manifestations, transmissibility, and laboratory studies of COVID-19: an observational study. *The Lancet Microbe*.

38. 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. *bioRxiv* 2020: 2020.03.25.009084.
39. Wu J, Chandra RK, Li P, Hull BP, Turner JH. Olfactory and middle meatal cytokine levels correlate with olfactory function in chronic rhinosinusitis. *The Laryngoscope* 2018; **128**(9): E304-e10.
40. General Office of National Health Committee. Notice on the issuance of a programme for the diagnosis and treatment of novel coronavirus (2019-nCoV) infected pneumonia (Trial version 3) 2020. [http://www.tjnk.gov.cn/att/0/10/04/15/10041580\\_832505.pdf](http://www.tjnk.gov.cn/att/0/10/04/15/10041580_832505.pdf) (accessed 26/4/2020)
41. Kohli P, Soler ZM, Nguyen SA, Muus JS, Schlosser RJ. The Association Between Olfaction and Depression: A Systematic Review. *Chemical senses* 2016; **41**(6): 479-86.

Accepted Manuscript

**Table 1:** Demographic and clinical characteristics of COVID-19 patients and controls

|                                | COVID-19 patients (n = 18) | Controls (n = 18)   | p value |
|--------------------------------|----------------------------|---------------------|---------|
| <b>Age (years)<sup>a</sup></b> | 28 ± 19 (18 - 59)          | 31 ± 17.5 (18 - 59) | 0.98    |
| 18 – 21                        | 5 (28%)                    | 5 (28%)             |         |
| 22 – 31                        | 5 (28%)                    | 7 (39%)             |         |
| 32 – 41                        | 4 (22%)                    | 2 (11%)             |         |
| 42 – 60                        | 4 (22%)                    | 4 (22%)             |         |
| <b>Gender</b>                  |                            |                     |         |
| Female                         | 11 (61%)                   | 13 (72%)            | 0.725   |
| Male                           | 7 (39%)                    | 5 (28%)             |         |
| <b>Smoking status</b>          |                            |                     |         |
| Smoker                         | 1 (6%)                     | 0 (0%)              | 1       |
| Non-smoker                     | 17 (94%)                   | 18 (100%)           |         |
| <b>Drinking status</b>         |                            |                     |         |
| Non-drinker                    | 11 (61%)                   | 14 (78%)            | 0.471   |
| Regular drinker                | 1 (6%) <sup>b</sup>        | 0 (0%)              | 1       |

|                                       |                     |                     |       |
|---------------------------------------|---------------------|---------------------|-------|
| Social drinker                        | 6 (33%)             | 4 (22%)             | 0.711 |
| <b>Past surgical history</b>          |                     |                     |       |
| Head injuries                         | 2 (11%)             | 0 (0%)              | 0.486 |
| Neurosurgery                          | 0 (0%)              | 0 (0%)              |       |
| Maxillofacial surgery                 | 1 (6%) <sup>c</sup> | 0 (0%)              | 1     |
| <b>Good past health</b>               | 16 (89%)            | 14 (78%)            | 0.658 |
| <b>Medical co-morbidities</b>         |                     |                     |       |
| Hypertension                          | 2 (11%)             | 2 (11%)             |       |
| Diabetes mellitus                     | 0 (0%)              | 0 (0%)              |       |
| Hyperlipidemia                        | 0 (0%)              | 0 (0%)              |       |
| Malignancy                            | 0 (0%)              | 1 (6%) <sup>d</sup> | 1     |
| Others                                | 0 (0%)              | 1 (6%) <sup>e</sup> | 1     |
| <b>History of zoster reactivation</b> | 0 (0%)              | 1 (6%)              | 1     |

<sup>a</sup>Age are median (IQR), n (%). <sup>b</sup>Patient works as a wine merchant. <sup>c</sup>History of tonsillectomy. <sup>d</sup>History of breast cancer. <sup>e</sup>Case of chronic hepatitis B carrier.

**Table 2:** Clinical, olfactory function, nasoendoscopy, and imaging findings of COVID-19 patients with olfactory dysfunction

|                                | Patient 1 | Patient 2         | Patient 4 | Patient 5        | Patient 14 | Patient 15 |
|--------------------------------|-----------|-------------------|-----------|------------------|------------|------------|
| <b>Patient characteristics</b> |           |                   |           |                  |            |            |
| Sex                            | F         | F                 | M         | F                | F          | F          |
| Age                            | 18        | 18                | 20        | 21               | 41         | 42         |
| <b>Clinical symptoms</b>       |           |                   |           |                  |            |            |
| Date of symptom onset          | 1 Apr 20  | 28 Mar 20         | 3 Apr 20  | 26 Mar 20        | 16 Mar 20  | 15 Mar 20  |
| Fever                          | Y         | N                 | Y         | N                | N          | N          |
| Chills                         | N         | N                 | Y         | N                | N          | N          |
| Cough                          | Y         | N                 | Y         | N                | N          | Y          |
| Sputum                         | Y         | N                 | N         | N                | N          | Y          |
| Shortness of breath            | N         | N                 | N         | N                | N          | Y          |
| Nasal complaints               |           | Y                 |           | Y                |            |            |
|                                | N         | (Rhinorrhea<br>a) | N         | (Rhinorrhea<br>) | N          | N          |

|                                                                |                    |           |                  |                  |                 |                |
|----------------------------------------------------------------|--------------------|-----------|------------------|------------------|-----------------|----------------|
| Others                                                         | N                  | N         | Y<br>(Headache)  | N                | Y<br>(Headache) | N              |
| <b>Olfactory dysfunction</b>                                   |                    |           |                  |                  |                 |                |
| Onset date of olfactory dysfunction                            | 4 Apr 20           | 29 Mar 20 | 3 Apr 20         | 26 Mar 20        | 23 Mar 20       | 23 Mar 20      |
| Interval between olfactory dysfunction and symptom onset (day) | 3                  | 1         | 0                | 0                | 7               | 8              |
| <b>Butanol threshold test (BTT) score</b>                      |                    |           |                  |                  |                 |                |
| First BTT score                                                | 1.5                | 0         | 1                | 3.5              | 2               | 2              |
| Date of first BTT                                              | 9 Apr 20           | 7 Apr 20  | 7 Apr 20         | 7 Apr 20         | 6 Apr 20        | 6 Apr 20       |
| Second BTT score                                               | 3                  | 1.5       | 3                | 3.5              | 0               | 3              |
| Date of second BTT                                             | 16 Apr 20          | 15 Apr 20 | 15 Apr 20        | 15 Apr 20        | 15 Apr 20       | 15 Apr 20      |
| <b>Smell identification test (SIT)</b>                         |                    |           |                  |                  |                 |                |
| SIT (score, out of 40)                                         | 26                 | 16        | 25               | 21               | 16              | 33             |
| SIT (category)                                                 | Moderate microsmia | Anosmia   | Severe microsmia | Severe microsmia | Anosmia         | Mild microsmia |
| Date of SIT                                                    | 10 Apr 20          | 7 Apr 20  | 7 Apr 20         | 7 Apr 20         | 6 Apr 20        | 6 Apr 20       |
| <b>Nasoendoscopy</b>                                           | Normal             | Normal    | Normal           | Normal           | Normal          | Normal         |
| <b>Computer tomography scan</b>                                |                    |           |                  |                  |                 |                |

|                                                     |   |   |   |   |   |   |
|-----------------------------------------------------|---|---|---|---|---|---|
| Paranasal sinuses (Lund-Mackay score)               | 2 | 1 | 2 | 0 | 0 | 0 |
| Olfactory cleft (opacification score) <sup>a</sup>  |   |   |   |   |   |   |
| Right                                               | 2 | 2 | 0 | 1 | 0 | 0 |
| Left                                                | 2 | 2 | 0 | 1 | 0 | 0 |
| Thorax (COVID-19 related lung changes) <sup>b</sup> | N | N | Y | N | Y | N |

<sup>a</sup>Olfactory cleft opacification score: complete opacification = 2; partial opacification = 1; Un-opacified = 0.

<sup>b</sup>Parenchymal ground-glass opacification.

**Table 3.** Cohort studies on olfactory disturbances in COVID-19 patients.

| Author                                     | Country | Sample size | Primary outcome(s)             | Methods of olfactory assessment | Prevalence of olfactory impairment (%) | Female (%)   | Proposed etiology of olfactory impairments |
|--------------------------------------------|---------|-------------|--------------------------------|---------------------------------|----------------------------------------|--------------|--------------------------------------------|
| Beltrán-Corbellini Á, et al. <sup>31</sup> | Spain   | 79          | Smell and taste disorder       | Questionnaire                   | 25/79 (31%)                            | 31/79 (39%)  | Damage of the olfactory sensory epithelium |
| Bénézit F, et al. <sup>23</sup>            | France  | 68          | Hyposmia and hypogeusia        | Questionnaire                   | 31/68 (45%)                            | Not reported | Pathology at the olfactory mucosa          |
| Burrer, et al. <sup>9</sup>                | USA     | 4707        | COVID-19 in healthcare workers | Consultation                    | 750/4707 (16%)                         | Not reported | Nil                                        |

|                                      |                                        |     |                                      |               |               |               |                                                                                  |
|--------------------------------------|----------------------------------------|-----|--------------------------------------|---------------|---------------|---------------|----------------------------------------------------------------------------------|
|                                      |                                        |     |                                      |               |               | d             |                                                                                  |
| Giacomelli A, et al. <sup>27</sup>   | Italy                                  | 59  | Olfactory and taste disorders        | Questionnaire | 14/59 (24%)   | 19/59 (32%)   | Transneural penetration through the olfactory bulb                               |
| Heidari F, et al. <sup>33</sup>      | Iran                                   | 23  | Anosmia                              | Consultation  | 23/23 (100%)  | 15/23 (65%)   | Nil                                                                              |
| Klopfenstein T, et al. <sup>20</sup> | France                                 | 114 | Anosmia                              | Consultation  | 54/114 (47%)  | 36/54 (67%)   | Nil                                                                              |
| Lechien JR, et al. <sup>7</sup>      | Europe (Belgium, France, Italy, Spain) | 417 | Olfactory and gustatory dysfunctions | Questionnaire | 357/417 (86%) | 263/417 (63%) | Viral invasion through ACE2 receptor to olfactory bulb or related regions of CNS |

|                                  |        |     |                                                          |                    |             |                |                                    |
|----------------------------------|--------|-----|----------------------------------------------------------|--------------------|-------------|----------------|------------------------------------|
|                                  |        |     |                                                          |                    |             | %)             |                                    |
| Lytras T, et al. <sup>28</sup>   | Greece | 40  | Prevalence of COVID-19 in repatriation flights to Greece | Consultation       | 2/5 (40%)   | Not rep-orte-d | Nil                                |
| Mao L, et al. <sup>8</sup>       | China  | 214 | Neurologic manifestation                                 | Consultation       | 11/214 (5%) | 127/214 (59%)  | Retrograde neuronal invasion       |
| Marzano AV, et al. <sup>24</sup> | Italy  | 22  | COVID-19 associated skin manifestation                   | Consultation       | 4/22 (18%)  | 6/22 (27%)     | Nil                                |
| Moein ST, et al. <sup>21</sup>   | Iran   | 60  | Olfactory dysfunction                                    | UPSIT <sup>a</sup> | 59/60 (98%) | 20/60 (33%)    | Damage of the olfactory epithelium |

|                                 |         |     |                                                                              |                                    |               |              |               |        |
|---------------------------------|---------|-----|------------------------------------------------------------------------------|------------------------------------|---------------|--------------|---------------|--------|
|                                 |         |     |                                                                              |                                    |               |              |               | %)     |
| Monti S, et al. <sup>26</sup>   | Italy   | 4   | Clinical characteristics of immunosuppressed patients with chronic arthritis | Consultation                       | 3/4 (75%)     | 4/4          | Nil           | (100%) |
| Spinato G, et al. <sup>32</sup> | Italy   | 202 | Alterations in smell or taste                                                | Questionnaire                      | 130/202 (64%) | 105/202      | Nil           | (52%)  |
| Toscano G, et al. <sup>29</sup> | Italy   | 5   | Guillain–Barré Syndrome associated with SARS-CoV-2                           | Consultation                       | 2/5 (40%)     | 1/5          | Nil           | (20%)  |
| Wolfel R, et al. <sup>30</sup>  | Germany | 9   | Virologic manifestations                                                     | Self-reported through consultation | 3/9 (33%)     | Not rep-orte | Not suggested | d      |

|                               |        |    |                          |               |             |             |                                       |
|-------------------------------|--------|----|--------------------------|---------------|-------------|-------------|---------------------------------------|
| Yan CH, et al. <sup>4</sup>   | USA    | 59 | Chemosensory dysfunction | Questionnaire | 40/59 (68%) | 29/59 (49%) | Pathology at the olfactory epithelium |
| Zayet S, et al. <sup>25</sup> | France | 62 | Anosmia and dysgeusia    | Consultation  | 32/62 (52%) | 38/62 (61%) | Nil                                   |

---

<sup>a</sup>UPSIT: University of Pennsylvania Smell Identification Test

**Table 4.** Case reports on olfactory disturbances in COVID-19 patients.

| Author                                       | Country           | Sex, age<br>(years) | Methods of olfactory<br>assessment   | Radiological<br>findings     | Proposed etiology of anosmia                                                                                                               |
|----------------------------------------------|-------------------|---------------------|--------------------------------------|------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|
| Eliezer M, et al. <sup>17</sup>              | France            | F, 40               | Odorant identification               | CT and MRI                   | Olfactory cleft obstruction                                                                                                                |
| Galougahi MK, et al. <sup>5</sup>            | Iran              | Not<br>specified    | Consultation                         | MRI of the<br>olfactory bulb | Nil                                                                                                                                        |
| Gane SB, et al. <sup>6</sup>                 | United<br>Kingdom | M, 48               | Consultation                         | Nil                          | Conductive loss at the olfactory cleft<br><br>Direct infection of the olfactory mucosa and destruction of the<br>olfactory sensory neurons |
| Gutiérrez-Ortiz C, et<br>al. <sup>18</sup>   | Spain             | M, 50               | consultation                         | Nil                          | Infection of the olfactory nerve; anti-ganglioside antibodies GD1b<br>IgG                                                                  |
| Jang Y, et al. <sup>19</sup>                 | Korea             | M, 42               | Consultation, visual<br>analog scale | Nil                          | Nil                                                                                                                                        |
| Ollarves-Carrero MF,<br>et al. <sup>22</sup> | Spain             | F, 40               | Consultation                         | Nil                          | Infection of the olfactory nerve                                                                                                           |

## Figure legends

**Figure 1:** Comparison of BTT scores between COVID-19 patients and healthy controls. Bars represent mean and standard error of mean (A). Comparison of mean BTT scores of COVID-19 patients and healthy controls stratified by age group (B). \*\* indicates statistically significant difference between means of groups.

**Figure 2:** Computed tomography scans of the nasal cavity and sinuses of COVID-19 patients with olfactory dysfunction showing opacified olfactory clefts indicating complete obstruction (A), partially opacified olfactory clefts (B) and patent olfactory clefts (C). Sinusitis was absent.

**Figure 3:** Representative histopathological and immunofluorescence staining results of nasal biopsy tissue sections. (A) H&E-stained section showed few intraepithelial neutrophils (white arrows), (B) Small numbers of lymphocytes, plasma cells and occasional neutrophils amongst mucous glands in the stroma (black arrows). Immunofluorescence staining of CD68 showing presence of scattered macrophages in the (C) epithelium and (D) stroma (green), the area in the boxes was magnified to demonstrate cytoplasmic staining of CD68 (white arrows). (E) Immunofluorescence staining of SARS-CoV-2 NP in the nasal mucosa and in magnified image (arrows). Localization of SARS-CoV-2 NP in CD68<sup>+</sup> cells by double immunofluorescence staining of CD68 (F, green) and SARS-CoV-2 NP (G, Red, using mouse anti-SARS-CoV-2 NP and Texas-Red conjugated Donkey anti-mouse secondary antibody), cell nuclei counterstained by DAPI in blue (H) and merged image (I).

**Figure 4:** Serial progression of BTT scores in patients with olfactory dysfunction.

**Figure 5:** Study selection

## Key points

COVID-19-related olfactory dysfunction is common (67%). Recovery can be slow (>3 weeks). Sinusitis and olfactory cleft obstruction were absent in most patients.

Immunohistochemical analysis of nasal biopsy revealed the presence of infiltrative CD68+ macrophages expressing SARS-CoV-2 antigen in the stroma.

Accepted Manuscript

Figure 1A

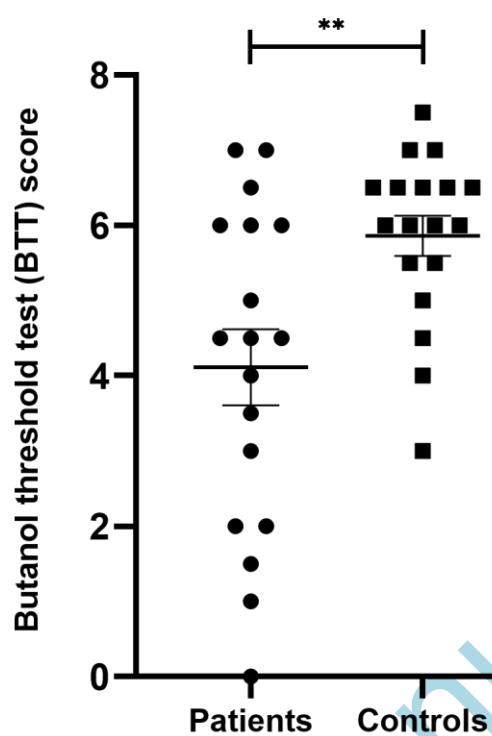


Figure 1B

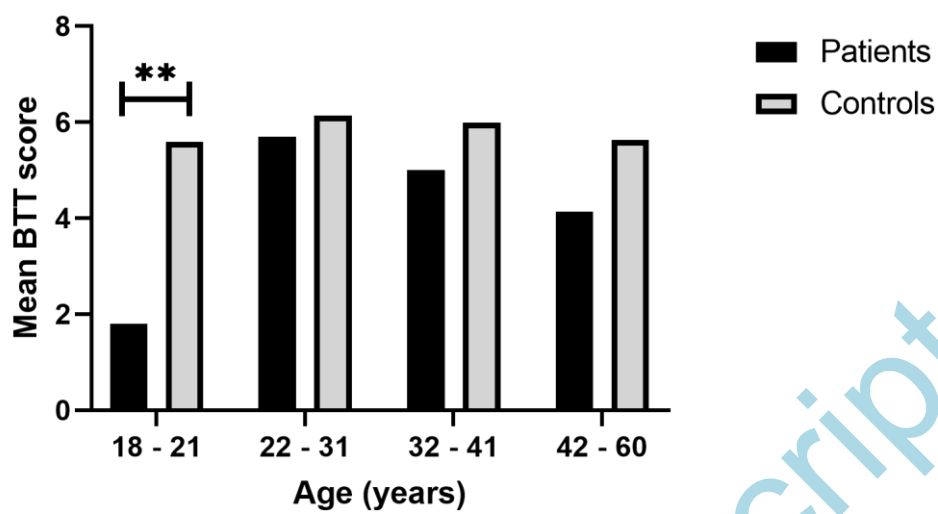
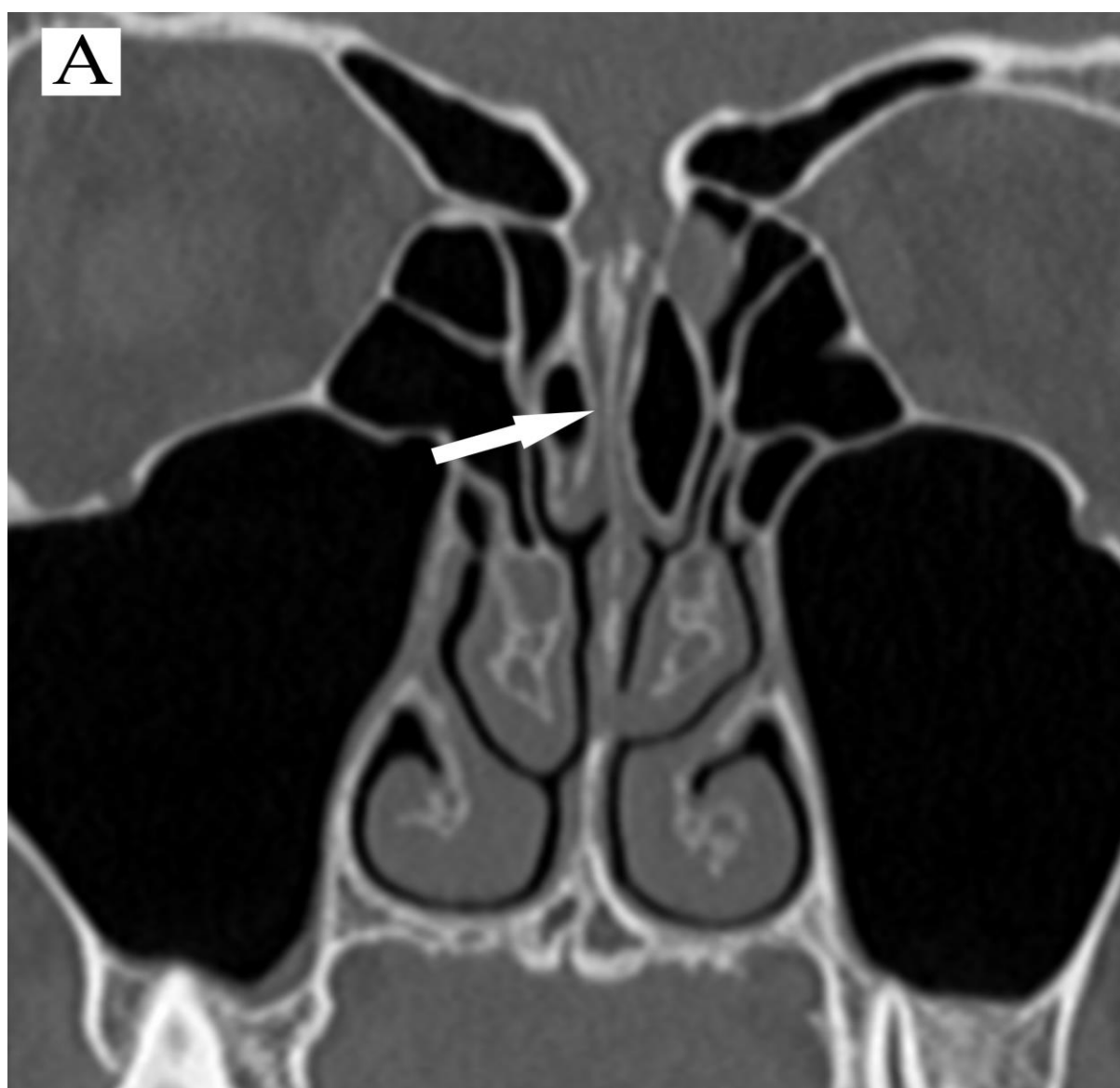
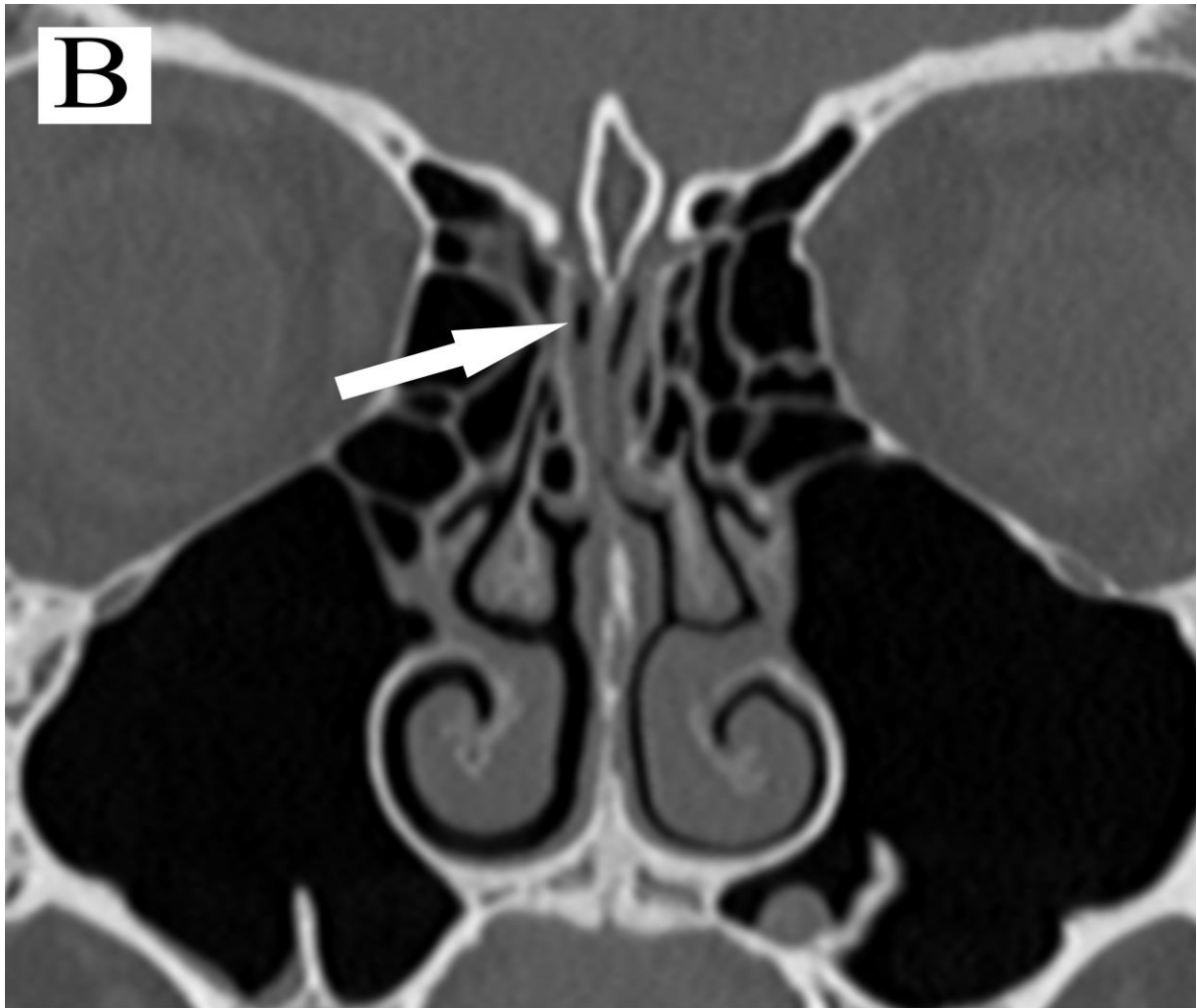


Figure 2A



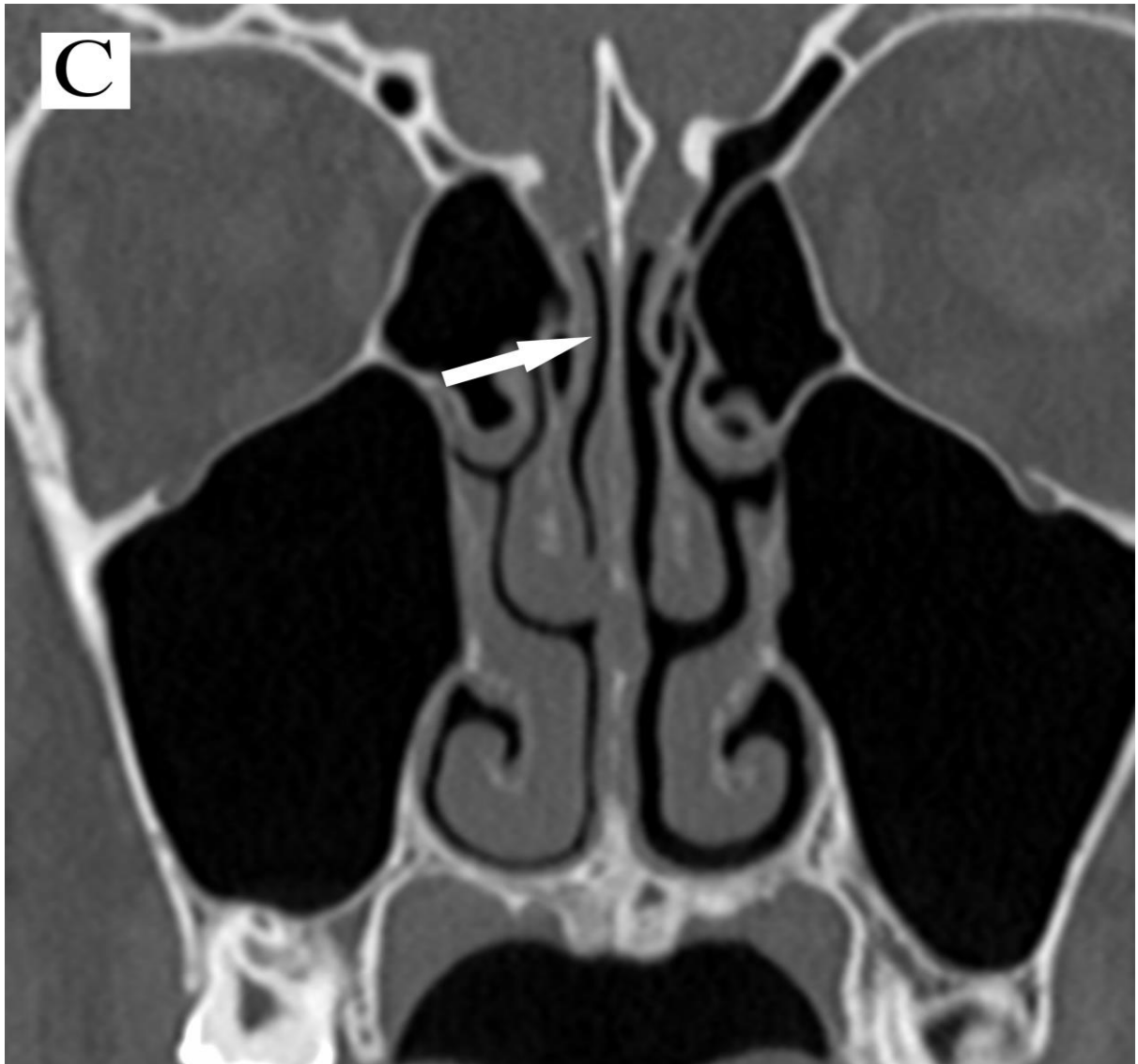
Accepted

Figure 2B



Accepted

Figure 2C



Accepted

Figure 3A

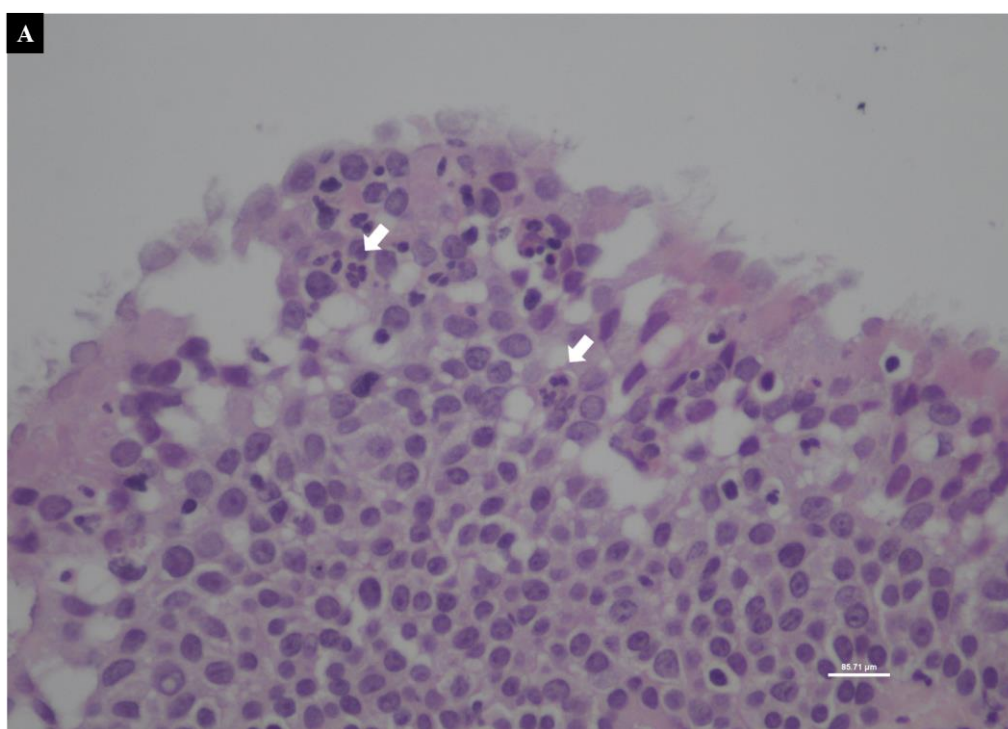


Figure 3B

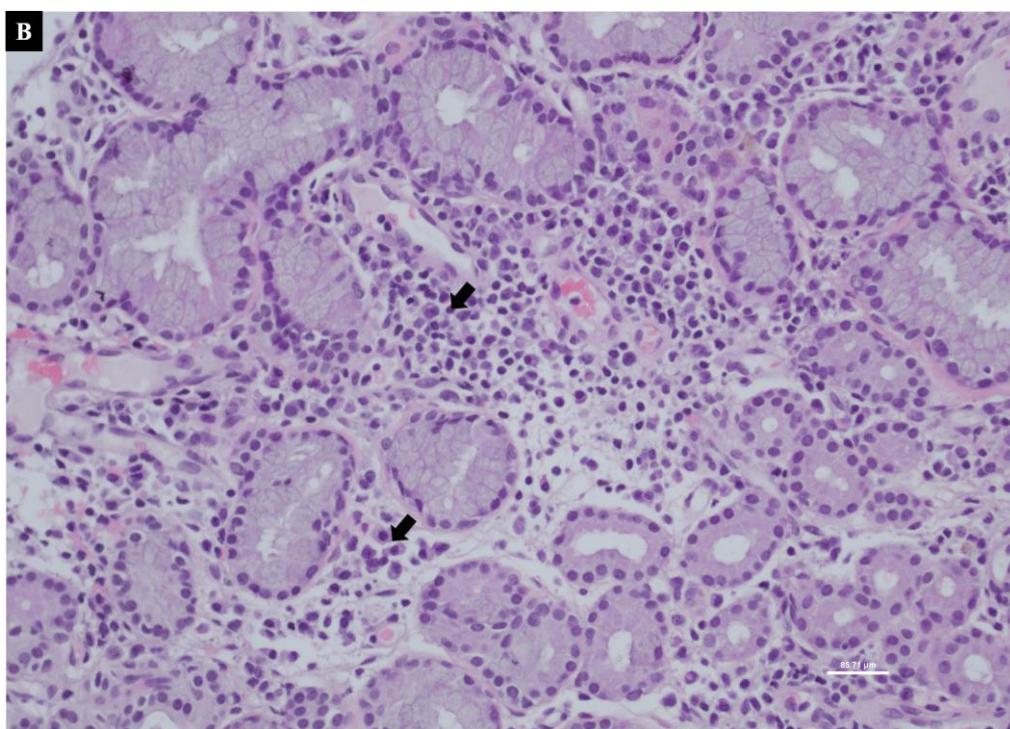


Figure 3C

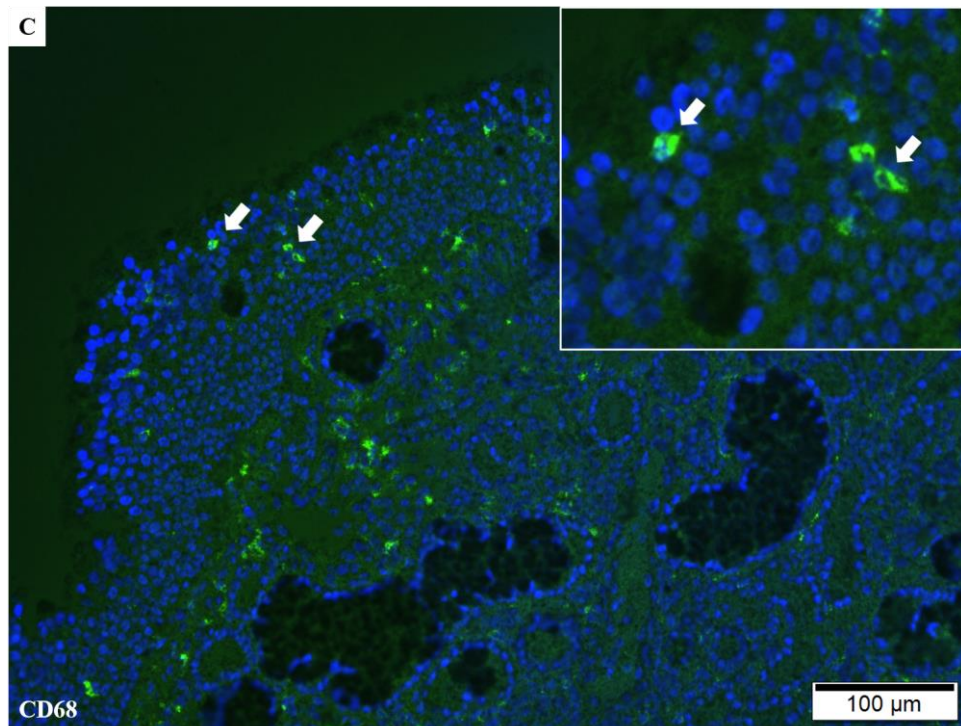


Figure 3D

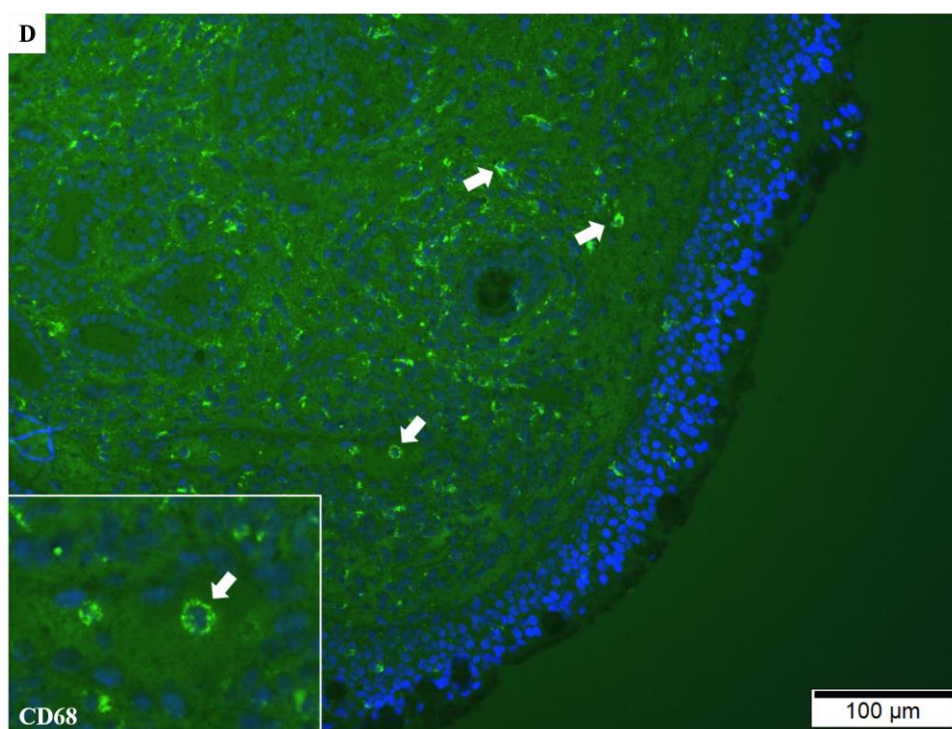


Figure 3E

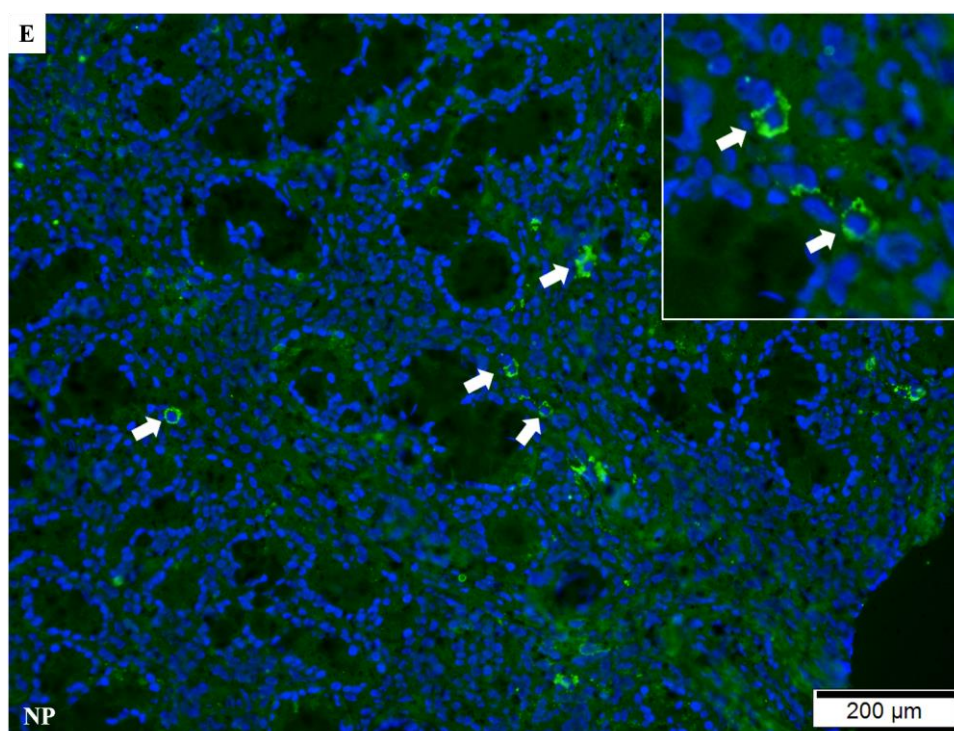


Figure 3F

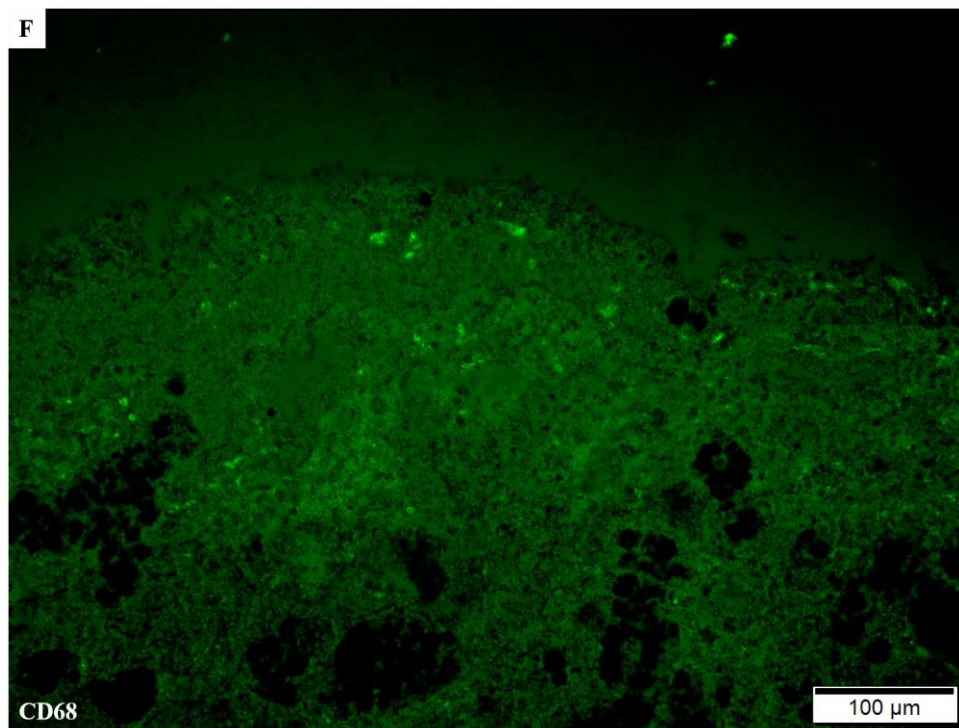


Figure 3G

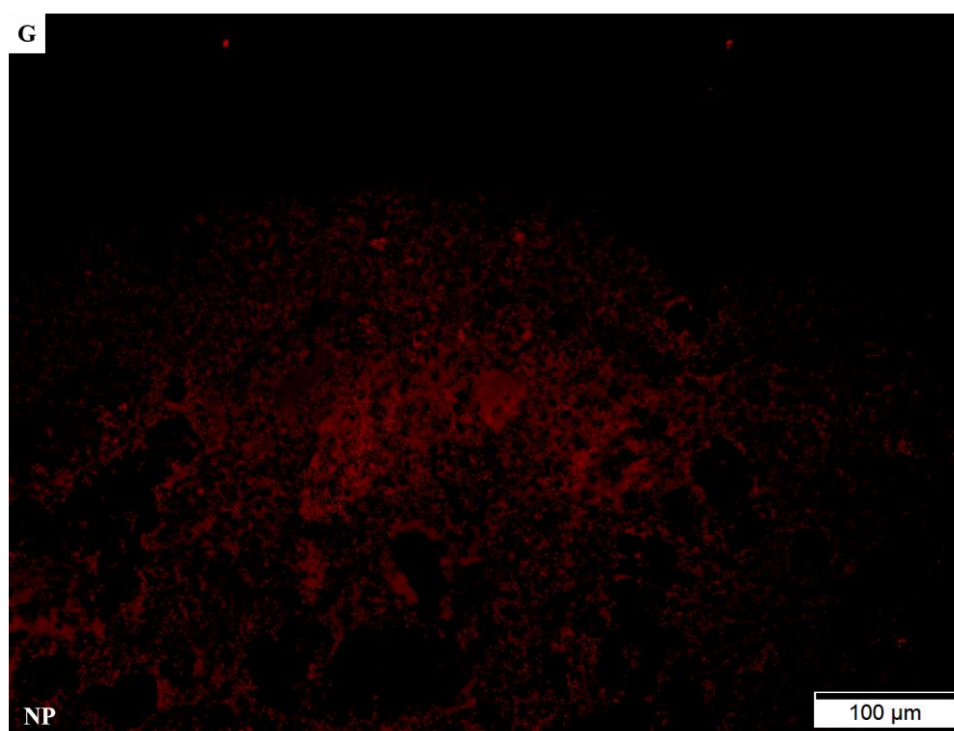


Figure 3H

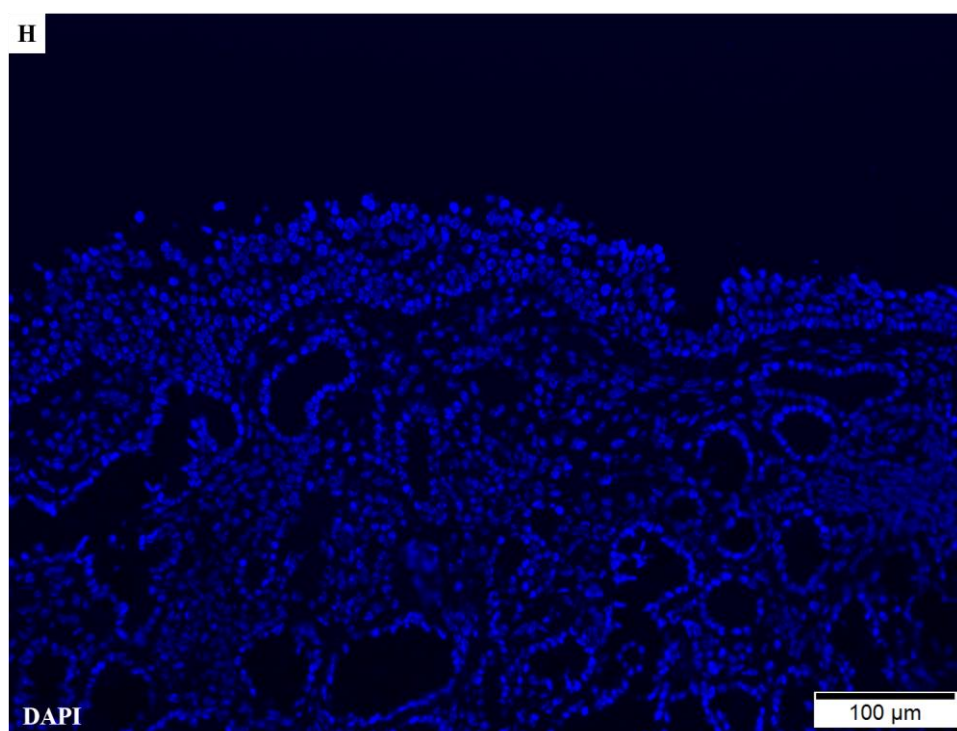


Figure 3I

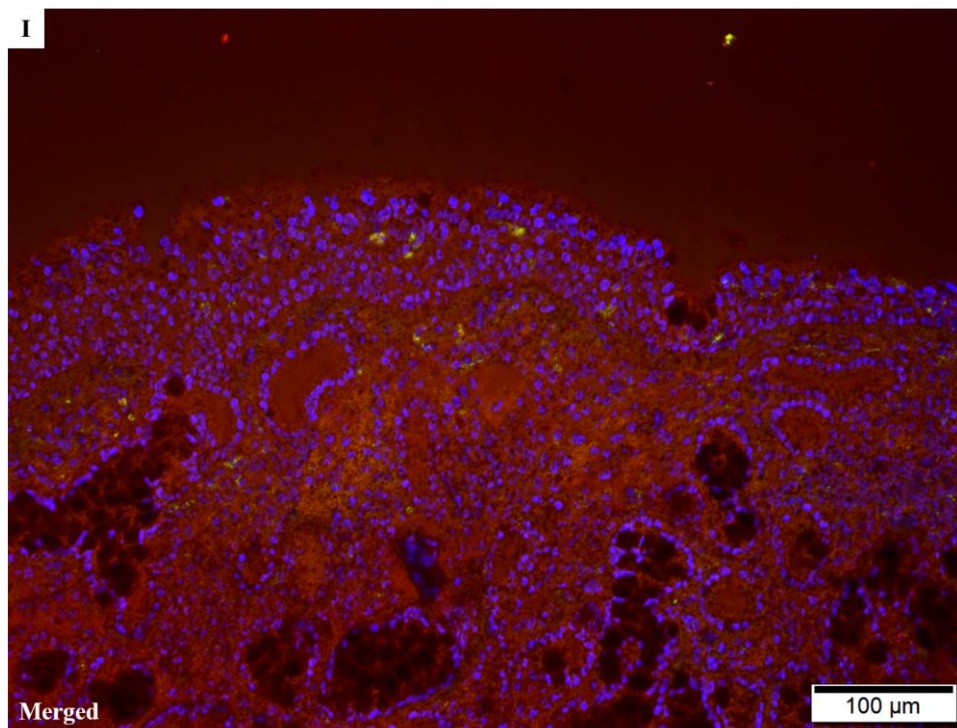
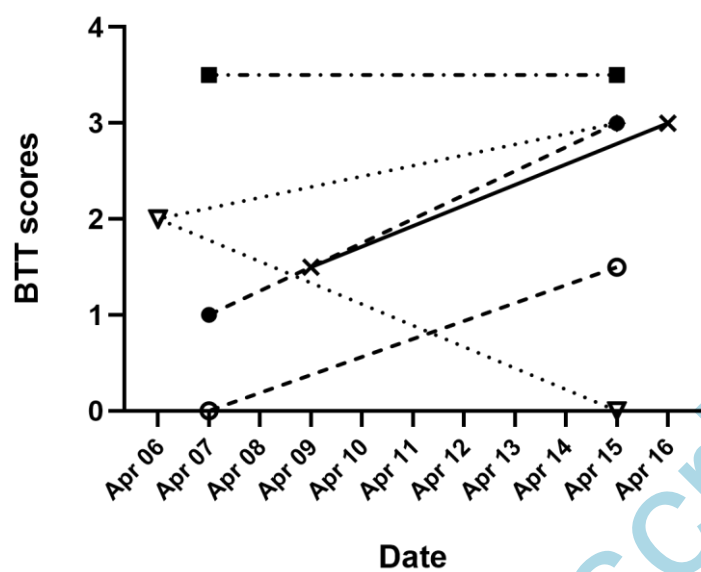


Figure 4



**Figure 5.** Study selection.

