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Persistent olfactory alterations in Post-COVID-19 condition

A systematic review and a cross-sectional study on patients from the OSI Ezkerraldea-Enkarterri-Cruces

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ABSTRACT

Introduction. The phenotype of Post-Covid-19 Condition (PC19C) has not precisely been described yet. Olfaction alteration is clearly part of the acute phase of the infection, but it remains unclear if it is part of the defining features of PC19C.

Primary objectives. To determine whether PC19C patients suffer from persistent olfactory alterations more frequently than the healthy population, comparing it to current literature. As well as, to objectify this damage with a validated and objective measurement tool such as the B-SIT (brief smell identification test).

Material and Methods. A systematic review and a cross-sectional study were performed. The databases used for the systematic review were Pubmed and SpringerLink. In total 39 papers were included in the review. For the cross-sectional study 40 PC19C patients and 40 controls that had not been infected by Sars-CoV-2 were tested. First, they filled out a questionnaire to rule out other reasons for olfaction alteration, and then, they realized the B-SIT. The results of the two groups were statistically compared by GraphPad Prism 8 and SSPS. For significance testing, the alpha level was set to 0.05 (α=0.05).

Results. There were no significant differences in the smelling performance of PC19C patients and the general population. Although hospitalized patients' results were significantly lower ($p = 0.04$). Literature supports our findings and presents other possible risk factors associated with long-lasting olfaction alteration, such as being a female, smoking, or younger age.

Conclusions. The prevalence of olfaction alteration in PC19C patients is not higher than in the general population. Hospitalized patients have higher smelling difficulties than non-hospitalized patients and the general population. Neuroplasticity is a possible explanation for olfaction recovery, as olfaction training treatments' success may entail.

KEYWORDS: Olfaction Alteration, Post-Covid-19 Condition

ABBREVIATIONS

PC19C: Post-Covid-19 Condition

COVID-19: Coronavirus Disease by the SARS-CoV-2 virus

OA: Olfaction Alteration

PVOD: Post-viral olfactory dysfunction

PD: Parkinson's Disease

AD: Alzheimer's Disease

B-SIT: Brief Smelling Identification Test

CC-SIT: Cross-Cultural Smelling Identification Test

UPSIT: University of Pennsylvania Smelling Identification Test

WHO: World Health Organization

CDC: Center for Diseases Control and Prevention

CNS: Central nervous system

ACE: Angiotensin-Converting Enzyme

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1. INTRODUCTION

1.1 BRIEF ANATOMY AND PHYSIOLOGY OF OLFACTION

1.1.1 Anatomy

1.1.1.1 The peripheral olfactory system

The nasal septum is mainly formed by the articulation of the septal cartilage anteriorly with the perpendicular plate of the ethmoid and the vomer bone, as well as the lacrimal bone, the nasal bone, and the frontal process of the maxilla. The top of each nasal fossa is the cribriform plate. The floor of the cavity is formed by the maxillary and the palatine bones. On both sides of the cavity, we can find from front to back, the maxillary, ethmoidal and sphenoidal sinus. The contact with the exterior is provided by the piriform aperture followed by the anterior nares, which continue with the choanae. These last ones enable the air through the nose to the nasopharynx (1). All along the nasal cavity, we can find three prominent structures: the superior, middle, and inferior turbinates. Some people may also have a fourth structure called the supreme concha(2).

Inside the nasal cavity, we can find four different types of epithelia: stratified, respiratory, transitional, and olfactory. The olfactory epithelium is located in the dorsal part of the nasal cavity, and it is formed by non-motile cilia of bipolar sensory neurons and its surrounding mucus, supporting or sustentacular cells, basal cells, Bowman's glands, epithelial cells, and the basal lamina (with blood vessels and autonomic nerves). The bipolar cells' axons are very thin and unmyelinated. All these axons come together in the fila olfactoria or olfactory nerve, covered by glial cells, which pierces the cribriform plate entering the ventral part of the olfactory bulb(2,3).

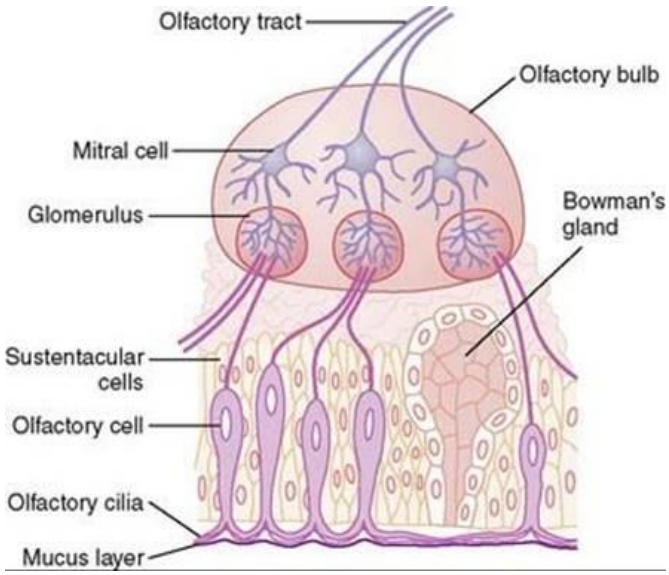

Figure 1. Olfactory epithelium and Olfactory bulb. From Guyton and Hall. Textbook of Medical Physiology, 13th edition(4).

The gene family coding olfactory receptors are the most abundant gene family among mammalians. We can find class I receptors and class II receptors, for water-borne odorants and volatile odorants respectively. Olfactory receptors are G-protein binding receptors(4). These receptors are not exclusive to the nasal epithelia and we can also find them, for example, in human leukocytes. Their interactions have an important influence on the innate and adaptative immune response, blood pressure control, cardiac function, neuronal systems, and especially concerning behavior(5).

1.1.1.2 The central olfactory system

The olfactory fossae are located in the anterior cranial fossa. At the bottom of this cranial fossa, we find the cribriform plate, on which the olfactory bulb is posed. The olfactory bulb is composed of six different layers: the olfactory nerve fascicles, the glomerular layer, the external piriform layer, the mitral cell layer, the internal plexiform layer, and the granule cell columns(2). In the second layer, neurons are organized in glomeruli that are odorant specific. This fact facilitates the recognition of one same odorant by different receptors in the epithelia, which are interconnected among them in the glomeruli(3).

Central olfactory functions are carried by one complex named rhinencephalon or primitive olfactory cortex. This rhinencephalon is composed of several structures: the olfactory bulb, olfactory tract, olfactory tubercle, olfactory striae, and piriform cortex, which is formed by the amygdala, the dentate gyrus, the fasciolar gyrus, and the supracallosal gyrus(2).

1.1.2 Physiology

Humans have a very primitive development of olfaction in comparison to other animals, for example, because the vomeronasal organ, which is a chemosensory organ linked with socio-sexual functions, or the nasal cul-de-sac for a more precise odorant identification, do not exist(2).

1.1.2.1 Peripheral olfaction

The airflow goes through the turbinates where the ciliate bipolar cells catch odorants and send information to the olfactory bulb. These bipolar cells respond to different odorants binding their cilia, depending on their localization inside the epithelia, although a proper description of the type of bipolar cells in each nasal area has not been done yet(2,3). The intracellular process is the following one: G-binding proteins in the cilia, when they get in contact with the odorant, produce an increase of cAMP inside the bipolar neuron, which, by opening a big quantity of Na^+ channels, produces a more positive intracellular voltage, and hence, the depolarization of the neuron, which sends the information towards the olfactory bulb(4).

1.1.2.2 Central olfaction

Bulbar cells are in charge of the transmission and the modulation of the afferent information, which takes the tracks towards more central structures via the mitral and tufted cells. The olfactory afferent information does not synapse at the thalamus and goes directly to the olfactory cortex(2,3). The first synapse usually occurs in the external plexiform layer of the olfactory bulb, where mitral and tufted cells take the afferent information. The axons that exit the bulb through the granular layer, go through the lateral, intermediate, and medial striae to the different structures

composing the rhinencephalon(2). The lack of this mediator synapse facilitates the entrance of any substance into the CNS(6).

1.1.3 The importance of olfaction loss

The loss of olfaction is categorized depending on its severity, which can be grouped into anosmia (total absence of smell) or hyposmia (diminishment of smell) in comparison to normosmia, which is the normal smell capacity(6). The consequences that the lack of olfaction can lead to are: altered food intake (insufficient or excessive), social isolation, memory problems, depression, unawareness of toxins, and gas exposures(3,6).

1.2 POST-COVID-19 CONDITION

COVID-19 was considered a pandemic by WHO in March 2020(7). At the moment this paper is being written (February 2, 2022), 376,478,335 confirmed cases, including 5,666,064 deaths, have been reported to WHO.

The symptoms accepted by WHO as reliable Sars-Cov-2 acute infection **predictors** in august 2021 were coughing, fever, fatigue, and shortness of breath. Nevertheless, olfaction and taste alterations have also been considered as such, due to their high specificity (90%) whenever the COVID-19 diagnosis is done(7,8). The mean prevalence of olfaction alterations (OA) in Covid-19 patients is 50.2%, which raises to 77% whenever objective measurement tests are used(9). Anosmia was the most frequent olfaction alteration (79.6%), followed by hyposmia (20.4%)(7). OA can be the only symptom of the infection(10). Regarding some authors, OA has been more frequently seen among female(7) and younger patients(6), whereas some others support that there is no difference related to age and gender(9).

Although the acute phase and its features have been of great knowledge worldwide, it is already more than a year that patients with long-lasting and incapacitating symptoms have been described. Nevertheless, there was no consensus or defined criteria for the diagnosis of these patients, and hence, this definition has been yearningly demanded by patients and clinicians(11–14).

On the 6th October 2021, the World Health Organization (WHO), published for the first time, a defining clinical case for this long-lasting syndrome. This definition is been given the name of “Post-Covid-19 Condition” (PC19C). It has been based on a Delphi consensus, by gathering the experience and evidence of several experts on the subject. The definition of PC19C has twelve domains and eighty-eight words, and it is the following one: “Post-COVID-19 condition occurs in individuals with a history of probable or confirmed SARS-CoV-2 infection, **usually 3 months from the onset** of COVID-19 with symptoms **that last for at least 2 months** and cannot be explained by an alternative diagnosis. Common symptoms include fatigue, shortness of breath, and cognitive dysfunction but also others that generally have an impact on everyday functioning. Symptoms may be new-onset, following initial recovery from an acute COVID-19 episode, or persist from the initial illness. Symptoms may also fluctuate or relapse over time. A separate definition may be applicable for children.” (15).

The CDC (Center for Diseases Control and Prevention) has published, at the same time, a slightly different definition un the same name (PC19C) which goes "Post-COVID conditions are a wide range of new, returning, or ongoing health problems people can experience four or more weeks after first being infected with the virus that causes COVID-19. Even people who did not have COVID-19 symptoms in the days or weeks after they were infected can have post-COVID conditions. These conditions can present as different types and combinations of health problems for different lengths of time. These post-COVID conditions may also be known as long COVID, long-haul COVID, post-acute COVID-19, long-term effects of COVID, or chronic COVID”(16).

Among the different studies that have been published in the last year, together with this last publication from the WHO and the CDC, a profile of PC19C patients has been defined. According to these publications, women, and those patients with more severe acute viral diseases are at a higher risk to suffer from this condition(15,17). Although some authors support that older patients have longer-lasting symptoms(18), some others suggest that PC19C is more prevalent among young adults(19,20). The role of ethnicity as a risk factor remains unclear(18,21). Most commonly described symptoms in this condition have been: fatigue (12–14,22), dyspnea (12,13,19,22), chest pain (19,22), anosmia, ageusia, headache (12,14,22), anxiety and depression (13,14,22) and

cognitive impairment(13,14). Other less common symptoms are arthralgia, insomnia(22), alopecia, and diarrhea (12), among many others.

According to Da Silva Junior P et al., after one month from the acute infection's resolution, the recovery rate for OA was 79%(7), which supports the idea of it being an acute covid-19 infection's symptom. Nevertheless, some studies have shown OA persistency from 4 to 12 weeks after the recovery from the acute phase(14).

Hence, the objective of this study is to determine whether olfaction alteration is a specific symptom of PC19C. As well as to quantify this alteration, and identify other risk factors that can lead to persistent olfaction loss.

2. HYPOTHESIS AND OBJECTIVES

2.1 HYPOTHESIS

Based on recent literature and the clinical development of Post-Covid-19 condition patients, olfactory alterations may relate more to the acute inflammatory phase, rather than to the long-term effects, and a full recovery is expected between 2 and 6 months after the infection.

2.2 OBJECTIVES

2.2.1 Primary objectives

1. To determine whether Post-Covid-19 condition patients suffer from persistent olfactory alterations more frequently than the healthy population.

2. To objectify this damage with a validated and objective measurement tool as the B-SIT (brief smell identification test).

2.2.2 Secondary objectives

1. To identify risk factors among Post-Covid-19 condition patients to develop persistent olfactory alterations.

2. To identify other risk factors, apart from Sars-CoV-2 infection, that may lead to persistent olfactory alterations.

3. MATERIAL AND METHODS

3.1 SYSTEMATIC REVIEW

3.1.1 Design of the review

A systematic bibliographic review was made regarding persistent olfaction alteration in PC19C, or in default in patients that showed to suffer from any kind, and different time lengths, of persistent symptoms. It is important to mention, that PC19C has not been an established term and phenotype until October 2021, which is why many of the texts included did not agree with the symptom cluster and/or the duration of the persistency.

3.1.2 Bibliographic research

3.1.2.1 Databases and research strategy

The research was made in *the Pubmed* database and *SpringerLink* databases. It was executed on the 16th of February 2022. The selected keywords for the research were: *"post-acute COVID-19 syndrome"* and *"smell"*. They were combined with the Boolean operator *"AND",* and the filters that were implemented were: papers that had been published within a period of a maximum of one year, only articles (no books), in English, Spanish or French, including Meta-Analysis, Reviews, Systematic Reviews, Randomized controlled trials, and Clinical Trials. In *SpringerLink*, the research area of "medicine and public health" was also included as a filter. In total, 56 papers were identified (9 from *Pubmed* and 47 from *SpringerLink*). One of them was duplicated. One of them was removed for being written in German.

3.1.2.2 Paper selection

Among the 54 papers that were initially screened to be in the systematic review a total of 14 were dismissed, 13 of them from the *SpringerLink* result group and 1 from the *Pubmed* group. Ten of these 14 were directly dismissed for their titles: 7 of them were associated with the acute COVID-19 disease, but not with the persistent symptoms, and the other 3 were associated with persistent symptoms, but exclusively focused on

the rheumatologic and musculoskeletal areas; 5 were dismissed after the reading of the abstract: 4 of them did not include the persistent symptomatology, 1 of them described an overlap syndrome with Kawasaki disease in children and the last one described the direct and indirect effects of the pandemic in the elderly population; and finally, the last one was dismissed after the reading of the whole text, since it was only focused on the neuropsychiatric approach of the symptomatology.

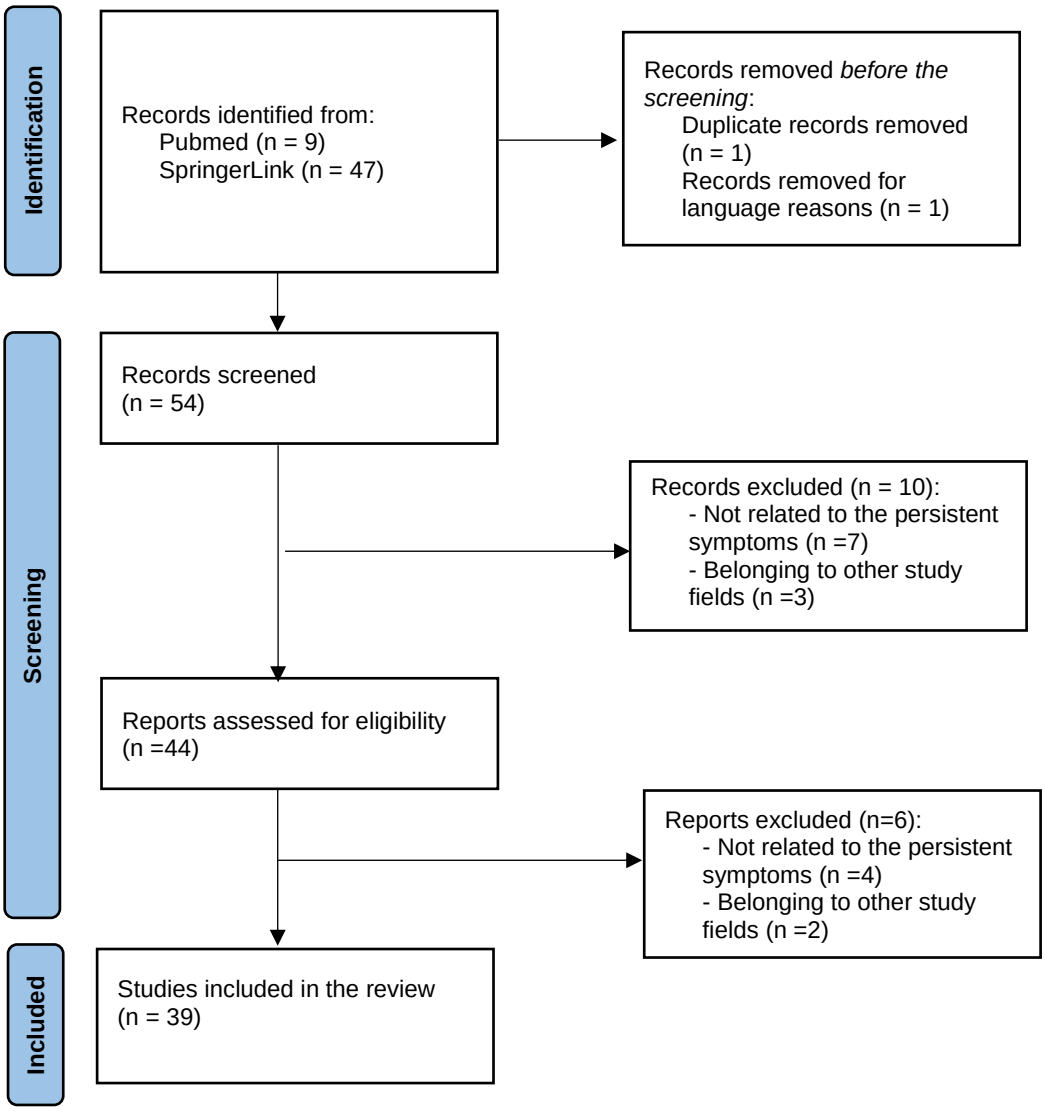

Figure 2. Flow diagram for the included studies (PRISMA 2020).

3.2 CROSS-SECTIONAL STUDY

3.2.1 Cases' and controls' recruitment

This study is an observational descriptive study on 40 patients with PC19C from Cruces University Hospital in Barakaldo, Spain. A comparative study has been made among these patients and 40 healthy control participants. Patients were recruited between 2nd March 2021 and 22nd of August 2021. This study included the first 40 patients from a larger study called "Persistent Covid: defining the phenotype with neurological affection". Participants were recruited orally in the outpatient clinic whenever they entered the study as part of their clinical assistance process for the Post-covid-19 condition.

Inclusion criteria were, a Covid-19 diagnosis confirmed by PCR or by detection of anti-Sars-Cov-2 IgG and/or IgM or a medical report that supports the diagnosis. Moreover, it is required that the patients suffer from any of the following symptoms after 12 weeks after the beginning of the infection: chronic physical and/or mental fatigue, palpitations, sensory disorders such as paresthesia or neuropathic pain, and/or dysautonomic disorders. Besides, the patients had to be aged 18 to 85 and they had to be able to understand the procedures of the study, as well as be able to communicate. Exclusion criteria were patients suffering from serious pathologies that could contraindicate the techniques in this project and patients hospitalized in the ICU during the acute phase. Furthermore, women going through pregnancy or lactation were excluded, as well as patients who suffered from severe trauma, alcoholism, image-based confirmed cerebral structural pathology, and patients carrying a pacemaker. Participation was voluntary and written informed consent was obtained manually. The clinical protocol was approved by the local ethics committee.

Controls were recruited among patients' companions at the outpatient clinic, as well as among volunteer participants recruited in the streets from San Sebastian (Gipuzkoa, Spain) attired by advertising panels. Inclusion criteria for these controls were not having any former Covid-19 diagnosis by PCR or by detection of anti-Sars-Cov-2 IgG and/or IgM or a medical report supporting it. Furthermore, they had to be aged from 18 to 85 and they had to be able to understand the procedures of the study, as well as be able to communicate. Furthermore, women going through pregnancy or lactation

were excluded, as well as patients who suffered from severe trauma, alcoholism, image-based confirmed cerebral structural pathology, and patients carrying a pacemaker. Participation was voluntary and written informed consent was obtained manually.

3.2.2 Olfactory analysis

These patients went through a baseline questionnaire and a Brief Smell Identification Test™ (BSIT® Sensonic © Philadelphia USA) test, to have an objective way of measurement of their olfaction capacity.

3.2.2.1 Questionnaire about olfactory disruptors

The questionnaire was based on manually searched scientific publications through PubMed, MSD manual, NIH: National Institute on Deafness and Other Communication Disorders' web page, and the following reference textbooks: Neurology in clinical practice, (pp 263-270)(Elsevier) and Principios de Neurología, (pp 195-202)(McGraw-Hill Interamericana. The aims of this questionnaire were, on the one hand, to rule out other causes of olfactory alterations apart from SARS-Cov2 infection, and on the other hand, to identify the subjective perception of their smelling and odor discriminating capacity. The questionnaire and its bibliography are included in Annex 1.

3.2.2.2 B-SIT (The Brief Smelling Identification Test^{TM})

The Brief Smell Identification Test (B-SIT) or Cross-Cultural Smell Identification Test (CC-SIT) is a 12 olfactory item test that follows a “scratch and sniff” system (Figure 1). This test was validated in March 1996(23). The booklet contains 12 sheets, each of them with one odor hidden in a brown label. The patient must scrap the label with a pencil to identify the odor and cross one of the four different options proposed for each item. Even if the patient doesn't identify the odor with one of the proposed answers, no item can be left blank. Once the test is finished, the patient must hand the booklet to the researcher, who will compare the answers to a scoring table that standardizes the number of correct answers regarding age and gender, and estimates the olfaction alteration level per patient. This scoring table is even able to identify

malingering, since, as all the items must be answered, just by probability, even a completely anosmic person would get 25% of the answers right. As a result, a lower score the 3 out of 12 would be suspicious (23). The odorants included in the test are the following: cinnamon, turpentine, lemon, chocolate, rose, paint thinner, banana, pineapple, gasoline, soap, and onion.

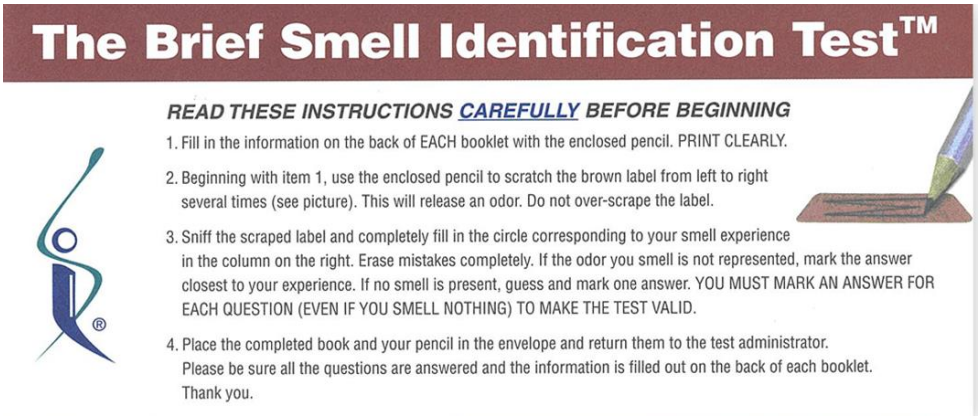

Figure 3. Brief Smell Identification Test™ (B-SIT®) from Sensonics International(23).

The CC-SIT or B-SIT was developed out of the UPSIT (the University of Pennsylvania Smell Identification Test) validated in 1996. The UPSIT test is a 40 item long test that was developed by Dr. Richard L. Doty, Director of the Smell and Taste Center at the University of Pennsylvania. This test, validated in 1984, proved to be able to detect very subtle olfactory alterations. During its validation process, they realized by multiple-regression studies, that age, gender, race, and smoking habits were the most influencing factors regarding olfaction, and nowadays, they are taken into account whenever an interpretation of UPSIT is performed(24). Despite its high accuracy, the UPSIT requires an investment of at least half an hour for its realization, and the odors that are included tend to be too specific regarding the eating habits of some countries, which becomes a problem when it may be used interculturally. The aim of developing the brief test was to be able to measure in a reliable manner olfaction alterations in a less than five minutes and more affordable test. Besides, the 12 odorants included in the B-SIT are supposed to decrease the cross-cultural discrepancies in terms of

nonequivalent exposure to the same odorant in different cultures(25). Although the brief test is more useful for daily practice than the UPSIT, where being short on time is a major issue, it is important to state that the reliability for the olfaction alteration measurements and the malingering detection is much higher with the UPSIT than with the B-SIT(23,25,26). This accuracy has been measured with the Spearman and Brown formula, which correlates the length of a test with its reliability, and the UPSIT scored 92% towards a 72% of the B-SIT(23). This lower accuracy for subtle alterations has a more remarkable impact when the sample for the study is small or patients are being assessed individually based on its results(25).

Other tests that have been used with a lower frequency, and mostly in the United States, for olfaction alteration identification have been: B-SIT-B, Q-SIT, Pocket Smell Test, Open Essence Smell Identification Test™, and Odor pen-based Sniffin' Sticks(25). Likewise, in Europe, the Scandinavian Odor Identification Test (SOIT) and the Smell Diskettes Olfaction Test (SDOF) have been more popular(27). It is important to underline that olfactory testing includes three areas: olfactory threshold, odor identification, and odor discrimination. Although the UPSIT and B-SIT only enable the identification, while other tests, such as the sniffin' sticks test, enable all 3 of them(27), the first two tests are much more frequently used in the international scientific community, mostly due to the lower impact of culture on the results(23).

For our study, B-SIT tests were performed in the outpatient clinic of IIS Biocruces Bizkaia together with another autonomic testing for the "Persistent Covid: defining the phenotype with neurological affection" study.

3.2.2.3 Statistical tools and analysis

Statistics were performed with GraphPad Prism8 and SSPS. For the quantitative variables (such as the number of males and females in each group, and the age distribution) parametric testing has been used, concretely the T student test. From this last test, means and standard deviations were obtained. As the variable used have been multiple, and most of them qualitative, a descriptive analysis of them, chi-square testing, and logistic regression have been performed. Regarding the exact punctuation of the test and the influence of the different variables in this punctuation, as a quantitative variable, a correlation matrix and linear regression were performed for its

study. Whenever the independent variables were studied separately concerning the performance at the B-SIT, chi-square was performed. For significance testing, the alpha level was set to 0.05 (α=0.05).

3.2.3 Ethical concerns

The confidentiality of the data involved in this project will be guaranteed by the single codification of each participant. Juan Carlos Gómez Esteban MD, Ph.D. from the Neurology Service at Cruces University Hospital and Principal Investigator of Neurodegenerative Diseases group at ISS Biocruces Bizkaia, will guarantee the good practice in the use of the Database. It is going to be guaranteed the compliance with the Biomedical Research Law 14/2007, the Personal Data's Protection Organic Law 15/1999 of the 13th of December, and the 1720/2007 Royal Decree of the 21st of December, which approves the Regulations for the Development of the Personal Data's Protection Organic Law 15/1999, and the 2016/679 European Union Regulations and the Board from the 27th of April 2016, this last one regarding natural people's protection in respect of the treatment and free circulation of personal data, so that the 94/46/CE Directive (General Regulations for Data Protection) gets abolished. As a result, no data that could identify any participant is going to be used or made public.

Ethical approval for this study was issued by the CEim (Comité de Ética de Investigación con medicamentos de Euskadi - Basque Ethical Committee for medication Research). Internal code: PI2020210.

4. RESULTS

4.1 RESULTS OF THE SYSTEMATIC REVIEW

As it has been previously mentioned, after having implemented the exclusion criteria for the articles in the systematic review, **thirty-nine** papers were included. Out of these thirty-nine: two were systematic reviews (5,13%), eight were reviews (20,51%), one was a not controlled clinical trial (2,56%), eighteen were cohort studies (46,15%), five were cross-sectional studies (12,82%), four were case reports (10,26%) and one was a test validation article (2,56%).

Among all these thirty-nine studies 6 stated that **olfaction alteration** remained an **acute** symptom that could not be included as part of PC19C (15,38%) (28–33), 8 of them did not mention olfaction alteration while speaking about persistent symptoms (20.51%)(34–41) and 25 of them described olfaction alteration to be reported as a **persistent** symptom(51.28%) (30,36,42–62).

In addition, 9 of these studies focused only on **hospitalized** patients (23,08%) and 5 only on non-hospitalized ones (12,82%). The rest studied both of them.

Regarding the 24 studies that involve olfaction and/or quality of life impact testing and direct field work with patients, 17 of them used non-validated testing methods (70.83%)(29–31,33,41,42,44,45,47,52–56,59,62,63) and the other 7, used **validated methods** (29,16%)(32,37,51,57,62,64,65).

These same twenty-four studies had very heterogeneous **chronology** for symptom testing: 7 studies performed their tests 1 to 3 months after the acute phase or hospitalization discharge(29,47,51,52,55,59,65), 2 studies after 4 months(56,64), 6 studies after 6 months(30,32,41,42,53,57), 5 studies after 7 to 9 months(33,37,43,44,54) and 4 studies after 11 to 12 months(31,45,62,63).

Among all the forty-four studies mentioned above, only Lucidi D. et al., contemplated other independent factors that could modify the olfactory performance, concretely **smoking**(53).

4.2 RESULTS OF THE CROSS-SECTIONAL STUDY

4.2.1 Descriptive statistics

Forty patients and forty controls participated in this study. The mean age of the patient group was 44,63±9,64 years, and the mean age of the control group was 40,8±14,63 years. Among patients 67,5% were women and 32,5% were men, whereas in the control group 72,5% were women and 27,5% were men. There were no statistically significant differences between the two groups regarding age and sex.

Regarding the several risk factors for olfaction alteration, 35% of the patient group and 35% of the control group were smokers. Only 3 of the participants of the study had olfaction alteration-related jobs (a plumber, a firefighter, and a constructor worker),

all of them from the patients' group. Among the patients, 30% had been hospitalized in hospitalization wards during the acute infection. Concerning concomitant illnesses and medication during the study, in the patient group, 30% of the patients had potentially olfaction alteration medication prescribed (66,67% are benzodiazepines, 16,67% are ACE inhibitors, 8,33% are statins, and 8,33% beta-blocker) in comparison to 20% of the controls (in this group 50% were benzodiazepines, 25% was anticonvulsant medication, 12,5% beta-blockers, and 12,5% ACE inhibitors). Equally 15% of the participants in both groups had other concurrent illnesses, such as sinusitis, rhinitis, or ongoing flu.

Attending to the effect of aging on smell, based on current literature (27), it was decided to categorize the patients into three age groups: 20-40, 40-60, and 60-80. Equally, gender-based and smoking habits-based categorization was made, although there's not enough evidence to prove the impact of these two factors on olfaction. The only risk factor that showed statistically significant differences was hospitalization among patients, where 25% of hospitalized patients showed OA in comparison to 10,71% of the non-hospitalized patients. That is why a categorization based on hospitalization during the acute phase was made.

The length of time that passed from the acute phase onset in each patient until the B-SIT performance was also studied. The mean time was 10,43±3,01 months. Only among hospitalized patients the mean time was 10,77±3,30 months.

4.2.2 Analytical statistics

Regarding the causal relationship among the different independent variables and the results in the B-SIT test, chi-square analysis was performed independently for "PC19C results vs control results", "age and B-SIT results", "gender and B-SIT results", "smoking and B-SIT results" and "hospitalization during the acute phase and B-SIT results". The set value for statistical significance was $p<0,05$. The only analysis that turned out to be significant was hospitalization and B-SIT results with a p-value of 0,04. Since the studied sample is parametric ($n>30$), a Pearson correlation coefficient and a linear regression were performed to analyze if the studied continuous independent variables (the amount of time since the onset of the infection until the test was made and age) had any impact on the B-SIT results, but both of them were

statistically non-significant. Logistic regression was performed in order to determine whether any of the studied categorical independent variables (gender, smoking habit, and having been affected by PC19C) had any impact on the B-SIT results, but all of them were again statistically non-significant.

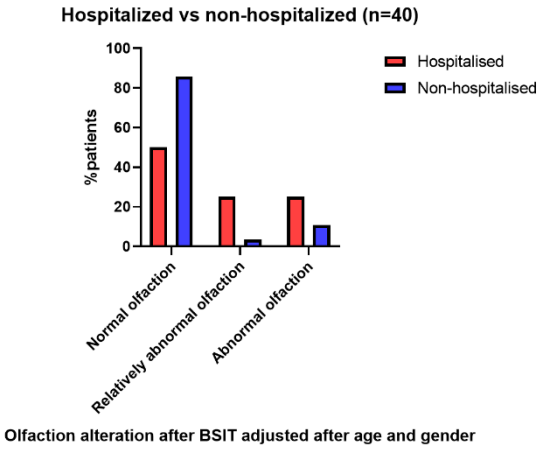

Figure 4. Comparison of the olfaction level between hospitalized and non-hospitalized patients.

According to the main hypothesis of this study, the difference in olfaction performance between the patient and the control group showed to be statistically non-significant, with a p-value of 0,40. **PC19C patients do not have a remarkable impact on their olfaction level, in comparison to the global population**. The mean punctuation for the B-SIT test among PC19C patients was 8,93±1,76, whereas in the control group was 8,40±2,00.

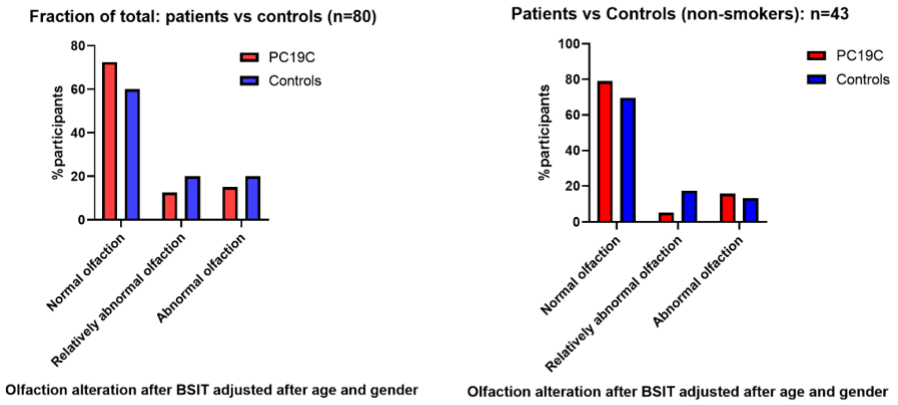

Figure 5. B-SIT results interpretation age and sex-adjusted for all the participants and only for the non-smoker participants.

Although **there is not a statistically significant relation** between smoking, age, gender, and B-SIT results, during the development of the study there are some **tendencies** that are observed among the different groups. Therefore, when it comes to olfaction, PC19C female patients aged 20 to 40 seem to be the most severely affected individuals. Moreover, as is shown in Figure 2, whenever smoking is removed as an independent variable, PC19C patients have more abnormal olfaction results than the control group, whereas the difference between those that tested for normal olfaction becomes smaller.

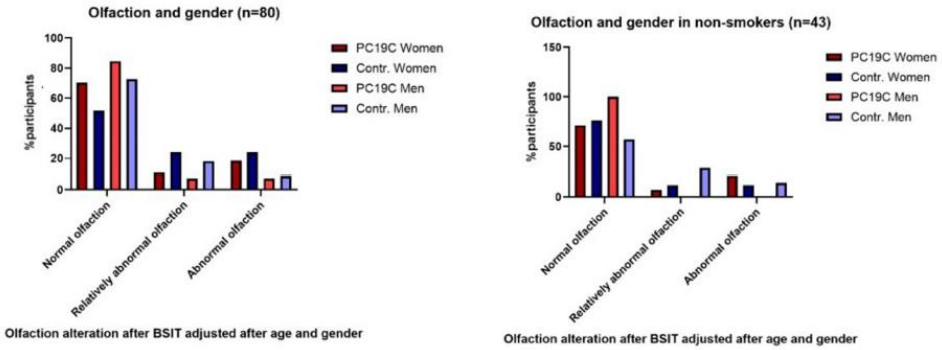

Figure 6. B-SIT results' interpretation age and sex-adjusted for olfaction and gender, and olfaction and gender among non-smokers.

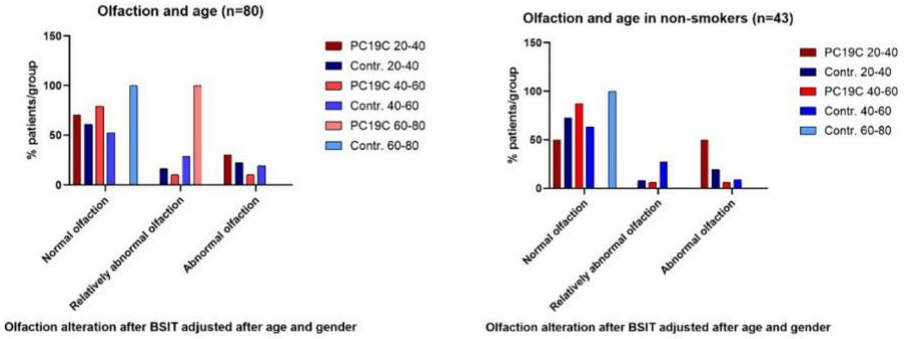

Figure 7. B-SIT results' interpretation age and sex-adjusted for olfaction and age, and olfaction and age among non-smokers.

5. DISCUSSION

5.1 PC19C AND PERSISTENT OLFACTION ALTERATION

Consistently with our results, several studies have shown that olfaction alteration remains an acute-phase symptom, not being especially prevalent among PC19C patients (28–33). On the other side, it is important to mention that olfactory dysfunction has also been described as a persistent symptom in some other studies included in our review (30,36,42–62). Among all the studies that have been considered in the systematic review, there is a variety between 7,3% and 100% of the patients that can remain with olfaction and taste alterations in the long term. Nevertheless, there are several reasons that can explain the heterogeneity of these results, such as the amount of time from the acute viral phase until the olfactory study is performed, the use of subjective testing methods rather than validated ones, and the heterogeneous composition of the sample in terms of age, gender, the severity of the acute disease and comorbidities. As has been mentioned in the results of the systematic review, only 29,16% of the studies that have been included used validated objective methods and only one of them took into account other olfactory disruptors, such as smoking.

Although the evidence in this study does not consider OA as a persistent symptom, it has to be clarified that the recovery period of every described acute symptom does not have the same length. That is why the time at which the tests are performed is so

important. According to this, as is explained in more detail in the results of the review, some studies report olfaction as a long-lasting symptom after only one month after the acute phase, while those that support more the non-persistent evolution of OA have usually been performed one year after the infection. These chronologic discrepancies are again due to the lack of specificity regarding the timing that defined PC19C. Moreover, Nehme M. et al. insisted on the idea that most of the persistent symptoms, including OA, decrease with time, especially 7 to 9 months after the infection(43). In addition to this, Lucidi D. et al. described the persistence of OA in a period of 1 to 3 months, and they stated that while 27% of the patients they studied remained symptomatic one to two weeks after the acute infection, only 5% of them did not experience any recovery in the following 3 months(53).

5.1.1 PC19C and persistent olfaction alteration among hospitalized patients

As it has been shown in our statistical analysis, it is almost unanimously accepted that patients that have been hospitalized during the acute phase have higher rates of long-lasting symptoms, among which we can find olfaction alterations(29,31,34,42,45,50,66). LaVergne et al. found that 5 months after recovery from the acute phase PC19C was observed in 23% of non-hospitalized patients, in comparison to 93% of hospitalized patients(50). In another study, Jacobson et al. showed that among non-hospitalized patients versus hospitalized patients, 46% and 73% of them, respectively, had some impairment in their daily activities(28). Conversely, in research performed with only hospitalized patients, six months after hospital discharge, only 15% of the patients showed impaired olfaction(32).

According to our results, hospitalized patients are more tending to present persistent olfaction alteration. Several studies support our findings. There is some controversy regarding whether the reason for this is the severity of the acute phase (45), the organ failure involved (44), the hospitalization itself (42,62), or all of them together (66). Zuschlag et al. suggest that among patients that have been hospitalized in the ICU, the sequelae are caused by the aggressive treatment received in the acute phase(31), such as high doses of steroids and long-lasting mechanical ventilation(49).

LaVergne et al. observed that 3 to 6 months after the acute infection, 85% of hospitalized patients had some remaining symptoms in comparison to 65% of non-

hospitalized ones. 93% of the hospitalized developed a PC19C, in comparison to 23% of the non-hospitalized(54). In another study, Jacobson et al. showed that among non-hospitalized patients versus hospitalized patients, 46% and 73% of them, respectively, had some impairment for their daily activities in the long term, including smelling, cooking, and eating, after the infection(29). In addition, Zuschlag et al. observed that one-third of the hospitalized patients in their study remained symptomatic one year after hospitalization (31).

Nevertheless, many studies focused on non-hospitalized patients have proved a high prevalence of persistent symptoms in these patients too (33), so other variables, apart from hospitalization, might have a strong impact on the development of PC19C.

5.1.2 PC19C, persistent olfaction alteration, age, sex, and smoking

Though we have not found significant statistical relation between age, sex, or smoking habit and the development of long-lasting OA, there is a clear tendency among our young, female, and smoker patients toward a more frequent persistent OA. But the literature is quite diverse in opinion.

In the same vein, some researchers support that age could be related to persistent OA, without clear evidence(43,49). Interestingly, others strongly state that anosmia and ageusia are related to younger people(53,66), as well as to being a woman(44,45,66) and that it is not correlated with the presence of other comorbidities(42). As Makaronidis et al. explain, although estradiol has proved to be protective against olfactory epithelial damage, women are less likely to recover from OA in PC19C (65). In contrast, some studies deny this association (30).

Luicidi et al. found that smoking was independently associated with a higher risk for persistent OD in PC19C patients. This could be explained, given by the fact that smoking increases the levels of the ACE2 receptors and it may facilitate the viral infection, as well as more severe infection and a higher risk of persistent OD(53). Although, many studies show that the representation of smokers among patients that develop the PC19C is lower in comparison to the general population(6).

5.1.3 PC19C and persistent olfaction alteration: pathophysiology

The big mystery is the etiology and pathophysiological root of OA in PC19C. Some researchers suggest a combination between the direct viral damage, a massive pro-inflammatory state, also known as a "cytokine storm"(44,48,60,67), and metabolic dysfunction, mainly involving mitochondrial activity(33).

As several studies have shown Sars-CoV-2 has a tropism for ACE2 receptors. These have been suggested to be the migration and damage mediators in several organs, including CNS. Even so, olfactory sensory neurons do not express ACE2, nor TMPRSS2, reflecting the possibility of other cell types being the entrance door for Sars-Cov-2 in the smelling nerve. The most implicated cells in this mechanism are the supranuclear cells. The infection of these cells causes sudden inflammation and damage to the olfactory epithelium. Rebholz et al. suggest four entry paths for the virus into the CNS: by anterograde synapsis through the peripheral nerves, directly from the nose to the CSF, through the vagal nerve (from lung or gastrointestinal branches), or the epithelium, and getting to the blood flow or lymph(6). The trigeminal and the vagal nerves, whose neurons contain ACE2 have also been described as possible entry pathways for the virus(67).

Meinhardt et al. proved the presence of Sars-CoV-2 RNA and protein in the nasopharynx and CNS, in autopsies of patients who died due to COVID-19, by immunohistochemistry, confocal and epifluorescence microscopy. In this study published in Nature neuroscience, they showed morphological and thromboembolic alterations in the CNS of infected patients during the acute phase (see images in Annex 2)(68). It has been proven that some pathogens take advantage of the olfactory tract as a migration mechanism, such as the poliovirus and prions at Creutzfeldt-Jakob(6).

In PC19C an overexpression of IL-6, IL-10, and IFN-gamma, as well as an increased CD8+ activity has been seen. All this proinflammatory state leads to the activation of the sympathetic nervous system, which together with the high ACE2 expression in the CNS, triggers a brainstem dysfunction, and therefore, autonomic clinical manifestations (61), which could also involve chemosensory impairment, such as OA (67). This inflammation may also lead to endotheliitis and brain-blood barrier dysfunction(66).

Nevertheless, Tortajada et al. and Horwitz et al. didn't find an association between the severity or the quantity of persisting symptoms and an elevation of C-reactive protein in PC19C patients(32,62).

Ryan et al, in a genetic approach of the etiology, found that patients that had more difficulties in recovery also had genetic alterations in the innate and adaptative immune systems. Patients with the more severe and long-lasting disease had overexpression of genes involved in immunometabolism and inflammation. They showed to have a high response based on Anti-Spike protein antibodies, at least 6 months after infection. In this study, they found an association between PC19C and some persistent changes in the patients' transcriptomes. The findings in PC19C patients were: on the one side downregulation of the genes regarding platelets, monocytes, and myeloid cells, and on the other side, upregulation in the granulocyte, NK cell, and CD4+ and CD8+ T lymphocyte line. In patients with neurological symptoms of PC19C, S100B was overexpressed, which is a neurological damage biomarker. The wide variety in peripheral immune system recovery rates may explain the diverse response and length of PC19C symptoms (30).

Kiatkittikul et al. did an FDG-PET and MRI-based study, where they found vasculitis and altered neuronal connectivity in all the brain lobes. FDG-PET showed higher uptake in multiple lymph nodes, which shows the possible autoimmune etiology of the disease. Patients that presented cognitive dysfunction made a remarkable improvement in the brain metabolic aspect, after a 6 months follow-up by FDG-PET (57).

Rebholz et al. stated that one of the reasons for anosmia having been observed in less severe acute COVID-9 cases might be due to the activation of a higher immune response that would damage nasal epithelia, but would also impair the virus to advance(6). Makarondis et al. found that seropositive patients had fewer chances to recover from OA than seronegative patients. Seropositives showed a recovery of 57,7% at 4 to 6 weeks, whereas seronegative was 72%(52,65).

5.2 OTHER PATHOLOGIES ASSOCIATED WITH OLFACTION ALTERATION

The most usual etiology for smell loss are upper respiratory infections, age-related loss, sinonasal pathology congenital disorders, or head trauma(69). Environmental chemicals, radiotherapy, chemotherapy, surgical procedures, other genetic, neurological, and psychiatric diseases, renal and liver diseases, and hypothyroidism are other less common causes of olfaction alteration(6).

20% of the adult population suffers from smell and taste loss. 10% of the population over 65 has an olfaction alteration and 62-80% over 80. Most of them are physiological, but they can also be related to many diseases(6).

Age-related OA is thought to be multifactorial, since many physiological changes (enzymatic changes in the nasal mucosa, loss of function of odorant receptor cells, and changes in neurotransmission and neuromodulation), together with cumulative damage due to environmental exposure can easily explain this lowered performance(70).

Between 61 and 83% of the patients with chronic rhinosinusitis present OA, due to both obstruction and inflammation in the nasal epithelia(69).

Congenital OA is extremely uncommon (1:10.000) in the general population. One of the most characteristic syndromes presenting with congenital anosmia ad hypogonadotropic hypogonadism is Kallmann syndrome(71).

The most commonly linked neurodegenerative diseases are Alzheimer's disease (AD), Parkinson's disease (PD), and Lewy body dementia. Smell loss precedes in many years (4-6 on average) the onset of cardinal symptoms in these diseases so that it can be used as an early predictor. 90% of early-stage PD and 85% of AD present olfaction alterations. PD patients with anosmia presented structural abnormalities in the amygdala, hippocampus, and primary and secondary olfactory structures at the MRI. Nevertheless, some studies carried out on patients with supranuclear palsy and corticobasal degeneration had no olfaction alteration. There might be a genetic relation between anosmia and PD, where the genes involved might be A53T, PINK1, and LRRK2. L-DOPA treatment did not show any improvement in smell. In AD,

cholinesterase inhibitors are related to a partial improvement in olfactory function. Other neurologic diseases that have shown olfactory alterations are Huntington's Disease and Amyotrophic Lateral Sclerosis(6).

Closer to the phenomenon we are studying, there is a condition called Post-viral olfactory dysfunction (PVOD) which has been seen in patients that survived Sars-CoV-1 and MERS-CoV. These patients are usually in their 30's, and the recovery rate that has been observed is between 32 and 66%, but it usually takes years. Two pathological mechanisms that have been observed in PVOD are: on the one hand, local inflammation of epithelium, and on the other hand, direct damage in the peripheral and the central nervous system involved in olfaction. PVOD patients had also experienced chronic fatigue, dyspnea, and insomnia, very similar to those that the PC19C patients are suffering from(6,66). OA related to upper respiratory infection has been linked to a lower degree of hyposmia, although higher rates of parosmia and phantosmia have been registered in comparison to other etiologies(69).

5.3 FACTORS THAT CAN ALTER THE RESULTS OF OLFACTORY TESTS

There is a big disagreement among different authors regarding the factors that can alter the results of odor perception tests. The only factor they all agree about is aging(24–27), in which a lower performance has been observed from the age of 60 on(27). JF Morley et al. showed that higher cut-off values are required in order to increase tests' sensitivity and specificity in women, suggesting that their olfaction might be sharper(25,26), whereas Delgado-Losada et al. didn't find any significant differences regarding gender(27). Some authors suggest that the performance gap between men and women may be related to estrogens(25,26).

While some studies state the effect of smoking on olfaction alteration(24) others affirm that the performance in several olfaction tests is the same for the smoker and non-smoker population(25,27). In addition to this, polypharmacy is thought to be one of the most common olfactory disruptors in the general population nowadays. Those drugs that have shown a bigger effect on olfaction are benzodiazepines, chemo-therapy, antibiotics, anti-inflammatory drugs, and gastrointestinal drugs. Among recreative drugs, cocaine has been the most involved one (6) The use of all of them has been screened among all the cases and controls in our study. The most commonly

used drug among our patients was benzodiazepines, and there was not a significant difference in the olfactory performance of these patients compared to those that did not use them.

Equally, ethnicity and disparate education level do not seem to provoke an impact on the performance level for olfactory tests(26).

Three of the major impairments in order to study neuronal damage in the olfactory tract via odor identifications test are: On the one hand, cultural and social differences regarding the exposure to each odorant(25). On the other hand, the fact that the human olfactory nerve has more than 400 types of G-protein binding odor receptors, and each of the receptors is able to be activated by more than one odorant. As a result, even if some receptors were damaged, the olfactory cortex would be able to identify the smell. This is due to a feature-detection process, in which other receptors are activated by this same odorant, not enabling to determine the existence of any damage in the olfactory tract. Finally, most of the odorants are composed of more than one chemical particle, so it is very hard to discriminate among the tracts that can be damaged based on the chemicals that these are able to identify(6,25).

At the B-SIT performance, Menon C et al. and Morley et al. found that among the 12 proposed items, turpentine was the least reliable odor, which was only correctly identified by the 66% of the participants, versus the 98% of correct answers that onion got, becoming the most reliable odorant(25,26).

After our results, and based on the number of errors that both cases and controls made during the test, we can observe that the least reliable odors because of being wrong in more than 80% of the cases and 70% of the controls is turpentine, while the most reliable ones are chocolate and onion, both of which have a lower mistake rate than 15% in both groups. One-way ANOVA was performed in order to see if any of the odorants' perception was significantly different between the two groups, but no differences were found.

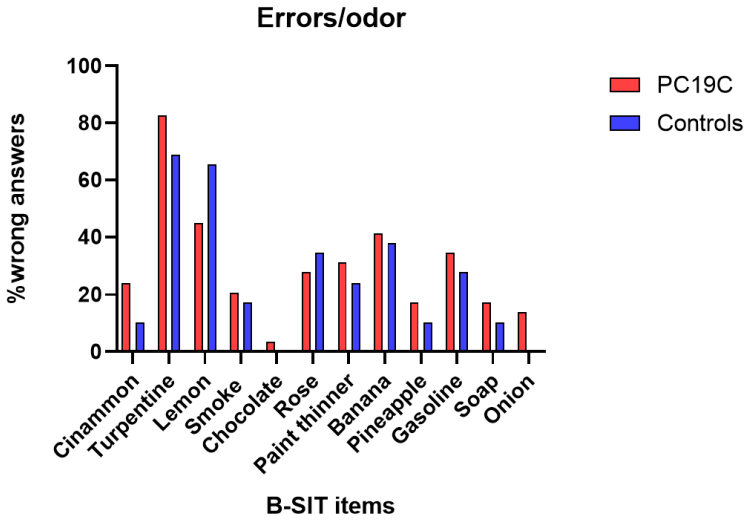

Figure 8. Percentage of wrong answers per each odorant in the B-SIT test.

The B-SIT test categorizes the patients into three categories: normal olfaction, relatively abnormal olfaction, and abnormal olfaction. B-SIT is resistant to sociodemographic and educational differences. Morley et al. found that turpentine was the lowest reliable odor, while onion was the highest one(25).

Chu et al. performed a study with UPSIT where all the patients studied with PC19C had an olfaction impairment (n=16). 50% of them had anosmia and parosmia, while 49% of them had isolated anosmia/ageusia. The most affected odorant was coffee. Parosmia is a marker of poor prognosis for smell recovery. An international survey analyzed that only 7% of the COVID-19 patients suffered from parosmia. Though it has also been seen, that parosmia is related to a good recovery after olfactory training in some studies performed with another post viral anosmia. Lower UPSIT scores are also associated with a more poor outcome regarding smell(64).

5.4 NEUROLOGICAL DYSFUNCTION IN PC19C

The most common neurologic alterations related to COVID-19 have been Bell's palsy, Guillain Barré syndrome, encephalitis, encephalomyelitis, seizures, and stroke. Patients presenting neurological manifestations during the acute phase have higher

chances to develop PC19C(48). Patients with Parkinson's Disease are more vulnerable to having long-lasting neurological manifestations.

Among neurological manifestations, headache has been the most frequent: holocraneal and continuous headache, without nausea or photo nor phonophobia. Other neurological manifestations are asthenia, instability, and cognitive impairments (such as memory troubles, executive functions deterioration, and naming problems(14). Cognitive impairment has been reported as an acute and chronic symptom of COVID-19. This mainly consists of memory (principally working memory) and concentration problems(67).

Baig et al. foresee that patients with long-lasting neurological symptoms will have significantly lower recovery chances(46).

5.5 OTHER CLINICAL MANIFESTATIONS OF PC19C

Apart from OA, PC19C includes a wide variety of symptoms. Caspersen et al. did not see a significant difference in long-lasting symptoms' frequency between the first and the second COVID-19 waves(45).

5.5.1 Chronic fatigue and dysautonomic syndrome

Fatigue is the most common symptom of PC19C. The risk factors related to chronic fatigue in PC19C are male gender, hospitalization during the acute phase, and comorbidities(13).

Dysautonomic symptoms are also common among PC19C patients. Some Postural orthostatic tachycardia syndrome (POTS) cases have been described. Other dysautonomic manifestations have been: dermographism, trembling, diarrhea, and facial blush(14).

5.5.2 Gastrointestinal, cardiological, and respiratory symptoms

Gastrointestinal symptoms have been detected 5 months after the acute infection in patients that suffered diarrhea during the acute phase(34).

Regarding respiratory sequelae, 2 months after the acute infection 53% of the patients in one study still showed CT-scan- based pulmonary abnormalities(66). Dyspnea as a persistent symptom has been associated with persistent structural lung changes(31).

Though ischemic and arrhythmic cases, as well as a few myocardiopathy cases, have been described in the acute infection. No cardiological or coagulation alteration is included in PC19C. That is why prophylactic anticoagulation is not recommended in patients with no clot risk(14).

5.5.3 Neuropsychological symptoms

Depression, anxiety, and higher substance abuse have been seen in COVID-19 and PC19C patients mainly due to a post-traumatic stress disorder state(67). Lemhöfer et al. showed that 50% of their patients mentioned some limitations in their daily life, being the most of them of psychological etiology and related to anxiety and depression(29). After Amdal et al. elderly patients suffered more often from neuropsychological problems in comparison to the younger ones in PC19C(28).

The other way around, neuropsychological symptoms during the acute phase are also related to a higher risk of developing PC19C(44).

5.5.4 PC19C in children

51% of the children in this study showed at least 1 long-lasting symptom. Children have lower chances to develop PC19C than adults, being more likely if they are older or have been symptomatic during the acute phase. The most affected age range was from 10 to 18 year-olds(55). In children, mostly adolescents, the most commonly persisting symptoms have been: chronic fatigue, anosmia, depression, weight loss, sleep disturbances, concentration troubles, rhinorrhea, abdominal pain, and diarrhea(59). They have also presented sequelae of neurological disorders such as sphincter dysfunction and facial muscle weakness and/or palsy. Though most the children do not develop long-lasting symptoms and very little data is available, because very few studies have been performed. In a study performed by Molteni et al. anosmia was the second most common PC19C symptom, which would appear in a later phase of the condition in comparison to the rest of the symptoms. OA has been

the debut of acute illness in 14% of children. And it has been described to be persistent for periods between 1 and 5 months(58). Being older, myalgia, and having been hospitalized during the acute phase were associated with a higher risk to develop PC19C(42).

5.6 PC19C AND VACCINATION

It is evidence-based to say that vaccination against Sars-CoV-2 prevents severe symptoms in the acute infection, but it is unclear the protective effect it may have in preventing PC19C. According to one study, performed with 2094 participants, 30,8% recovered fully from their symptomatology after vaccination, 4.7% improved, 28,7% remained the same and 3,3% worsened(36). According to Munblit et al., vaccination may help reduce the risk to develop PC19C, by helping the immune system to eliminate a higher amount of virus and avoiding a pathological immune response, but the long term response to the vaccination effects is yet to be seen(60)

5.7 THERAPEUTICAL APPROACH FOR OA IN PC19C

Olfactory training has shown successful outcomes in OA due to chemosensory alterations in other post-viral and metabolic pathologies(49). Although there are other therapeutical possibilities, such as medical (corticosteroids, theophylline, alpha-lipoic acid, and oral zinc) and surgical treatment, they have not shown a long-lasting effect. The most effective option has been olfactory training, where neuroplasticity is supposed to be the key target of the treatment(6,65,69).

In general, there are no evidence-based treatments and interventions defined for PC19C patients. A multidisciplinary intervention must be taken into action: neuropsychological therapy, physical rehabilitation (including musculoskeletal, respiratory, and cardiological rehabilitation) and pharmacological means may be necessary (48).

5.8 LIMITATIONS OF THIS PROJECT

We had several limitations while the development of this project. First of all, the project started in March 2021, and there was not an agreed definition of PC19C until October 2021. This produces several problems for establishing inclusion and exclusion

criteria for participants, as well as for the determination of objectives of the study. In addition to this, some of the odorants in the B-SIT test were unknown or mistaken by the participants, despite their olfaction performance. And finally, the sample size was limited (n=80).

6. CONCLUSIONS

Although, olfaction alteration is a determinant symptom of COVID-19, its prevalence among PC19C patients is not higher than in the general population.

Hospitalized patients have higher smelling difficulties than non-hospitalized patients and the general population. This hospitalized patients also present several other disruptive symptoms that may be associated with the severity of the disease and the aggressive treatment received during hospitalization.

Further study is needed in order to reassure that there is no permanent damage in those CNS structures involved in olfaction, as a result of the viral infection or the inflammatory response, being neuroplasticity a possible explanation for olfaction recovery, as olfaction training treatments' success may entail.

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ANNEX 1: QUESTIONNAIRE ABOUT OLFACTORY DISRUPTORS

PRE B-SIT QUESTIONNAIRE

DATE:

NAME:

GENDER:

☐	Male
☐	Female
☐	Other

Age:

☐ <20

☐ 20-40

☐ 40-50

☐ 50-60

☐ 60-80

☐ >80

Make a cross if you have ever consumed any of the following drugs or toxics:

	Antiepileptic drugs
	Antiarrhythmic drugs
	Antihypertensive Drugs
	Benzodiazepines
	Statins
	Antithyroid drugs
	Antibiotics (such as: ampicillin, azithromycin, ciprofloxacin, tetraciclins)
	Chemotherapy
	Head and Neck Radiation Therapy
	Cocaine

Do you or have you ever smoked? If it is so, how often and for how much time have you smoked?

Make a cross if you suffer from any of the following health conditions:

	Recent airway infections
	Sinusitis
	Parkinson's Disease
	Epilepsy
	Alzheimer's Disease
	Huntington's Disease
	ALS (amyotrophic lateral sclerosis)
	Any psychiatric condition
	Hypothyroidism
	Kidney diseases
	Liver diseases

Cross YES or NO if you have worked during most of the time of your professional career in any of the following occupations: foundry, photographic laboratory, mining, construction, a job involving pigments and paint, hairdressing, furriery, exterminators, drug preparation, thermometer reparation, a job involving barometers or machines related to mercury, minerals and metal processing, battery fabrication, machining, match fabrication, cement fabrication, pyrotechnics, printing, glass processing, gasoline and tank preparation or agriculture.

	YES
	NO

Mark with a cross if you suffered from any of the following symptoms during the acute phase of the COVID-19 infection:

	Fever
	Chills
	Cough
	Sputum production
	Shortness of breath

	You have been hospitalized

From 1 to 10 how would you mark you olfaction before suffering from COVID-19:

From 1 to 10 how would you mark your olfaction just after recovering from the acute phase of the infection:

From 1 to 10 how would you mark your olfaction today:

Date when you started with COVID-19 symptoms:

Recovery date of the acute infection:

Date when you perceived olfaction alterations for the first time *(if you never perceived any olfaction alteration make a cross)***:**

SIGNATURE OF THE PARTICIPANT:

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ANNEX 2: IMAGES FROM THE ARTICLE FROM MINHARDT ET AL. IN NATURE NUROSCIENCE

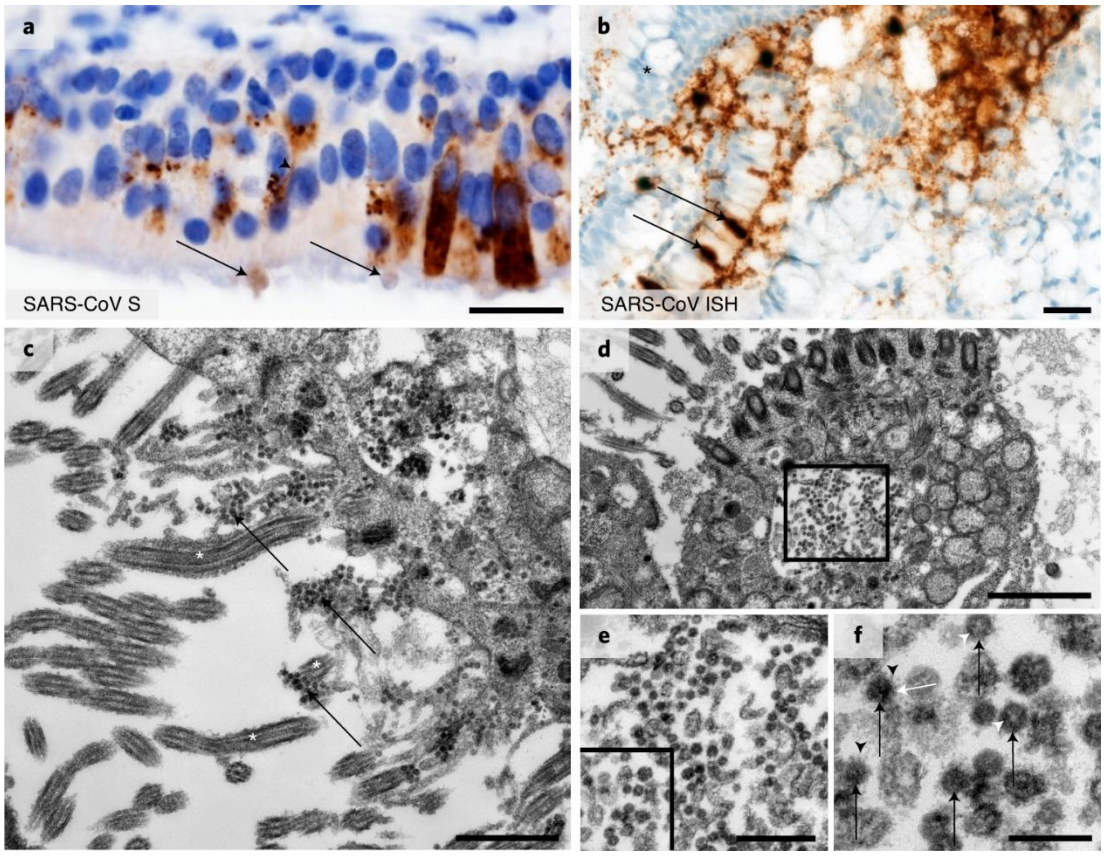

Figure 9. Immunohistochemistry- in situ hybridization- and electron microscopy-based detection of SARS-CoV within the olfactory mucosa. a, CoV antigen detected by anti-SARS-CoV S protein antibodies (brown, individual P30) exhibits a cytoplasmic, often perinuclear, signal for CoV-positive cells resembling epithelial cells and cells harboring dendrite-like projections (arrowhead) with tips (arrows), which morphologically qualify as OSNs. b, SARS-CoV-2 RNA ISH showing intense signals in the mucus layer and cells (arrows) of the epithelium (asterisk) (brown, individual P15). c–f, Ultrastructural images of re-embedded FFPE material showing numerous extracellular CoV particles (c, arrows) attached to kinocilia (c, white asterisks) and intracellular CoV particles (d–f, increasing magnification) in a ciliated cell (individual P15, punch biopsy from the area in b). In e and f, intracellular CoV particles are located within cellular compartments of different sizes and are similar in their size and substructure. In f, at high magnification, five particles in this region show a particularly well-recognizable substructure (black arrows) that includes characteristic surface projections (black arrowhead), a heterogeneous and partly granular electron-dense interior, most likely representing RNP (white arrowheads), and a membrane envelope (white arrows). Scale bars: 20 µm (a), 50 µm (b), 1 µm (c), 2 µm (d), 500 nm (e) and 200 nm (f). **From Meinhardt et al. 2021** (68)

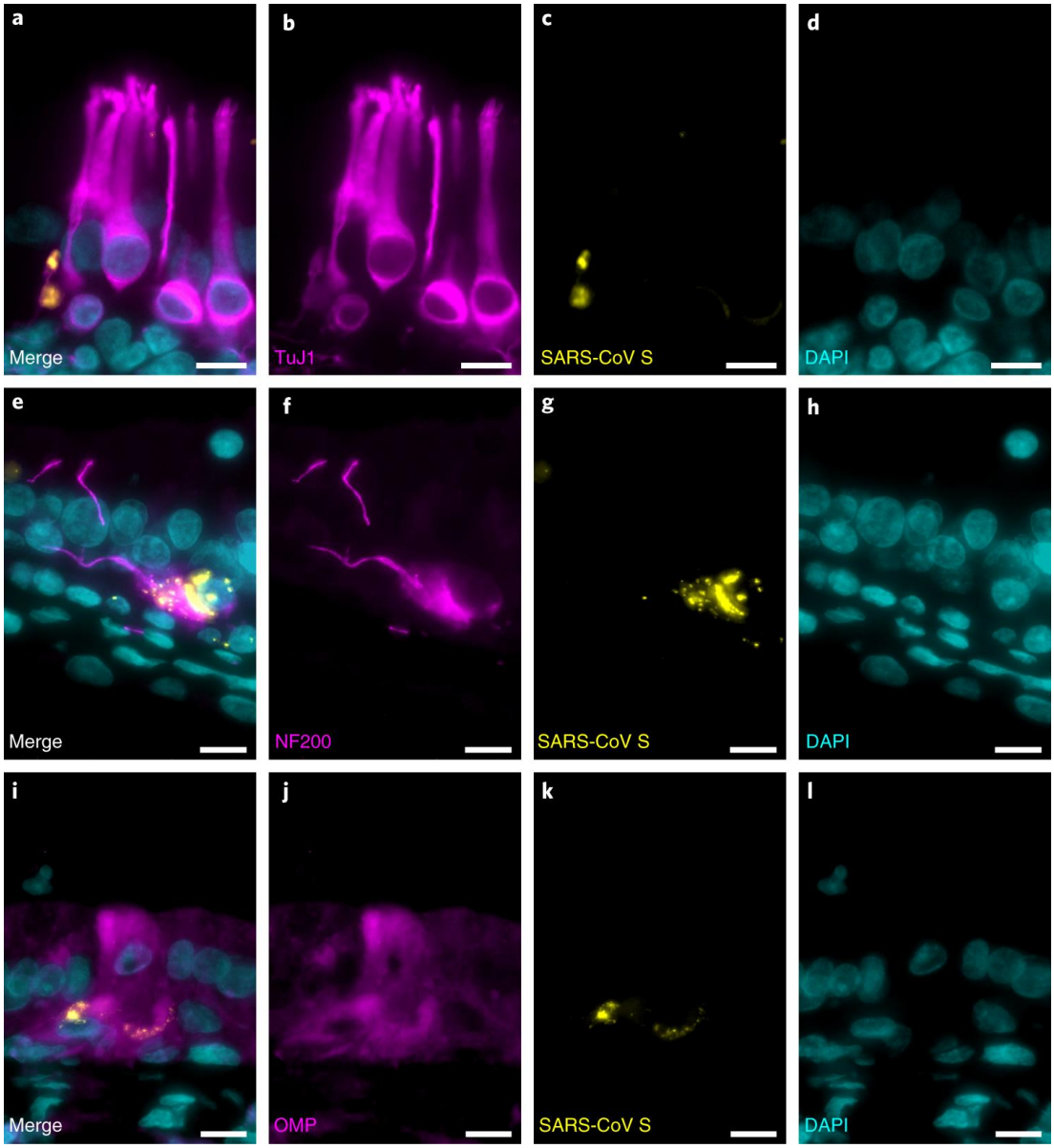

Figure 10. Colocalization of SARS-CoV spike protein with neural/neuronal cells in distinct olfactory mucosa samples from individuals with COVID-19. a–l, Representative maximum-intensity projections of confocal (a–d and i–l) or epifluorescence (e–h) microscopy images of olfactory mucosa showing intracytoplasmic staining for SARS-CoV S protein within TuJ1+ (a–d, individual P27), NF200+ (e–h, individual P27) and OMP+ (i–l, individual P27) OSNs. Staining for TuJ1, NF200 and OMP (magenta, Alexa Fluor 488) marks cells of neuronal origin, staining for SARS-CoV S protein (yellow, Alexa Fluor 555) visualizes the presence of SARS-CoV and DAPI staining (petrol) identifies all cell nuclei (n = 3 individuals with COVID-19 (P27, P30 and P32) were analyzed; n = 2 individuals without COVID-19 served as controls; shown are representative images from P27). Scale bars, (all panels) 10 µm. **From Meinhardt et al. 2021** (68)**.**