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What smell and taste disorders by SARS-CoV-2 do we know? Predictive value of the Venezuelan Olfactory Test and RT-PCR molecular analysis in COVID-19 infection*

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Abstract

Background: Smell and taste disorders are reported very frequently and at an early stage in SARS-CoV-2 infectious disease. These symptoms could be sensitive and specific to establish possible severity of the infection, and may suggest the flow of decisions as to further therapy.

Objective: We asked whether smell and taste impairment are earlier and more sensitive symptoms than the RT-PCR molecular assays for SARS-CoV-2 detection.

Methods: Subjects (N=275) with a probable COVID-19 diagnosis were classified as follows: Symptomatic with chemosensory dysfunction, symptomatic without chemosensory dysfunction, and asymptomatic. Validated unbiased testing of the chemosensory dysfunction was performed by means of the Venezuelan Olfactory Test and taste test. Nasal swabs and blood samples were analyzed by RT-PCR molecular analysis a rapid diagnostic test to detect the SARS-CoV-2 virus and viral antibodies, respectively. Smell and taste testing and RT-PCR were performed every 3 to 5 days to patients until full recovery.

Results: Out of 144 patients that were positive for SARS-CoV-2: 45.83% had COVID-19 symptoms, smell and taste disorders; 23.61% had COVID-19 symptoms but not smell or taste disorders, and 30.55% were asymptomatic. Mild hyposmia and hypogeusia were frequently associated with SARS-CoV-2 symptoms. Recovery from chemosensory dysfunction occurred between day 3 and 14. RT-PCR becomes negative after 21 days. The Venezuelan Olfactory Test and taste test has a 61.68% positive predictive value, 45.83% sensitivity, and 68.7% specificity for SARS-CoV-2.

Conclusions: Smell and taste disorders are associated symptoms with SARS-CoV-2 infection, but not a predictor of the disease, as compared to the molecular RT-PCR test.

Key words: COVID-19, Smell, Taste, diagnosis, RT-PCR, olfactory test, anosmia

Introduction

Since the beginning of the pandemic SARS-CoV-2 infection, treatment of patients with COVID-19 focused on the management of fever, cough, shortness of breath, and respiratory failure. Importantly, there is a growing set of observations that suggest additional symptoms including a wide range of concurrent neurological manifestations, such as anosmia, disgeusia, seizures, stroke, confusion, encephalopathy, and total paralysis [1 – 6]. Noticeable, up to 20% of COVID-19 patients require intensive care unit (ICU) admission due to their neurological issues, and COVID-19 patients in ICU who have neurological deficits, are at a higher risk of mortality [7].

Primary findings from Mao et al. established neurological manifestations of hospitalized patients with SarS-CoV-2 infection in Wuhan, China [5]. Awareness of olfactory and gustatory impairment as a potential early symptoms of COVID-19 infection, e.g., "loss of taste and smell" was soon added to the list of symptoms that may appear 2 to 14 days after exposure to SARS-CoV-2 virus. For instance, the American Academy of Otolaryngology–Head and Neck Surgery (AAO-HNS) released the COVID-19 Anosmia Reporting Tool for Clinicians [8 -10]. Strikingly, anosmia was the initial symptom in around 27% of cases and was present in 73% of cases prior to laboratory diagnosis of COVID-19 [11]. As a consequence, at early stages of the SARS-CoV-2 infection, the smell and taste disorders seem to have predictive value as to the evolution of the infection [12].

The presence of smell alterations associated with a viral infection is not new in otorhinolaryngology; many viruses may cause olfactory dysfunction due to an inflammatory process of the nasal mucosa and the development of rhinorrhea. Among the viral agents associated with these alterations are rhinovirus, parainfluenza, Epstein-Bar, and some coronavirus [13, 14]. So far, the physiopathology of smell and taste disorders in the SARS-CoV-2 infection is still under scrutiny.

The SARS-CoV-2 virus may entry into the cells through the host angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease family member II (TMPRSS2) receptors expressed in all kinds of cells of the respiratory epithelium of the olfactory mucosa [15]. This early finding suggested that the olfactory damage mechanism was non-neural in nature. However, recent findings from Meinhardt et al., have describe at least two other possible ways of SARS-CoV-2 infection to CNS. A transmucosal pathway which appears to conduct viral particles axon associated from the olfactory neuroepithelium into the brain through the cribiform plate to olfactory bulb and more discrete nuclei of the olfactory pathway; and invading directly olfactory sensory neurons [16, 17]. Otherwise, the damage to the sense of taste appears to be directly on the taste receptor and as the result of the production of cytokines that irritate the trigeminal and glossopharyngeal nerves that transmit sensory signals to the central nervous system [18].

Prevalence of olfactory (52.73%) and gustatory (43.93%) dysfunction has been established in recent meta-analysis studies of data, taking into account self-report and validated surveys [11]. However, only 4 studies have a confirmed diagnosis of SARS-CoV-2 infection by RT-PCR molecular methodology [11]. Other meta-analysis of data determined prevalence of smell and taste impairment of around 31% in severe and 67% inmild-to-moderate symptomatic patients. The loss of smell and taste preceded other symptoms in 20% of cases and it was concomitant in 28% of individuals [19]. In many of these studies analyzed, patients were not followed up systematically until recovery, nor was the relationship proven by SARS-CoV-2 RT-PCR test, or COVID-19 antisera rapid diagnostic tests [11, 19]. As a consequence, these factors may have led to an overestimation of the symptom as predictive factor of the disease.

Taking into a consideration that previous reports were based mainly in surveys and phone interviews, we asked whether smell and taste disorders determined by an unbiased validated testing are predictive and associated with SARS-CoV-2 infection. COVID-19 diagnosis was clinically established and confirmed with SARS-CoV-2 RT-PCR molecular and immunoglobulin detection by rapid diagnostic testing methodology. Smell and taste multiple evaluation was accompanied with the follow up and comprehensive observation of the patient's recovery from the COVID-19 infection. We have addressed whether smell and taste disorders are an early and more sensitive biomarker than the RT-PCR test for diagnosing the SARS-CoV-2 infection.

Materials and methods

Population

A sample of 275 individuals between 19 and 65 years was examined between March and August 2020 (Table 1). Data reported in this study take into a consideration subjects that completed all analysis until recovery criterion was reached. The following were collected for all subjects at the beginning of the study protocol: 1) Clinical record, including age, gender, epidemiology background on the basis of travel and contact with positive cases, questions about suggestive respiratory signs and other symptoms, presence or not of smell and/or taste disorders. 2) General otorhinolaryngology (ORL) physical examination, Venezuelan Olfactory Test (VOT) [20-22], and Basic Taste Test evaluation. 3) Nasopharyngeal swab for SARS-CoV-2 by RT-PCR molecular analysis and detection of SARS-CoV-2 antibodies by rapid diagnostic test (RDT).

In this study, data was collected from patients coming to the walking clinic which declared smell and taste discomfort around the time when cases of COVID-19 started to be known in several hospitals and health centres in Venezuela. Relevance of these symptoms were lately acknowledged and associated with other more common symptoms of SARS-CoV-2 infection. Sampled population were recruited and followed-up every 3 to 5 days,

to verify their overall clinical condition. Smell and taste tests were carried out, blood samples were drawn to perform RDTs, and nasopharyngeal swabs for RT-PCR tests, until they met the recovery criterion. The recovery criterion is met when one of the following conditions is fulfilled: 1) The relative smell and taste test score is at the highest level and the RT-PCR test is negative. 2) It has been established that there is no permanent smell or taste disorder.

Clinical assessment

The clinical assessment of patients with probable COVID-19 diagnostic followed the guideline suggested by the National Institute of Health of the United States of America (NIH-USA SARS-CoV-2 assessment guidelines).

Common symptoms found in patients with diagnosis of SARS-CoV-2 infection

Symptomatic with smell and taste dysfunction by Venezuelan Olfactory Test (VOT) (SyMVOTT+, N=107)

General symptoms as described: headache, cough, fever, general malaise, chest pain, dyspnea, myalgia, arthralgia, shivering, hyporexia, nausea, vomiting, diarrhea and any other symptom described in the literature that might be present and/or suggest a SARS-CoV-2 infection. Positive smell and taste dysfunction according to the VOT grading.

Symptomatic without smell and taste dysfunction by VOT (SyM-VOTT-, N= 61)

General symptoms as in the previous paragraph. Negative smell and taste dysfunction according to the VOT grading

Asymptomatic (AsyM, N= 107)

Patients who did not present with any prior symptoms or signs associated with the coronavirus.

VOT and taste test

The VOT is a smell test that was adapted for Venezuela from the smell identification test of the University of Pennsylvania (20). The Venezuelan short test is based in 10 odorants that are commonly recognized by Venezuelans and which were validated during a prior exploratory study [21]. On the basis of identification and discrimination of odors presented to patients, the VOT provides a relative grading scale as follows: Normosmia (8-10), mild hyposmia (6-7), moderate hyposmia (5-4), severe hyposmia (2-3), and anosmia (0-1).

The taste test consists in recognizing the 5 universally accepted basic tastes [22]. If the patient cannot recognize the tastes, he is diagnosed with ageusia, and hypogeusia if the patient recognized up to 4 tastes.

Rapid Diagnostic Test (RDT) to determine expression of antibodies generated to SARS-COV-2 virus antigens

Diagnostic tests Nº IFU-COVID3-01 (Nhui deep blue Medical Technology co. Batch: 20200307) were used to determine IgG/IgM antibodies. This kit uses a recombinant SARS-CoV-2 antigen conjugated with colloidal gold, which can interact with antibodies circulating in blood or in a serum or plasma preparation.

Table 1. SARS-CoV-2 sampled population. Distribution by gender and age.

	Males		Females	
	151		124	
Age Range	N	%	N	%
Age Range	N	%	N	%
18-24	27	9.81	16	5.81
25-31	41	14.90	32	11.63
32-38	31	11.27	28	10.18
39-45	15	5.45	19	6.90
46-52	16	5.81	9	3.27
53-59	17	6.18	10	3.63
60-66	4	1.45	10	3.63

Sample Collection for RT-PCR Testing for SARS-CoV-2 detection

Samples were obtained from patients by nasopharyngeal swab strictly following the biosecurity protocol. Then, they were placed in YOCON viral transport medium, batch Y25200101, and kept at a temperature between 2 and 8oC until they reached the reference laboratory. Samples collected for RT-PCR testing for SARS-CoV-2 detection were processed by the Virology Service of the "Rafael Rangel" National Hygiene Institute of the Ministry of the People's Power for Health in Caracas, Venezuela.

Ethical approval

Collection and analysis of data were approved by the Bioethical committee of the "Carlos Arvelo" University Military Hospital of Caracas. All studies were conducted in compliance with the Declaration of Helsinki, and all participants signed an informed consent. The current study included participants for which there was full information on multiple SARS-CoV-2 measures and key outcomes, including psychosocial factors, chronic medical conditions, and socio-demographic factors.

Statistical analysis

We asked whether there is a relationship between the SARS-CoV-2 infection and the presence or absence of symptoms. Chi squared (χ^2) tests were used to find the most frequent type of symptom under COVID-19 infection; and to evaluate the relationship among type of olfactory disorder, age and gender. In the case of the association between two variables, when the result of the χ^2 test was positive, that is to say, the variables were

Table 2. Association of chemiosensorial impairment with RT-PCR molecular analysis.

	SyVOTT+	SyVOTT-	ASyM	N	SyVOTT+	SyVOTT-	ASyM
					Standardized residuals		
PCR+	66	34	44	144	**2.56***	0.37	**-2.88***
PCR-	41	27	63	131	**-2.56***	-0.37	**2,88***
n =	107	61	107				

$\chi^2 = 9.19$, df = 2, $p < 0.05$. * Standardized residuals: positive value indicates significance at $p < 0.05$ of association between chemiosnesorial dysfunction and positive RT-PCR results for SARS-CoV-2 virus infection.

dependent on, or associated to one another, a standardized residuals analysis was carried out. This analysis allows to determine in a significant manner which cell or frequency contributed more to the rejection of the null hypothesis in the χ^2 test. Additionally, it allows to find out which cells deviated significantly from the expected value. Any deviation value higher than ±1.96 from the normal distribution is considered significant. χ^2 tests and standardized residuals analyses were carried out in R with the chisq.test function of the stats.package [23].

A post-hoc power analysis was performed for χ^2 tests performed with G* Power 3.1.9.4 statistical package [24]. Power analysis (1-β) for the χ^2 tests with $\alpha = 0.05$ and size effect index (w) was also calculated for further comparisons. W is not associated with contrasting distributions H0 and H1. The effect size index W as used to determine probability of rejecting either H0 when is fair to reject it. The W index values ranks W = 0 (none association) and $Wmax = \sqrt{1 - r}$ highest value of association between variables where *r* is the lowest value of divergence in the contingency. Pearson's χ^2 test and Fisher's exact test were used to evaluate between-group differences in two categorical variables. Descriptive statistics was used in the analysis by age group and type of chemosensory impairment. The predictive value, sensitivity and specificity of diagnostic tests used were calculated on the basis of the Wilson score method using the OpenEpi, version 3 software. Diagnostic Test Open code Calculator.

Results

Population demography

The most frequent age group among study subjects was the 25 - 38 years old, average age of the population was 33.63 ± 5 years old, and male (54.90 %) were more frequent than female (45.09 %) subjects (Table 1).

Frequency of SARS-CoV-2 symptoms in the sampled population

RT-PCR molecular tests were performed on the 275 subjects who had gotten also smell and taste tests. RT-PCR+ for SARS-CoV-2 was detected in 144 subjects, while 131 were RT-PCR- (Table 2). In the RT-PCR+ group, clinical symptoms (SyM) and dysfunctional smell and taste (SyMVOTT+ group) were found in 45.83% (66/144 subjects), in contrast symptoms without chemosensory deficits (SyMVOTT-) was determined in 23.61% (34/144). Interesting, in the studied population RT-PCR+ without symptoms (ASyM+) were found in 30.55% (44/144) subjects. Surprisingly, 41 over 131 patients with COVID-19-associated clinical symptoms and smell and taste impairment (SyMVOTT+) were negative for the RT-PCR SARS-CoV-2 test (41/131; 31.30%). In addition, 20.61% subjects with clinical symptoms did not have chemosensory impairment (SyMVOTT-; 27/131). In this RT-PCR- group, 48.10% were asymptomatic subjects (63/131; AsyM) (Table 2). These findings pointed out that olfactory and taste disorders were more frequent among the COVID-19 symptomatic population.

The higher number of RT-PCR+ patients with (66/144) and without (34/144) olfactory and taste disorders, both with symptoms of the infection indicates that the relative effectiveness of RT-PCR tests in confirming the COVID-19 infection is 75%. In contrast, if the large number of PDR- (197/275; 71.63%) individuals in the whole sample is taken into account, the relative effectiveness of this test is 28% (results not shown). This finding confirms reduced reliability of PDR tests for diagnosing coronavirus infection.

The frequency of subjects in the SyMVOTT+, SyMVOTT- and ASyM groups as a function of the presence of chemosensory symptoms and the positive molecular test results was heterogeneous ($\chi^2 = 9.19$, df = 2, $p < 0.05$). These findings were suggestive that general symptoms and chemiosensorial dysfunction were associated, with smell and taste impairment in the case of SARS-CoV-2 infection (Table 2). These clinical outcome is consistent with results from standardized residual analysis. The chemosensory dysfunction is very frequent in RT-PCR+ subjects and, in this sample, very few subjects who also were RT-PCR- were asymptomatic (Table 3, Figure 1). Post-hoc power analysis ($\lambda = 9.61$, df = 2, $p < 0.05$) was indicative of $p = 0.798$, ($\alpha = 0.2$), this result suggests that our sample is sufficient to support our findings. On the other hand, olfactory and taste disorders are infrequent in the general population (Table 3).

Table 3. Predictive value of VOT and PCR for diagnosing SARS-CoV-2 infection.

	SyVOTT+	(SyVOTT-) + (ASyM)	N
RT-PCR+	66	78	144
RT-PCR-	41	90	131

χ^2- = 13.796, df = 2, p <0.05

Table 4. Clinical grading of SARS-CoV-2 disease and a positive Venezuelan Olfactory Test and Taste Test (VOTT+).

Clinical Grading of COVID-19 Disease	VOTT+ (N= 66)
Mild	65 (98.48%)
Moderate	1 (1.51%)
Severe	0
Critical	0

Predictive value of VOTT

When the frequency of patients with olfactory and taste disorders that were diagnosed with the VOTT was compared to the RT-PCR tests positive results, the positive predictive value of the VOTT was 61.68%, while the negative predictive value was 53.57%. These results suggested that VOTT+ patients had a 0.62 probability of being RT-PCR+ for SARS-CoV-2. On the other hand, the sensitivity and specificity for COVID-19 diagnosis of the Venezuelan Olfactory Test and basic taste test was 45.83% and 68.7%, respectively. These findings supported that these tests allow to detect olfactory and taste disorders in 46% of healthy individuals and in 69% of patients who were also clinically SARS-CoV-2 positive.

SARS-CoV-2 affectation and chemosensory dysfunction

Coronavirus infection produces different degrees of affectation, as described in the methods section. In this research, it was observed that 98.48 % of the 66 subjects (65/66) with chemosensory impairment (VOTT+) had mild clinical signs and 1.51% (1/66) moderate signs (Table 4).

Characteristics of VOTT+, SyMVOTT +, and SyMVOTT- patients

Clinical signs as a consequence of SARS-CoV-2 infection are quite variable. The number of SARS-CoV-2 infected patients presenting with chemosensory disorder only (VOTT+) was 18/98 (18.6%). In contrast, there were 48/98 (43.9%) SyMVOTT+ (Table 5). A heterogenous distribution was significant according to Chi square analysis ($\chi^2 = 13.79$, df = 2, $p < 0.01$) consistent with significant standardized residuals. Under this context a post-hoc power analysis also has shown $\lambda = 16.58$, df = 2, $p < 0.05$. with a $p = 0.908$ ($\alpha = 0.1$), this finding suggests that our sample is sufficient to support our findings.

The group of VOTT+ patients without any other accompanying symptom (no concomitants), who referred olfactory and taste disorder as their only symptom before the molecular diagnostic and before being admitted to the hospital was 18/97 (18.55%). When differentiated on the basis of the type of chemosensory dysfunction, 7 presented with olfactory disorder only, 4 of them anosmia, and 3 with mild hyposmia. Combined smell and taste disorders at various degrees were present in 10 subjects, and only 1 had hypogeusia. All these findings suggest a disorder variability that may indicate the presence of coronavirus infection and not only the anosmia or the ageusia. In this group of patients, chemosensory disorders were an early biomarker of the coronavirus disease.

Chemosensory dysfunction was a symptom in 31.25% of the subjects of the SyMVOTT+ group before being admitted to the hospital. In contrast, 68.75% of the patients of the sample that did not declare the disorder before being admitted to the hospital, were positive for olfactory or taste disorder when the VOT and taste test were used. This confirms the need to use a standardized objective taste for detecting the chemosensory disorder during the hospital stay. On the other hand, the onset of the chemosensory dysfunction in the SyMVOTT+ group occurred between the 3rd and 5th day in 70% of the cases.

Other associated symptoms in the SyMVOTT+ group vary. Among them, headache, myalgia, arthralgia, shivering, odynophagia, and hyporexia in 48% of patients; fever, headache, and general malaise in 31%, dry cough and chest pain in 8% dyspnea, and only fever or myalgia in 8% and 2%, respectively. Similarly, in the SyMVOTT- patients, which accounted for 32.98% of the sample, there was fever, headache, myalgia, arthralgia, and shivering in 46.87% of them; headache, arthralgia and myalgia in 15%, as well as chest pain, dyspnea in 15%, rhinorrhea, fever, nasal congestion, and dysphonia in 12.5%; and cough, general malaise, fever, and shivering in 9%. As relates the variety of symptoms in SyMVOTT+ and SyMVOTT- groups, no differences were observed in the frequency of presentation that would warrant an additional classification in the SARS-CoV-2 infection.

Age, gender and VOTT

In this study, when the presence of chemosensory dysfunction in the subjects of the sample was taken into account, the frequency was similar among them regardless gender or age (Table 6). Furthermore, smell and taste disorders combined were present in 40.90% of VOTT+ patients. The severity of the disorder is anosmia and ageusia in 30% of the cases, while mild hyposmia and hypogeusia were observed in 59.25% of the cases.

The olfactory disorder alone, was present as anosmia (12.12%), severe hyposmia 6.06%, mild hyposmia 25.75%, and moderate hyposmia (6.06%). It may be concluded from the analysis of the

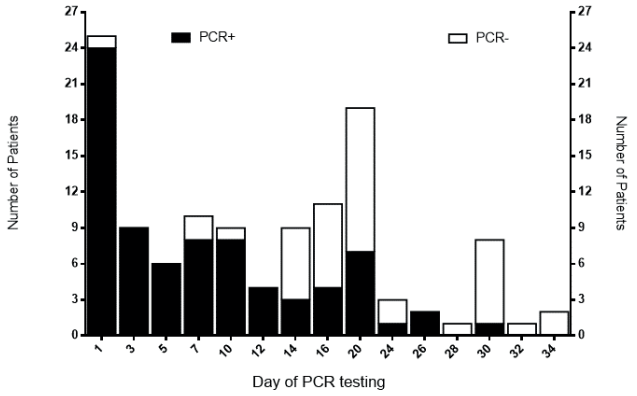

Figure 1. RT-PCR molecular analysis under SARS-CoV-2 infection. RT-PCR molecular analysis was performed multiple times to each patient until testing become negative. Each bar represent the number of patients RT-PCR+ (black) and RT-PCR- (white) through time (days of PCR testing).

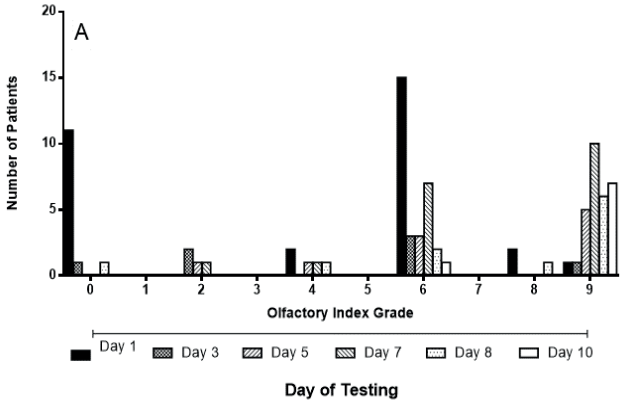

Figure 2. Olfactory impairment and evolution of SARS-CoV-2 infection. Venezuelan Olfactory Test was performed until Olfactory Index Grading reached normosmic value (8-10). A. Each bar represents number of subjects at specific grade of olfaction. Bar symbol represents day of testing (Day 1 -10).

sample that the presence of both disorders is the prevailing clinical sign, and of those two, the most frequent olfactory disorder is mild hyposmia (32 patients). At the same time, anosmia was the second olfactory disorder in terms of frequency (17 patients). In contrast, hypogeusia as the only clinical sign is not frequent in coronavirus infection.

Time course of SARS-CoV-2 grading by means of RT-PCR Test

The frequency analysis of the RT-PCR molecular test for SARS-CoV-2 was carried out for all individuals of the protocol in a systematic manner from day 1 to day 34 post infection. The viral gene expression was very frequent from day 1 to day 7, as it was present in 47/78 (60.25%) subjects; it decreased between day 10 and 14, and from day 16 to day 20, when it was evident in 15/78 (19.23%) and 11/78 (14.10%) subjects, respectively. Only 3 individuals had a positive RT-PCR test result on day 26 and only one did so until day 30. The frequency of VOTT+RT-PCR+ 66/275, and VOTT-RT-PCR+ 78/275 individuals is different from that of VOTT+RT-PCR- 41/275, VOTT-RT-PCR- 90/275 subjects (Fisher´s exact test $P < 0.0184$). The frequency of RT-PCR+ molecular tests is higher between days 1 and 14 and lower between day 16 and 24. It was observed that the maximum time interval for viral gene expression was 21 days (Figure 1).

Time course of SARS-CoV-2 grading by means of VOT

The assessment of chemosensory dysfunction as a function of time by means of the Venezuela olfactory test allowed to establish a group of subjects with anosmia 11/31 (35.48%) and another with mild hyposmia 15/31 (48.4%) on day 1. The number of individuals positive for mild hyposmia and normosmia in the VOT increased in frequency with time, as follows: day 5, 8/10 (80%); day 7, 17/19 (89.5%); day 10, 8/8 (100%); day 14 8/10 (80%); and day 20, 15/16 (93.75%). Between day 5 and day 20, the sense of smell of 66/142 (46.47%) subjects had been restored (normosmia), as evidenced by the VOT test. The average recovery interval was 8 to 10 days, with a minimum of 3 days and a maximum of 20 (Figure 2). Only one patient did not recover the sense of smell during the time of the study.

Discussion

The recent onset of this SARS-CoV-2 zoonosis in human populations has led to a significant number of studies aimed at understanding the variety of symptoms as part of the etiology of the disease [25]. Establishing a following up protocol of patients with diagnosis of SARS-CoV-2 viral infection, we have determined by using an unbiased smell and taste testing, association between olfactory and taste dysfunction concurrent with symptoms of mild COVID-19.

Taking into a consideration results from systematic reviews and meta-analysis recently published by Tong et al. [11] and Borsetto et al. [19], to the best of our knowledge, our findings have demonstrated the predictive value of a standardized smell test for the assessment of the sense of smell and taste in individuals with respect to a molecular diagnostic of SARS-CoV-2 infection by RT-PCR test.

Our findings established evolution of the chemosensory disorder of COVID-19 subjects until the sense of smell and taste were fully restored. In addition, viral genes analysis by means of the SARS-CoV-2 RT-PCR test and coronavirus antibodies expression were carried out in symptomatic and asymptomatic patients until criteria of full recovery was reached. Anosmia and ageusia were otorhinolaryngological and neurological signs of the coronavirus disease [26].

Currently, the physiopathology of anosmia due to COVID-19 is

Table 5. General symptoms and chemosensory dysfunction in SARS-CoV-2 infection.

	SyVOTT+	SyVOTT-	VOT+		SyVOTT+	SyVOTT-	VOT+
				N		Standardized residuals	
PCR+	48	32	18	98	**-3.28***	**-0.14**	**-3.14***
%	43.9	37.8	18.6				

χ^2-=13.796, gl = 2, p< = 0.01. * Standardized residual values at p < 0.05. It is worth mentioning that the analysis shown in this table was carried out with all the patients that had symptoms associated with COVID-19 (98/144 positive cases) excluding asymptomatic cases (44/144).

Table 6. VOT+ and taste + by age and gender.

	Males			Females		
	Age Range (years old)			Age Range (years old)		
	18-35	36-51	>52	18-35	36-51	>52
VOTT*		%			%	
Anosmic	0	3.0	1.5	1.5	3.0	3.0
Mild Hyposmia	7.6	1.5	1.5	7.6	6.1	1.5
Moderate Hyposmia	3.0	0	0	1.5	0	1.5
Severe Hyposmia	1.5	0	0	1.5	3.0	0
Hypogeusia	6.5	0	1.5	1.5	0	0
Both	7.6	6.0	4.5	13.6	3.0	6.1

VOTT* : Venezuelan Olfactory Test and Taste grading.

apparently better understood; however, there is still the question why patients with moderate to severe COVID-19 infection have less olfactory affectation [27-29]. Findings of this study show that 98% of patients with smell and taste disorders had mild COVID-19. These results are in agreement with a recent report of self-described olfactory dysfunction in mild forms of SARS-CoV-2 infection that did not require hospitalization [28]. For instance, the chemosensory dysfunction established in this study by means of self-evaluation underestimates the incidence of this symptom; therefore it would be advisable to examine whether anosmia is less prevalent in more severe forms of the infection and, consequently, the mechanism that leads to the physiopathological process that gives rise to this affectation [29-31].

The definition of a mild or severe form of SARS-CoV-2 infection may imply there is an immune response with more or less contention power thus leading to a milder or more serious viral infection, respectively [32]. Therefore, it is possible that, in the mild forms of the coronavirus infection, a more intense and faster immune response will produce more local inflammation that evolves into inflammatory processes involving the neuroepithelium and olfactory bulb [33-34]. For instance, expression of IgA antibodies as a primary response of defense in the olfactory and throat mucosa may generate at primary blockage of the SARS-CoV-2 infection [33].

A recent report has established highly conserved common sequences of the human olfactory receptor and viral particles which may be blocked by the IgA antibody as a result of the viral infection. In doing so, mild symptoms of COVID-19 may occur and a transient anosmia and dysgeusia is establish [17]. Furthermore, a sustained binding of the IgA, and concomitantly IgM and IgG antibodies may explain an anosmia or dysgeusia sequalae. As a result of common sequences recognized by the IgA antibody in olfactory receptor neurons [32]. In contrast, in low intensity late immune responses, patients with mild symptoms in ears, nose and throat; later, may involve higher and lower airways with respiratory compromise [33, 34].

These two hypotheses have not been experimentally corroborated. However, noticeable, patients with severe or critical forms have a higher immunoglobulin concentration in the serum and nasal secretions than patients with mild forms, because the severe respiratory compromise which has been justified as being a cytokine storm mechanism.

Since the beginning of the pandemic, COVID-19 symptoms reports have been about the respiratory tract affectation, with serious and fatal complications in a certain percentage of cases. The first reports involving a nervous system affectation were as-

sociated to a sudden decrease in the sense of smell, sometimes accompanied by a decrease in the sense of taste, which patients recovered from regardless whether they had a positive infection outcome or not. Initial reports were based on telephone interviews or online self-assessments by means of a questionnaire. As a result, the incidence of the chemosensory disorder was 19.4%, 64%, and 85.6% [11, 19].

These findings confirmed the initial observation of Lechien et al. who were the first to mention the sudden loss of the sense of smell in SARS-CoV-2 infected patients. Subsequent reports expanded on the vision of this author and confirmed the association between the infection by this virus and the chemosensory disorder. Self-assessment of the senses of smell and taste has been proposed as an index of SARS-CoV-2 transmission that could be of value for projecting the need of hospital care units required for compromised patients [30]. However, as it is commonly the case in these smell and taste self-assessment studies, sensitivity and specificity are compromised.

Applying standardized tests to SARS-CoV-2 infected patients has allowed to determine the level of involvement of the sensory function in the progression of the viral pathology. In a previous study, an UPSIT test for the Persian population applied to a reduced number of patients in Iran established that the olfactory disorder is variable, as there was a reduced rate of anosmic subjects (25%), while the largest percentage had moderate and severe microsmia (60%). Therefore, it was suggested that this degree of sensitivity was not enough to be able to consider the smell test as an indicator of the progression of the infection (30).

The senses of smell and taste form a physiological system that is affected by COVID-19; however, the degree of affectation seems to be mild. This has been shown by the studies carried out using as objective evaluator the chemosensory test of the Clinical Research Center of Connecticut, which has shown, just like Moein et al., findings, that a very small number were anosmic, while most had moderate and mild hyposmia (80%). At the same time, the taste test established that the taste affectation was mild to moderate. All patients maintained a normal degree of discrimination and a subjective overall recovery of 66%; but up to 88% presented with a certain degree of chemosensory disorder [31].

In contrast with these results, in this study, the results of several smell and taste tests showed the progressiveness of the disorder and time to recover from the onset of the infection. Contrary to what was seen in prior studies [26-31], in our sample, mild hyposmia and anosmia accounted for the highest share with a progressive recovery. The onset of the infection and its evolution allowed to make an evaluation until the recovery of the patient.

Evidence of SARS-CoV-2 infection and its demonstration by virtue of new technologies have given rise to molecular tests with variable sensitivity and specificity. Findings suggesting an asymptomatic infection in significantly frequent cases of negative RT-PCR test results imply several possibilities, which include that individuals have either not been exposed to the infection or they received a reduced viral load. At the same time, the characteristics of the sample, as a result of collection, storage, preservation, and processing, could also contribute to the high number of negative results. The time course of the infection, and the effectiveness of molecular tests to prove the infection, suggest an order in the events of infection progression that range from 5 to 7 days for the first clinical signs to become evident. The sensitivity of the molecular diagnosis increases in a delayed manner, as a function of the increase in the concentration of viral particles that could reach a maximum level on the 7th day and up to the following 14 days, to then decrease gradually as a result of the immune response of the patient.

In contrast, in this study, the RT-PCR molecular test leads to suggest a minimum time of 1 day from the date of admission of the patient to the protocol and up to 14 days. This is the first study that established a multiple system for following up the progression of the chemosensory disorder and detecting the infection; by means of the molecular test or the SARS-CoV-2-specific antibodies detection test.

In this research, we corroborate the spontaneous recovery of most patients. Only one patient still has anosmia-type smell disorder; and in her case, other tests were carried out to establish the actual cause of such disorder. In this regard, there is similarity with what has been reported in the literature about recovery time. Surprisingly, we did not find mechanistic explanation in regards of persistence of chemosensory dysfunction and RT-PCR test negative outcome. Eventhough, recent findings from Root-Bernstein may be an alternative explanation [17].

The SARS-CoV-2 spike mutation has been described as mediating the infection in human cells. Korber et al. listed 13 mutations that are deemed to be within a wider phylogenetic spectrum that could change with geography and with time, and which could give some populations certain selective advantages as to the transmission or resistance to the infection. In the case of Europe, the spike mutation D614G was dominant and began spreading in February 2020 and had such aggressive impact on the population. There is evidence of recombinant strains circulating locally and that are indicative of infections by multiple strains [35,36]. Rodríguez-Morales et al. described 3 genomic sequences of coronavirus mutations that may explain the behavior of the disease in Venezuela, in specific zones like Zulia, (where there has been significant migratory activity of people to and from Colombia and Brazil) and where the severity of the infection has been higher than in other geographic zones of the country [37]. This hypothesis could explain why infections are mild or asymptomatic in some areas and severe in others, or why the penetrance of chemosensory dysfunction is lower than in other reports.

Conclusions

We may conclude that there is significant presence of smell and taste disorders, as clinical signs, in the course of the mild coronavirus disease. Therefore, every patient who presents with symptoms, must be isolated and screened for the disease by means of appropriate diagnostic tests. Patients evolve towards the resolution of smell and taste disorders spontaneously and recover in a period of time between 3 days and 5 weeks, with an average of 8 to 10 days. Rapid diagnostic tests are not useful for diagnosing the coronavirus infection, but only for following it up. We would suggest including the olfactory test and taste test as part of the battery of tests complementary to the RT-PCR test for diagnosing SARS-CoV-2 and following up the progression of the disease.

Authorship contribution

RP, MM and CAG: Conceptualization and Ideas; formulation or evolution of overarching research goals and aims. Supervision Oversight and leadership responsibility for the research activity planning and execution, including mentorship external to the core team. RP, JJN, WR, NP, EL, ASG, SG, AH, AG, KD, MM, MDJ, SP, LR, LLP, AA, MM: Investigation and Conducting a research and investigation process, specifically performing the experiments, or data/evidence collection. CAG, AH, MM, and RP: Formal analysis: Application of statistical, mathematical, and formal techniques to analyze or synthesize study data. PR and CAG equally contributed in analyzing data and wrote the manuscript.

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Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Conflict of interest

The authors of this investigation declare no conflicts of interest.

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