High prevalence of long-term olfactory, gustatory, and chemesthesis dysfunction in post-COVID-19 patients: a matched case-control study with one-year follow-up using a comprehensive psychophysical evaluation*

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Abstract

Background: Using an age and gender matched-pair case-control study, we aimed to estimate the long-term prevalence of psychophysical olfactory, gustatory , and chemesthesis impairment at least one year after SARS-CoV-2 infection considering the background of chemosensory dysfunction in non-COVID-19 population.

Methodology: This case-controlled study included 100 patients who were home-isolated for mildly symptomatic COVID-19 between March and April 2020. One control regularly tested for SARS-CoV-2 infection and always tested negative was matched to each case according to gender and age. Chemosensory function was investigated by a comprehensive psychophysical evaluation including ortho- and retronasal olfaction and an extensive assessment of gustatory function. Differences in chemosensory parameters were evaluated through either Fisher's exact test or Kruskal-Wallis test.

Results: The psychophysical assessment of chemosensory function took place after a median of 401 days from the first SARS-CoV-2 positive swab. The evaluation of orthonasal smell identified 46% and 10% of cases and controls, respectively, having olfactory dysfunction, with 7% of COVID-19 cases being functionally anosmic. Testing of gustatory function revealed a 27% of cases versus 10% of controls showing a gustatory impairment. Nasal trigeminal sensitivity was significantly lower in cases compared to controls. Persistent chemosensory impairment was associated with emotional distress and depression.

Conclusion: More than one year after the onset of COVID-19, cases exhibited an excess of olfactory, gustatory , and chemesthesis disturbances compared to matched-pair controls with these symptoms being associated to emotional distress and depression.

Key words: anosmia, case-control studies, COVID-19, SARS-Cov-2, taste disorders

Introduction

Self-reported chemosensory changes are amongst the most frequent symptoms of the acute phase of coronavirus disease 2019 (COVID-19) [1–5]. Recently, modelling techniques even suggested that daily olfactory testing could be effective in monitoring the COVID-19 epidemic and in reducing the costs related to molecular and antigen test screening and surveillance [6]. Moreover, smell and taste disturbances are among the most common symptoms in COVID-19 long-haulers both in patients who suffered by mild-to-moderate disease [7–9] and in those requiring hospitalization [10]. However, many studies on long-COVID syndrome have improperly neglected the importance of chemosensory dysfunction [11,12]. This mirrors the experience of patients who believe their persistent olfactory symptoms is being overlooked by the healthcare system [13].

It has previously been reported that self-assessment of smell and taste may inaccurately estimate the real prevalence of chemosensory disorders [14,15]. Several studies have demonstrated a discrepancy between subjective and psychophysical evaluation of alterations in smell and taste in COVID-19 patients [16,17]. Nevertheless, compared to the self-reported impairment in chemosensitivity which has a baseline parameter of comparison consisting in the subjective perception of smell or taste preceding the onset of COVID- 19, psychophysical studies may suffer from suboptimal specificity. The prevalence of olfactory dysfunction in the general population according to psychophysical tests is estimated to be up to 21% [18,19]. Moreover, in older adults the prevalence of psychophysical olfactory impairment in the setting of no self-reported deficit is 15% [20].

Thus, the aim of the present age and gender matched-pair case-control study was to estimate and characterize the long-term prevalence of olfactory, gustatory, and chemesthesis impairment through comprehensive psychophysical evaluation in COVID-19 patients while considering the background of chemosensory dysfunction in non-COVID-19 population. We also correlated our results with the self-reported chemosensory abilities to understand whether we could rely on them to follow-up the long-term smell and taste impairments.

Materials and methods

This case-controlled study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the ethic committees for clinical experimentation of Treviso and Belluno provinces (ethic vote: 780/CE) and Friuli Venezia Giulia Region (CEUR-2020-Os-156). Informed consent was obtained verbally and writing.

Subjects

A regional interdisciplinary task force of healthcare workers was created in response to the COVID-19 pandemic to monitor all patients with polymerase chain reaction (PCR) confirmed severe acute respiratory syndrome coronavirus 2 (SARS- CoV-2) infection and identified the home-isolated COVID-19 patients. Cases were randomly sampled from home-isolated, mildly symptomatic COVID-19 subjects living in Trieste municipality who tested positive during March and April 2020. Cases selection was done through a computerized procedure which assigns to each patient the same probability of being included in the study. To facilitate matching to controls recruited from the medical staff, only cases between the ages of 21 and 70 were sampled. Patients were considered mildly symptomatic if they had less severe clinical symptoms, with no evidence of pneumonia, SpO2 ≥ 94%, not requiring hospitalization, and therefore considered suitable for being treated at home. Patients were contacted by telephone and invited to participate in the psychophysical evaluation of the chemosensory functions until the desired sample size was reached.

Controls were recruited from Hospital staff of two Hospitals (Trieste University Hospital and Treviso General Hospital) who, according to institutional surveillance, were at least biweekly tested for SARS-CoV-2 with PCR on nasopharyngeal and throat swabs. Controls were enrolled on a voluntary basis among those who consistently tested negative for SARS-CoV-2 infections and matched 1:1 to cases by sex and age (±3 years).

Subjects with a history of previous craniofacial trauma, surgery, or radiotherapy in the oral and sinonasal area, chronic rhinosinusitis, pre-existing olfactory or gustative dysfunction, were excluded from the study. Subjects with evidence of acute or chronic rhinosinusitis on nasal endoscopy were also excluded.

Questionnaires

Symptoms reported by cases during the acute phase of COVID-19 and their persistence at the time of the psychophysical evaluation were assessed through ad hoc questions and structured questionnaires, including the ARTIQ (Acute Respiratory Tract Infection Questionnaire) and the SNOT-22 item "Sense of smell or taste", scored on a six point Likert scale, as previously reported [1]. Both cases and controls completed the 5-item World Health Organization Wellbeing Index (WHO-5) questionnaire, a short, generic global rating of subjective well-being, as described by Topp et al. [21]. Individuals scored each of five using a six-point Likert scale reflecting the previous fortnight. The total score, ranging from 0 to 25, is expressed as a percentage, where 0% represents the worst possible well-being, 100% represents best possible well-being, a score <50 is indicative of emotional distress, while a score ≤28 indicates likely depression [21].

Evaluation of nasal patency

To evaluate nasal obstruction, each participant was asked to self-report their nasal patency using a 100 mm Visual Analogue

Scale (VAS), ranging from 0 (absence of nasal obstruction) to 100 (complete nasal obstruction). The Peak Nasal Inspiratory Flow (PNIF) was assessed using the In-Check portable inspiratory flow meter (GM Instruments, Irvine, UK), and a tight-fitting anaesthetic mask. Patients performed three trials at maximal effort while sitting. The highest flow rate (litres per minute [L/min]) of these three measurements was recorded.

Endoscopic evaluation of the olfactory cleft

The olfactory cleft endoscopy scale (OCES) was used to score endoscopic assessment of the olfactory cleft in all subjects, as previously described [22].

Self-assessment of chemosensory perception

Prior to psychophysical olfactory tests, each participant was asked to self-report their chemosensory perception, namely odour perception ("How would you rate your sense of smell?"), flavour perception ("How would you rate your fine taste, e.g., during eating and drinking?), and taste perception ("How would you rate your basic taste: sweet, sour, salty, bitter?) using a 100 mm Visual Analogue Scales (VAS), ranging from 0 (no perception) to 100 (excellent perception).

Psychophysical evaluation

Evaluation was performed in silent and well-ventilated rooms. To avoid chemosensory desensitization, all participants were instructed not to eat, drink, smoke, or brush their teeth up to 2 hours before participation in the measures.

Orthonasal olfactory function

Orthonasal olfactory function was measured using the validated extended Sniffin' Sticks test (SST) battery (Burghart Messtechnik, Wedel, Germany) including phenylethyl-alcohol (PEA) odour thresholds (T), odour discrimination (D), and odour identification (I) [23]. The maximum score for each of the 3 subsections of the SST is 16. Results are combined for a composite TDI score (range 1–48) and categorised as functional anosmia (TDI ≤ 16.0), hyposmia (16.25 - 30.5), or normosmia (TDI ≥ 30.75)(24). The test has been validated and shown to have high test-retest reliability [25]. Testing was performed in accordance with the standardised testing protocol (see Supplementary methods for more details) [23].

Gustatory psychophysical evaluation

Gustatory evaluation was performed using the validated taste strips test (Taste Strips, Burghart Messtechnik, Wedel, Germany) impregnated with four different concentrations of each of the tastes "sweet", "sour", "salty" and "bitter" performed strictly according to a standardised protocol (see Supplementary methods for more details) [26]. Based on a forced-choice, the taste strips score (TSS) was calculated (0–16 points), and used to define hypogeusia (≤9 points) and normogeusia (≥10 points) [26].

Retronasal olfactory function

Retronasal olfactory function was tested using 20 powdered tasteless aromas (Givaudan Schweiz AG, Dubendorf, Switzerland) as described by Yoshino et al. [27]. For each trial, participants were blindfolded and occluded their nostrils. before delivering each stimulus, in powdered form (approximately 0.05 g), to the mid-dorsal section of the participant's anterior tongue. The tongue was withdrawn into the mouth, the nostrils were unblocked, and participants then exhaled through their nostrils. After exhalation, participants identified the odour from a list of four verbal descriptors. The total score ranged between 0 and 20 and was based on the sum of correctly identified flavours.

Nasal trigeminal chemesthesis

Each participant was asked to sniff freshly prepared 70% acetic acid solution and indicate the intensity of the stinging on a VAS ranging from 0 (no perception) to 100 (extremely strong perception). In order not to compromise the participants' performance in other tests, this examination was placed at the end of the sequence of psychophysical tests.

Statistical analysis

Considering that the one-year prevalence of self-reported olfactory impairment in patients with previous COVID-19 was 18% [28] and that self-assessment of chemosensory function was observed to underestimate the psychophysical prevalence hyposmia/anosmia [16,17], 100 cases and 100 matched controls were necessary to estimate a difference of 10% in the prevalence of hyposmia/functional anosmia (TDI ≤ 30.5) in cases (p1=25%) compared to controls (p0=15%) [29–31], fixing the a priori probabilities a=0.05 and b=0.20. Qualitative variables (e.g., prevalence of symptoms) were reported as percentage with corresponding 95% confidence intervals (CI) according to Clopper-Pearson; differences across groups were evaluated through Fisher's exact test. Quantitative variables were reported as mean or median values with CI or interquartile rage (IQR), respectively, and differences were tested through two-tailed t-test or Mann-Whitney test. Cases were then classified as "Never anosmic", "Recovered" and "Persistent"; differences in chemosensory were evaluated through Kruskal-Wallis test and pairwise comparisons were conducted through a post-hoc analysis according to Holm.

Results

Psychophysical assessment of chemosensory function took place after a median of 401 days (IQR: 388-413) from confirmed diagnosis. Sociodemographic and clinical characteristics of 100 cases and 100 controls are summarized in Table 1. Median age was 49 years and 61.0% were males, in both groups. No significant differences were observed between cases and

Table 1. Sociodemographic and clinical characteristics of cases and controls.

	Controls		Cases		Fisher's exact test
	n	% (95% CI)	n	(%)	
Sex					
Men	61	61.0 (50.7-70.6)	61	61.0 (50.7-70.6)	
Women	39	39.0 (29.4-49.2)	39	39.0 (29.4-49.2)	
Age (years)					
<45	37	37.0 (27.6-47.2)	36	36.0 (26.6-46.2)	
45-54	33	33.0 (23.9-43.1)	33	33.0 (23.9-43.1)	
≥55	30	30.0 (21.2-40.0)	31	31.0 (22.1-41.0)	
Smoking habits					
Never	60	60.0 (49.7-69.7)	61	61.0 (50.7-70.6)	P=0.6363
Former	17	17.0 (10.2-25.8)	21	21.0 (13.4-30.3)	
Current	23	23.0 (15.2-32.5)	18	18.0 (11.0-26.9)	
Drinking habits					
Never	57	57.0 (46.7-66.9)	64	64.0 (53.8-73.4)	P=0.3855
Ever	43	43.0 (33.1-53.3)	36	36.0 (26.6-46.2)	
Comorbidities (number)					
0	78	78.0 (68.6-85.7)	71	71.0 (61.1-79.6)	P=0.3304
1-2	22	22.0 (14.3-31.4)	29	29.0 (20.4-38.9)	
Comorbidity					
Immune suppression	0	0.0 (0.0-3.6)	2	2.0 (0.2-7.0)	P=0.4975
Diabetes	2	2.0 (0.2-7.0)	1	1.0 (0.0-5.4)	P=1.0000
Obesity	2	2.0 (0.2-7.0)	12	12.0 (6.4-20.0)	P=0.0101
Cardiovascular disease	17	17.0 (10.2-25.8)	9	9.0 (4.2-16.4)	P=0.1400
Cancer	0	0.0 (0.0-3.6)	1	1.0 (0.0-5.4)	P=1.0000
Chronic respiratory disease	2	2.0 (0.2-7.0)	6	6.0 (2.2-12.6)	P=0.2790
Kidney disease	0	0.0 (0.0-3.6)	0	0.0 (0.0-3.6)	P=1.0000
Liver disease	0	0.0 (0.0-3.6)	1	1.0 (0.0-5.4)	P=1.0000

controls regarding both smoking and drinking habits and the number of comorbidities. However, obesity was significantly more prevalent in cases compared to controls (12.0% versus 2.0%, P=0.0101). At the time of study enrollment, 91.0% of controls complete the vaccination course, while none of the cases received their second SARS-CoV-2 vaccination dose.

According to retrospective self-rating of "sense of smell or taste" during the acute phase of the disease at the time of the psychophysical evaluation, 69.0% of cases reported a new onset of smell or taste impairment during the acute phase of the disease. 33.0% reported a persistently altered sense of smell or taste at the time of the psychophysical evaluation. This was the most prevalent long-lasting symptom followed by tiredness (25.0%), muscle pain (14.0%), and shortness of breath (12.0%) (Supplementary Table 1).

No significant differences were observed between cases and controls on nasal patency self-evaluation by VAS, while the maximal PNIF was significantly higher in controls compared to cases. Moreover, OCES scores were significantly higher in cases (Supplementary Table 2) with 9 of them having clear drainage and/or swelling partially narrowing the olfactory cleft.

Self-reported sense of smell, taste, and flavour did not significantly differ between cases and controls (Table 3). However, as expected, among cases those reporting a persistent post-COVID-19 alteration in the sense of smell or taste exhibited significantly lower scores on the VAS scales of smell, taste, and flavour (Supplementary Table 3).

Orthonasal olfactory function

7.0% were functionally anosmic, 39.0% of cases were hyposmic, and 54.0% were normosmic according to the TDI score. The

Table 2. Olfactory and gustatory function in case and controls according to TDI score and TSS.

	Controls		Cases		Fisher's exact test
	n	% (95% CI)	n	(%)	
TDI score					
≤16.0	0	0.0 (0.0-3.6)	7	7.0 (2.9-13.9)	p<0.0001
16.25-30.5	10	10.0 (4.9-17.6)	39	39.0 (29.4-49.2)	
≥30.75	90	90.0 (82.4-95.1)	54	54.0 (43.7-64.0)	
TSS					
≤9	10	10.0 (4.9-17.6)	27	27.0 (18.6-36.8)	P=0.0032
≥10	90	90.0 (82.4-95.1)	73	73.0 (63.2-81.4)	

TDI, Threshold, Discrimination, Identification; TSS, Taste Strips Score.

Table 3. Psychophysical evaluation and self-rating of chemosensory function in cases and controls.

	Controls	Cases	Mann-Whitney test
	Median (Q1-Q3)	Median (Q1-Q3)	
Chemosensory psychophysical evaluation			
Orthonasal			
Thresold	9.0 (6.8-10.8)	7.0 (4.0-9.8)	p=0.0004
Discriminant	13.0 (12.0-14.0)	11.0 (9.0-13.0)	p<0.0001
Identification	14.0 (13.0-15.0)	12.0 (10.0-14.0)	p<0.0001
TDI	35.0 (32.9-38.6)	31.5 (24.5-35.1)	p<0.0001
Taste strip score	13.0 (11.0-14.0)	11.0 (9.0-12.0)	p<0.0001
Retronasal score	18.0 (16.0-19.0)	16.5 (13.0-18.0)	p=0.0002
Acetic acid VAS score	100 (98.0-100)	98.0 (91.0-100)	p=0.0032
Self-reported perception (VAS 0-100)			
Self-rating of taste perception	85.0 (74.5-95.0)	89.0 (70.0-100)	p=0.4550
Self-rating of odour perception	83.0 (67.5-94.5)	83.0 (60.0-95.0)	p=0.6916
Self-rating of flavour perception	85.5 (75.0-94.0)	85.0 (66.0-99.0)	p=0.7816

prevalence of hyposmia in controls was 10.0% with none being functionally anosmic (P<0.0001) (Table 2). There was a statistically significant difference between cases and controls across all orthonasal olfactory sub-tests including threshold, discrimination, and identification (Figure 1 and Table 3). A subset analysis according to self-reported chemosensory impairment during the acute phase of the disease and at the time of psychophysical evaluation, showed that cases with persistent subjective chemosensory impairment exhibited the poorest median scores for all olfactory tests (Figure 2 & Supplementary Table 3). However, cases recovered from subjective chemosensory alteration also exhibited a TDI score significantly lower than controls (Figure 2 & Supplementary Table 3). Accordingly, the prevalence of olfactory dysfunction increased from controls to cases who never reported a COVID-19 associated olfactory impairment, those reporting recovery and then those reporting persistent dysfunction (Supplementary Table 4). A moderate positive linear correlation (r=0.57) was observed in cases between self-rating of odour perception and TDI score (Supplementary Figure 1).

The variation of olfactory subset score in each olfactory group was evaluated (Supplementary Figure 2). Anosmic cases showed the greatest reduction in all the subset scores, resulting in the lowest TDI score. In controls, identification and discrimination scores declined on average by approximately 2 points each in hyposmic patients compared to normosmic (Δ_D=1.8 points, Δ_I=2.0 points); conversely, the threshold score showed a more consistent decrease (Δ_T=6.3; p<0.0001). Similar variations have been observed in cases (Δ_T=4.8 points, Δ_D=2.7 points, Δ_I=2.0 points; p<0.0001), suggesting that threshold is most impaired by SARS-CoV-2 infection.

Among cases, the OCES score was not significantly different

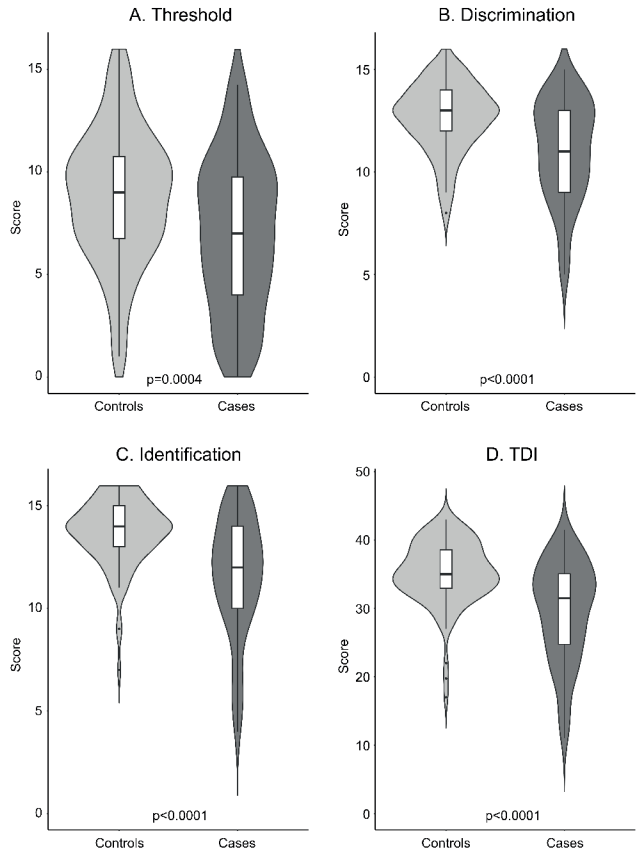

Figure 1. Violin and bloxplot representing ratings for threshold (A), discrimination (B), identification (C), and combined TDI score (D) in cases and controls. TDI, Threshold, Discrimination, Identification.

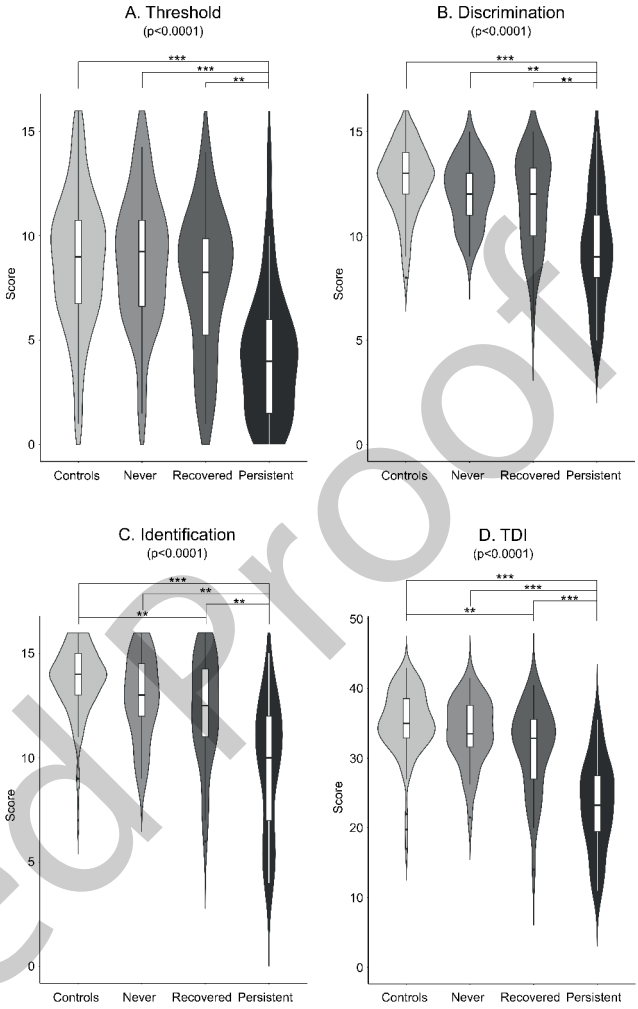

Figure 2. Violin and bloxplot representing ratings for threshold (A), discrimination (B), identification (C), and combined TDI score (D) in cases and controls. Cases are classified according to the self-reported altered sense of smell or taste in never (patients with COVID-19 not associated with sudden onset of chemosensory dysfunction), recovered (patients recovered from chemosensory dysfunction that arose suddenly during COVID-19), persistent (patients still self-reporting an altered sense of smell or taste that arose suddenly during COVID-19). * p<0.05; ** p<0.01; *** p<0.001. TDI, Threshold, Discrimination, Identification.

between subjects with a TDI ≤30.5 and those with a TDI>30.5. Conversely, cases with a TDI indicating olfactory impairment had a significant lower PNIF compared to cases with normal olfactory function (Supplementary Table 5).
While in controls a decline in olfactory function was observed in older age groups, no relationship was observed in cases between olfactory scores and age (Supplementary Table 6).

Gustatory function

A significant difference in taste perception was observed between cases and controls (P<0.0001) (Figure 3 & Table 3). When considering separately the four taste qualities, statistically significant differences were evident for sour, bitter, and sweet taste (Supplementary Table 7). Patients self-reporting a persistently altered sense of smell or taste scored poorest (Supplementary Figure 3 & Supplementary Table 3). Based on TSS, 27 cases (27.0%; 95% CI:18.6-36.8%) and 10 controls (10.0%; 95% CI: 4.9-17.6%) had hypogeusia (P=0.0032) (Table 2). When combining the results of the orthonasal olfactory testing with TSS, 15 cases (15.0%; 95% CI: 8.6-23.5%) had combined olfactory and gustatory dysfunction compared with 2 controls (2.0%; 0.2-7.0%; P=0.0015), while 18 controls (18.0%; 95% CI: 11.0-26.9) and 58 cases (58.0%; 95% CI: 47.7-67.8), respectively, had olfactory or gustatory dysfunction (P<0.0001) (Supplementary Table 8). A weak positive linear correlation (r=0.29) was observed in cases between self-rating of taste perception and TSS (Supplementary Figure 1).

Retronasal olfactory function and nasal chemesthesis

The retronasal identification test revealed a median score of 16.5 and 18.0 in cases and controls, respectively (P=0.0002) (Figure 3 & Table 3). The subset analysis of cases showed that particularly cases with persistent subjective chemosensory impairment exhibited a significantly lower score for retronasal

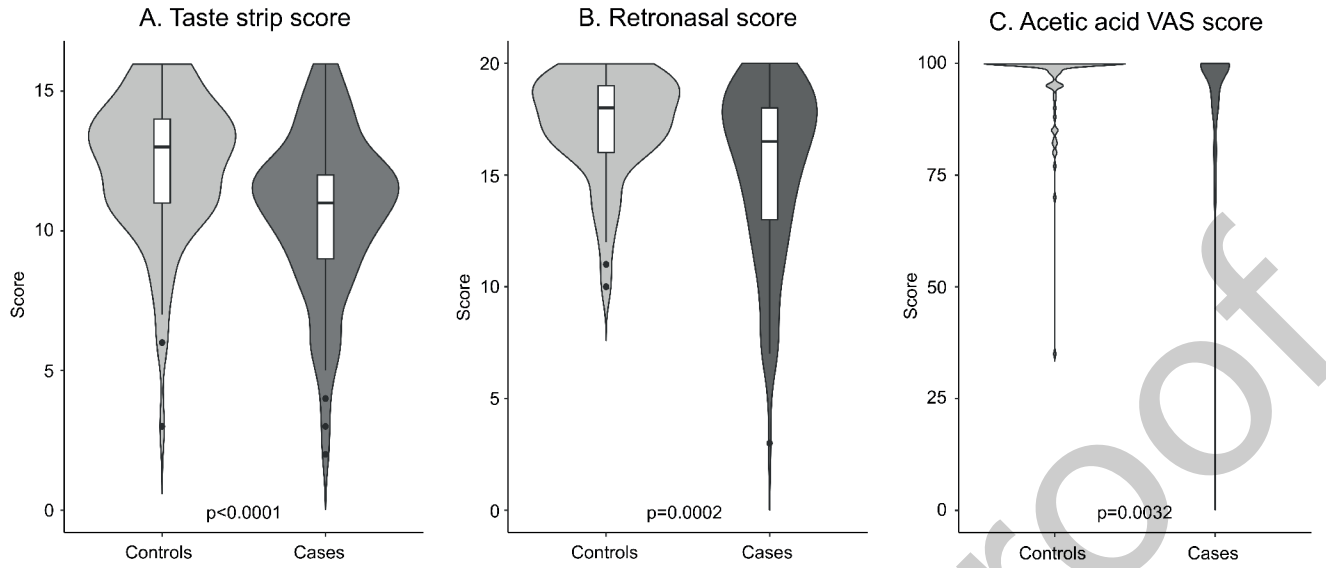

Figure 3. Violin and bloxplot representing ratings for taste (A), retronasal smell (B), and acetic acid VAS (C) in cases and controls. VAS, Visual Analogic Scale.

identification compared to controls (Supplementary Figure 3 & Supplementary Table 3). A correlation analysis revealed no significant correlation between self-rating of flavour perception and retronasal score (Supplementary Figure 1). The estimation of the trigeminal sensitivity by VAS after sniffing a 70% acetic acid solution revealed significant lower VAS scores in cases compared to controls (P=0.0032) (Figure 3; Table 3; Supplementary Figure 3 & Supplementary Table 3).

WHO-5

Fourteen cases (14.0%; 95% CI: 7.9-23.4%) and three controls (3.0%; 95% CI: 0.6-8.5%) reported depression, whereas distress was found in 22 cases (22.0%; 95% CI: 14.3-31.4%) and 7 controls (7.0%; 95% CI: 2.9-13.9%). In cases, the prevalence of depression increased with decreasing orthonasal smell, being 7.4% (2.1-17.9%) in 54 patients with TDI>30.5 and 42.9% (9.9-81.6%) in those (n=7) with TDI<16 (Figure 4; P<0.0001).

Discussion

At a median time of approximately 13 months from confirmed diagnosis, patients with previous COVID-19 exhibit a high prevalence of chemosensory changes. The psychophysical evaluation of orthonasal smell identified 46% and 10% of cases and matched controls, respectively, having olfactory dysfunction, while testing of gustatory function revealed gustatory impairment in 27% of cases versus 10% of controls. Overall, 58% of cases versus 18% of controls had an olfactory or gustatory dysfunction, with 7% of cases being functional anosmic.

In the literature, the prevalence of smell impairment associated with SARS-CoV-2 infection ranges from 0% to 98% with lower frequencies being captured by subjective ratings. Based on self-reported symptoms, 69% of cases reported an altered sense of smell or taste during the acute phase of COVID-19, consistent with previous reports showing that about 2/3 patients develop a chemosensory impairment following SARS-CoV-2 infection [1,28]. The D614G mutation is responsible for the enhanced cell binding of the SARS-CoV-2 spike protein to the ACE2 protein and is proposed to be responsible for the higher rate of chemosensory impairment observed in Western Countries compared to that observed in East Asian cohorts in the early phase of the pandemic [32,33]. Italy had the first sampled case of the full G614 haplotype and had shifted to all G614 samples prior to March 1, 2020 [34]. Thus, the first wave of COVID-19 pandemic in Italy was dominated by G614 variant which may be responsible for the higher rate of altered sense of smell observed in the present study. Both single nucleotide polymorphism that differ in frequency between populations and splice variants of the ACE2 entry protein and TMPRSS2 serine protease, the key host-specific cellular moieties responsible for the cellular entry of the virus, may modulate the susceptibility to develop olfactory loss following SARS-CoV-2 infection [35].

Several authors have highlighted that self-rating of the olfactory function is imprecise and may underestimate the prevalence of olfactory dysfunction [36–38]. A psychophysical evaluation is indispensable to measure the degree of chemosensory dysfunction, distinguish between smell and taste impairment [39] and measure residual olfactory function as this assessment may serve as a prognostic predictor [40]. This is most relevant in the long-term evaluation of patients with olfactory dysfunction, as it has been observed that subjects tend to overestimate the

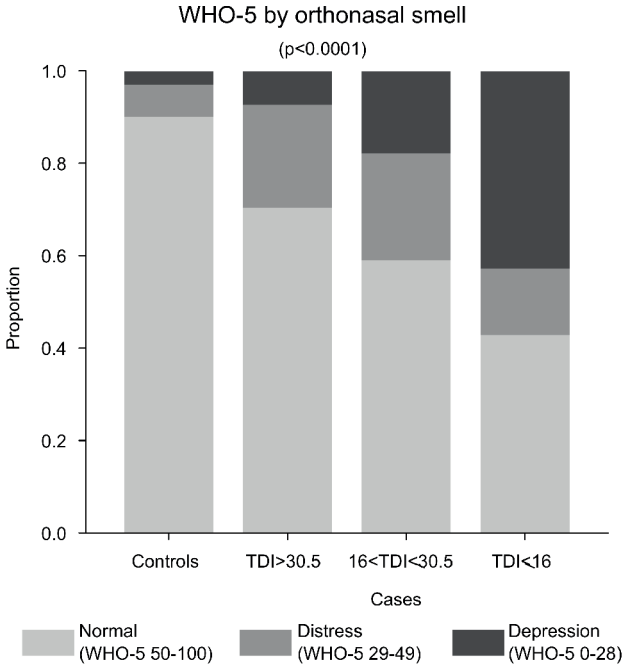

Figure 4. WHO (Five) well-being index in controls and cases and according to TDI (P<0.0001). TDI, Threshold, Discrimination, Identification.

extent of their recovery with self-reporting compared with psychophysical testing of chemosensory function [16,41–43]. We observed a progressive increase in the proportion of subjects with olfactory dysfunction from controls to cases who never reported a COVID-19 associated olfactory impairment, to those who recovered an olfactory deficit towards those who reported persistent dysfunction. This trend was also seen when considering the median TDI and retronasal score. These observations suggest that SARS-CoV-2 infection leaves an olfactory "scar" in many of the infected subjects and support the findings that subjective evaluation of the olfactory function overestimates the recovery of the sense of smell as individuals seem to adjust to olfactory loss [44].

In the present series, a persistent psychophysical gustatory impairment was confirmed in 27% of cases, substantially lower than confirmed olfactory dysfunction. According to a recent meta-analysis a greater overlap exists in the frequency of smell and taste disturbances during COVID-19, with pooled worldwide prevalence being 43.0% and 44.6%, respectively [45]. However, these data were mainly based on the patient's self-reported smell and taste perception with very few studies using validated tests of gustatory function. Those that used validated psychophysical tests found a prevalence of hypogeusia of 26% during the acute phase of the disease [46,47]. If self-assessment of 'smell' may underestimate the real prevalence of smell impairment, self-assessment of 'taste' may, on the contrary, overestimate the prevalence of true gustatory dysfunction [48]. Impaired retronasal olfaction can be indeed misinterpreted as taste dysfunction [49]. While the expression of ACE2 receptor in sustentacular cells of the olfactory neuroepithelium (OE) was consistently demonstrated in several studies [35], the expression of the ACE2 receptor in taste buds was confirmed only in a very recent investigation demonstrating the colocalization of ACE2 receptor, SARS-CoV-2, and type II taste cell marker [50]. This and other studies provide psychophysical evidence that patients with COVID-19 do have an impaired gustatory perception [46,47]. However, as alternative hypothesis, olfactory dysfunction following SARS-CoV-2 infection could be associated indirectly with impaired gustatory dysfunction. These senses share specific brain projection areas and mutually amplify each other, a mechanism that may fail in COVID-19 associated olfactory dysfunction [51]. Conversely, few patients had an isolated hypogeusia whose prevalence was not significantly different from that observed in matched controls.

Chemesthesis is still unexamined in most psychophysical studies on chemosensory impairment in COVID-19 [47,52,53]. In the present series, cases exhibited a significantly reduced sensitivity of the intranasal trigeminal system compared to matched controls. This confirms previous self-reported observations showing that chemesthesis is significantly reduced following SARS-Cov-2 infection [54,55]. Although these findings suggest that all the three major chemosensory modalities may be targeted by SARS-CoV-2, the mechanisms for chemesthesis dysfunction are yet to be elucidated. However, the olfactory loss could indirectly affect a reduction in chemesthesis in a similar way to the above on the interaction between smell and taste. The olfactory and trigeminal system are indeed closely associated and interact by reciprocally suppressing and enhancing each other [56].

In our previous study we observed that prevalence of loss of smell during SARS-CoV-2 infection and its short-term evolution were unrelated to the state of nasal patency and that most patients did not report nasal obstruction [57]. Here significant differences in PNIF and endoscopic evaluation of the olfactory cleft were found between groups. However, the presence of an OCES >0 in cases was not significantly correlated with a TDI ≤30.5 which seems to exclude a conductive problem as the underlying cause of hyposmia/anosmia in most patients with post-COVID olfactory dysfunction. Conversely, a lower PNIF was recorded in cases with a TDI ≤30.5 suggesting that sub-optimal nasal patency may contribute to the olfactory impairment.

Emotional distress and depression were more prevalent in cases compared to controls. Although distress could relate to other features of long-COVID, the correlation between well-being olfactory impairment suggests that olfactory impairment may play a causative role. Consistently, other authors found that

among all symptoms experienced by COVID-19 patients, chemosensory disturbances were most dominantly associated with depressed mood and anxiety [58]. Olfactory impairment impacts on social communication, work and sexual life and has been previously shown to be associated with decreased quality of life outside COVID-19 context [59].

As odour threshold at least partially reflects the functionality of the peripheral olfactory system [19], the finding that odour detection was the olfactory task most compromised in COVID-19 long haulers is consistent with the hypothesis that SARS-CoV-2 targets the OE. Why a proportion of patients experiences a transient loss of smell while some have not fully recovered over one year later remains unknown. It has been proposed that in the first case only the supporting cells are targeted by SARS-CoV-2 while in the second case the stem cell compartment of the OE, i.e., horizontal basal cells and globose basal cells, is targeted by the virus. Both these cell populations exhibit the molecular makeup that makes these cells prone to SARS-CoV-2 infection, i.e., ACE2 receptor and TMPRSS2 serine protease, which are, conversely, not expressed by the olfactory sensory neurons [60]. A longitudinal study comparing brain scans acquired from subjects before and after SARS-CoV-2 infection, showed that olfactory and gustatory cortical systems have reduced thickness and volume of the grey matter [61]; it is not clear however if this results from lack of stimulation, or may represent a neuro-invasive effect of SARS-CoV-2 on brain area involved in olfaction and taste, thus affecting cognition. The high prevalence of persistent olfactory dysfunction highlights a potential limitation of using olfactory testing as a means of surveillance; although relatively rare, such surveillance may fail to detect cases of reinfection.

The present study is the most comprehensive study that has been conducted regarding COVID-19-related chemosensory dysfunction as it includes a long-term evaluation, a control arm, both self-rated and psychophysical testing of ortho- and retronasal olfaction, assessment of gustatory function and of well-being. The absence of psychophysical evaluation prior to or during the acute phase of the disease is the main limitation of the present study. Only patients with mild disease were recruited and this study should be replicated in patients previously hospitalized for severe COVID-19. In order to reduce the burden of the evaluation, the measurement of PNIF was performed only in basal conditions. An assessment before and after using a nasal decongestant could have been more informative to discriminate between anatomical and mucosal abnormalities.
For the same reason, the evaluation of the trigeminal sensitivity was done using a VAS analogue scale after sniffing highly concentrated acetic acid solution. We believe that this aspect should be better investigated through more in-depth investigations that include the use of ascending concentrations and a lateralization test. Finally, as the recovery time from non-COVID-19 post-infectious olfactory dysfunction can be 2-3 years [62], these data should be considered as intermediate findings.

Conclusion

More than one year after the onset of coronavirus disease 2019, cases exhibited an excess of olfactory and gustatory, and chemesthesis disturbances compared to matched controls. A substantial proportion of cases self-reporting resolution of olfactory dysfunction were found to have persistent chemosensory impairment, at higher rates than matched controls. Persistent chemosensory impairment is associated with emotional distress and depression.

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Authorship contribution

PBR had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: PBR, TH, CH, JP, GT. Acquisition of data: PBR, GS, CF, AA, RM, EZ, EC, KC, SF. Analysis and interpretation of data: PBR, TH, CH, MD, AM, DB, LAV, MT, NG, GT. Drafting of the manuscript: and Critical revision of the manuscript for important intellectual content: PBR, TH, CH, MD, JP, GT. Statistical analysis: JP. Supervision: PBR, TH, CH, MD, JP, GT.

Conflict of interest

The authors declare that they have no conflicts of interest.

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This manuscript contains online supplementary material

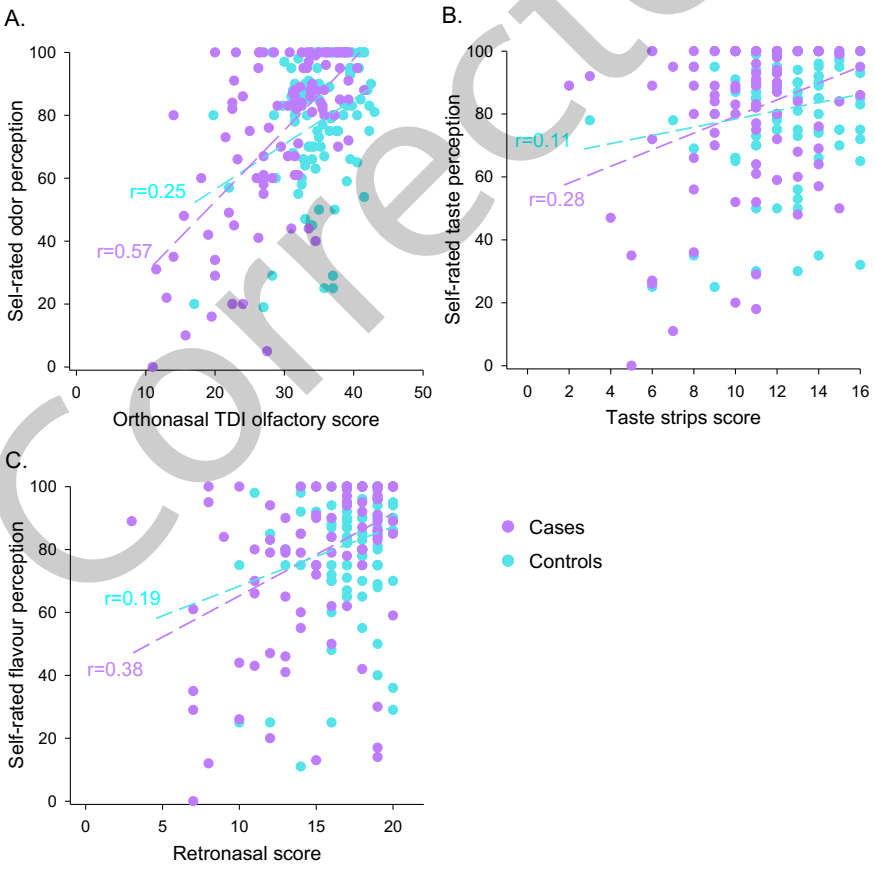
SUPPLEMENTARY MATERIAL

Orthonasal olfactory function

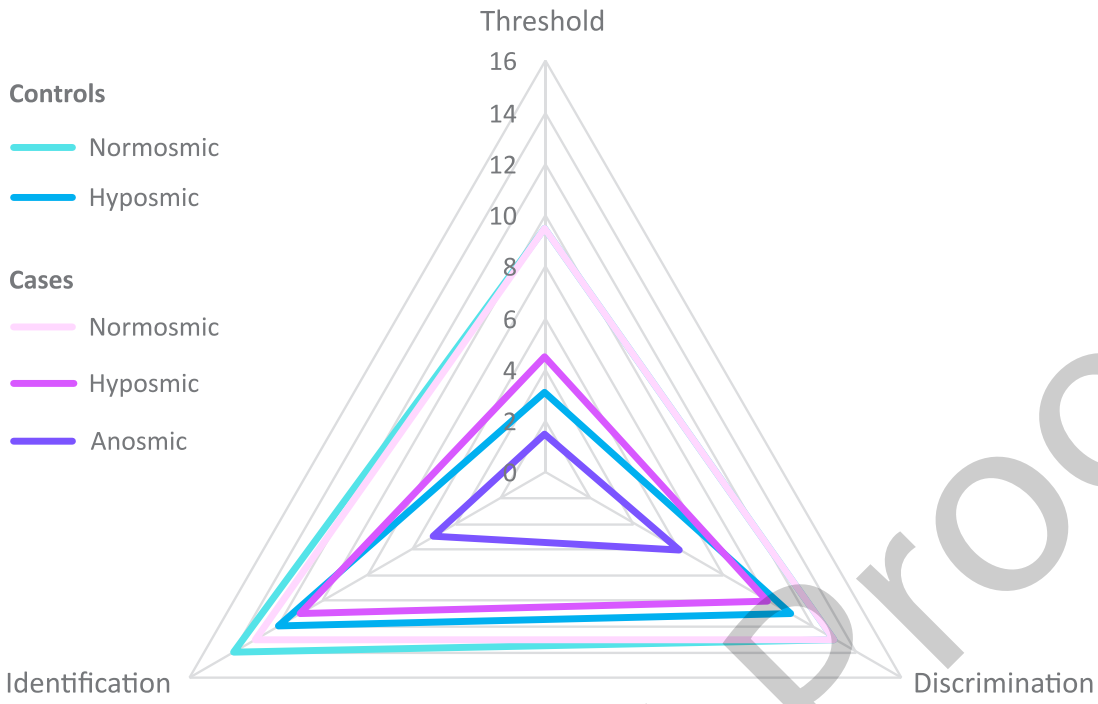
The test is based on pen-like odour-dispensing devices. For odour testing the cap of the pen was removed for approximately 3 seconds and the felt-tip was presented approximately 2 cm in front of the subjects' nostrils. Testing started with the threshold subtest, where 16 dilutions were used. The participants received three odourised pens, with one containing the odour (phenyl ethyl alcohol, PEA - a rose-like smell) and the others containing solvent, propylene glycol, alone. Triplets were presented in increasing odour concentrations, starting with the lowest one. After identifying the correct (odour containing) pen twice in a presented triplet, a reversal of the staircase was started until the participant could no longer correctly identify the odour containing pen. The Threshold score was the mean of the last four out of seven staircase reversals. For the Discrimination, also 16 triplets were presented, with two pens containing the same odour and the third a different one, which the participant should identify. The last subtest performed was the Identification, where 16 pens with different odours were presented. Individuals were asked to choose the object that described the odour the best using multiple forced choice from flash cards where the name of the objects were written.

Gustatory psychophysical evaluation

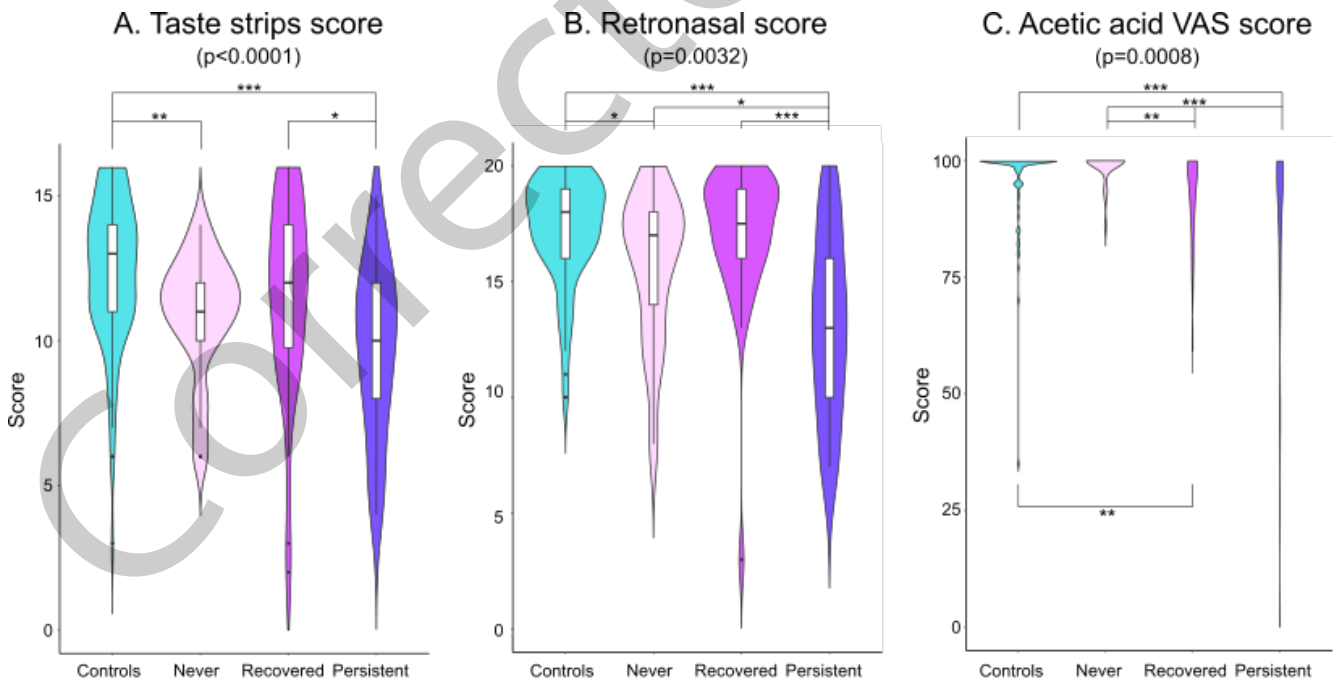
The taste test is based on filter paper strips with a length of 8 cm and a tip area of 2 cm^2 being impregnated with four different concentrations of each of the tastes "sweet" (strips A–D), "sour" (strips E–H), "salty" (strips I–L), and "bitter" (strips M–P). Subjects were invited to move the strips from the left to the right side of the tongue. Participants had to identify the taste from a list of four descriptors: sweet, sour, salty, and bitter (multiple forced choice). Based on the answers of the patients, a taste strips score (TSS) was calculated (0–16 points), used for the identification of hypogeusia (≤9 points) and normogeusia (≥10 points). The first strips in each category (A, E, I, M) have the highest and the subsequent strips have lower taste concentration. For a standardized and reproducible performance of the taste test, the order of taste strips must be respected. At first taste strips with low taste concentrations were presented. According to the increasing concentrations in each category, the strips with the highest concentrations were administered at the end of the test.



Supplementary Figure 1. A. Correlation between self-rating of odour perception and orthonasal olfactory score; B. Correlation between self-rating of taste perception and taste strips score; C. Correlation between self-rating of flavour perception and retronasal score.



Supplementary Figure 2. Olfactory subset score in each olfactory category by cases and control status. Anosmic cases showed the greatest reduction in all the subset scores, resulting in the lowest TDI score. In controls, identification and discrimination scores declined on average by approximately 2 points each in hyposmic patients compared to normosmic (Δ_D=1.8 points, Δ_I=2.0 points); conversely, the threshold score showed a more consistent decrease (Δ_T=6.3; p<0.0001). Similar variations have been observed in cases (Δ_T=4.8 points, Δ_D=2.7 points,Δ_I=2.0 points; p<0.0001), suggesting that threshold is most impaired by SARS-CoV-2 infection.



Supplementary Figure 3. Violin and bloxplot representing ratings for taste strip test (A), retronasal score (B), and acetic acid test (C) in cases and controls. Cases are classified according to the self-reported altered sense of smell or taste in never (patients with COVID-19 not associated with sudden onset of chemosensory dysfunction), recovered (patients recovered from chemosensory dysfunction that arose suddenly during COVID-19), persistent (patients still self-reporting an altered sense of smell or taste that arose suddenly during COVID-19). * p<0.05; ** p<0.01; *** p<0.001.

Supplementary Table 1. COVID-19-Related Symptoms in cases during the acute phase of the disease and at >12-months follow-up.

	Acute phase				>12-month follow-up			
	Mild		Severe		Mild		Severe	
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)
Any symptoms	26	26 (18-36)	74	74 (64-82)	37	37 (28-47)	16	16 (9-25)
ARTIQ								
Dry cough	37	37 (28-47)	9	9 (4-16)	2	2 (0-7)	0	0 (0-4)
Coughing up mucus	7	7 (3-14)	2	2 (0-7)	0	0 (0-4)	0	0 (0-4)
Blocked nose	18	18 (11-27)	6	6 (2-13)	2	2 (0-7)	1	1 (0-5)
Fever	55	55 (45-65)	16	16 (9-25)	0	0 (0-4)	0	0 (0-4)
Headache	25	25 (17-35)	10	10 (5-18)	1	1 (0-5)	1	1 (0-5)
Sore throat	26	26 (18-36)	1	1 (0-5)	0	0 (0-4)	0	0 (0-4)
Muscle pain	42	42 (32-52)	20	20 (13-29)	12	12 (6-20)	2	2 (0-7)
Joint pain	38	38 (28-48)	19	19 (12-28)	8	8 (4-15)	1	1 (0-5)
Chest pain	18	18 (11-27)	4	4 (1-10)	1	1 (0-5)	0	0 (0-4)
Sinonasal pain	4	4 (1-10)	4	4 (1-10)	2	2 (0-7)	0	0 (0-4)
Loss of appetite	26	26 (18-36)	7	7 (3-14)	1	1 (0-5)	0	0 (0-4)
Problem breathing	17	17 (10-26)	7	7 (3-14)	2	2 (0-7)	1	1 (0-5)
Wheezing	4	4 (1-10)	2	2 (0-7)	1	1 (0-5)	1	1 (0-5)
Shortness of breath	19	19 (12-28)	8	8 (4-15)	10	10 (5-18)	2	2 (0-7)
Other symptoms								
Felt tired	44	44 (34-54)	29	29 (20-39)	20	20 (13-29)	5	5 (2-11)
Red eyes	7	7 (3-14)	4	4 (1-10)	1	1 (0-5)	0	0 (0-4)
Diarrhoea	27	27 (19-37)	3	3 (1-9)	2	2 (0-7)	0	0 (0-4)
Nausea	19	19 (12-28)	1	1 (0-5)	1	1 (0-5)	0	0 (0-4)
Vomit	7	7 (3-14)	1	1 (0-5)	0	0 (0-4)	0	0 (0-4)
Abdominal pain	13	13 (7-21)	0	0 (0-4)	1	1 (0-5)	0	0 (0-4)
Insomnia	13	13 (7-21)	4	4 (1-10)	7	7 (3-14)	1	1 (0-5)
Dizziness	13	13 (7-21)	3	3 (1-9)	3	3 (1-9)	0	0 (0-4)
Smell or taste impairment [a]	14	14 (8-23)	55	55 (45-65)	21	21 (13-30)	12	12 (6-20)
Smell only	4	4 (1-10)	6	6 (2-13)	8	8 (4-15)	1	1 (0-5)
Taste only	0	0 (0-4)	3	3 (1-9)	2	2 (0-7)	0	0 (0-4)
Both smell and taste	10	10 (5-18)	46	46 (36-56)	11	11 (6-19)	11	11 (6-19)

[a] Assessed through SNOT-22. "Slight", "Mild" and "Moderate" impairment were classified as "Mild"; "Severe" and "As bad as it can be" were classified as "Severe". ARTIQ, Acute Respiratory Tract Infection Questionnaire

Supplementary Table 2. Nasal patency, PNIF, and OCES score in cases and controls

	Controls		Cases		Mann-Whitney test
	Median (Q1-Q3)		Median (Q1-Q3)		
Nasal patency	80.0 (65.5-94.0)		86.0 (68.0-98.0)		P=0.3102
Maximal PNIF	120.0 (100.0-150.0)		110.0 (90.0-137.5)		P=0.0354
OCES (n - %)					
0	100	(100)	91	(91.0)	P=0.0032
1	0	(0.0)	5	(5.0)	
2	0	(0.0)	4	(4.0)	

PNIF, Peak Nasal Inspiratory Flow; OCES, Olfactory Cleft Endoscopy Scale

Supplementary Table 3. Psychophysical testing and self-rating of chemosensory function in cases and controls according to evolution of impairment of sense of smell and taste.

	Controls	Cases			Kruskal-Wallis test
		Never (n=31)	Recovered (n=36)	Persistent (n=33)	
	Median (Q1-Q3)	Median (Q1-Q3)	Median (Q1-Q3)	Median (Q1-Q3)	
Chemosensory psychophysical evaluation					
Orthonasal					
Threshold	9.0 (6.8-10.8)	9.3 (6.5-10.8)	8.3 (5.3-10.0)	4.0 (1.5-6.0)	p<0.0001
Discriminant	13.0 (12.0-14.0)	12.0 (11.0-13.0)	12.0 (10.0-13.5)	9.0 (8.0-11.0)	p<0.0001
Identification	14.0 (13.0-15.0)	13.0 (12.0-15.0)	12.5 (11.0-14.5)	10.0 (7.0-12.0)	p<0.0001
TDI	35.0 (32.9-38.6)	33.5 (31.5-37.8)	32.9 (27.0-35.6)	23.3 (19.5-27.5)	p<0.0001
Taste strip score	13.0 (11.0-14.0)	11.0 (10.0-12.0)	12.0 (9.5-14.0)	11.0 (8.0-12.0)	p<0.0001
Retronasal score	18.0 (16.0-19.0)	17.0 (13.0-18.0)	17.5 (16.0-19.0)	13.0 (10.0-16.0)	p<0.0001
Acetic acid VAS score	100 (98.0-100)	100 (100-100)	97.0 (89.0-100)	95.0 (80.0-100)	p<0.0001
Self-reported perception (VAS 0-100)					
Self-rating of taste perception	85.0 (74.5-95.0)	95.0 (83.0-100)	94.5 (86.0-100)	60.0 (35.5-80.0)	p<0.0001
Self-rating of odour perception	83.0 (67.5-94.5)	95.0 (83.0-100)	88.0 (80.5-99.5)	46.5 (30.0-60.5)	p<0.0001
Self-rating of flavour perception	85.5 (75.0-94.0)	95.0 (80.0-100)	90.5 (84.0-99.5)	52.5 (32.0-80.0)	p<0.0001

Supplementary Table 3. Psychophysical testing and self-rating of chemosensory function in cases and controls according to evolution of impairment of sense of smell and taste.

Supplementary Table 4. TDI and TSS in controls and in cases according to evolution of impairment of sense of smell and taste.

	Controls		Cases						Fisher's exact test
			Never (n=31)		Recovered (n=36)		Persistent (n=33)		
	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	n	% (95% CI)	
TDI score									
≤16.0		0.0 (0.0-3.6)	0	0.0 (0.0-11.2)	1	2.8 (0.1-14.5)	6	18.2 (7.0-35.5)	P<0.0001
16.25-30.5	10	10.0 (4.9-17.6)	6	19.4 (7.5-37.5)	10	27.8 (14.2-45.2)	23	69.7 (51.3-84.4)	
≥30.75	90	90.0 (82.4-95.1)	25	80.6 (62.5-92.5)	25	69.4 (51.9-83.7)	4	12.1 (3.4-28.2)	
TSS									
≤9	10	10.0 (4.9-17.6)	5	16.1 (5.5-33.7)	9	25.0 (12.1-42.2)	13	39.4 (22.9-57.9)	P=0.0018
≥10	90	90.0 (82.4-95.1)	26	83.9 (66.3-94.5)	27	75.0 (57.8-87.9)	20	60.6 (42.1-77.1)	

TDI, Threshold, Discrimination, Identification; TSS, Taste Strip Score

Supplementary Table 5. PNIF and OCES score in cases according to TDI score.

	Cases TDI≤30.5		Cases TDI>30.5		Mann-Whitney test
	Median (Q1-Q3)		Median (Q1-Q3)		
Maximal PNIF	100.0 (90.0-120.0)		120.0 (90.0-140.0)		P=0.0435
OCES (n - %)					
0	40	(87.0)	51	(94.4)	P=0.4321
1	3	(6.5)	2	(3.7)	
2	3	(6.5)	1	(1.9)	

PNIF, Peak Nasal Inspiratory Flow; OCES, Olfactory Cleft Endoscopy Scale. Note: combining OCES 1-2, difference is not significant (P=0.2947).

Supplementary Table 6. Psychophysical testing of olfactory function (median values) in cases and controls according to age group (years).

	Cases				Controls			
				p-value				p-value
Age groups (years)	<45	45-54	≥55		<45	45-54	≥55	
Orthonasal								
Thresold	6.75	7.00	7.50	0.9356	9.50	9.00	7.50	0.0260
Discriminant	11.00	11.00	11.00	0.9904	13.00	12.00	13.00	0.4477
Identification	12.00	12.00	12.00	0.4409	14.00	14.00	13.00	0.0073
TDI	29.75	31.50	31.50	0.9242	35.50	35.25	33.75	0.0157
Retronasal score	17.00	17.00	16.00	0.7956	19.00	17.00	17.00	0.0017

TDI, Threshold, Discrimination, Identification; VAS, Visual Analogic Scale.

Supplementary Table 7. Mean scores in gustatory function obtained by taste strips test in cases and controls.

	Controls	Cases	t-test
	Mean (95% CI)	Mean (95% CI)	
Sweet	3.5 (3.3-3.6)	3.1 (2.9-3.3)	P=0.0014
Salty	2.9 (2.7-3.1)	2.7 (2.5-2.8)	P=0.0754
Sour	2.7 (2.5-3.0)	2.2 (2.0-2.4)	P=0.0003
Bitter	3.4 (3.2-3.6)	2.9 (2.6-3.2)	P=0.0093

Supplementary Table 8. Prevalence of subjects with hyposmia/anosmia (TDI≤30.5) and hypogeusia (TSS≤9).

	Controls	Cases	Fisher's exact test
TDI≤30.5	10.0 (4.9-17.6)	46.0 (36.0-56.3)	P=0.0004
TSS≤9	10.0 (4.9-17.6)	27.0 (18.6-36.8)	P =0.0032
TDI≤30.5 AND TSS≥10	8.0 (3.5-15.2)	31.0 (22.1-41.0)	P <0.0001
TSS≤9 AND TDI≥30.75	8.0 (3.5-15.2)	12.0 (6.4-20.0)	P =0.4804
TDI≤30.5 AND TSS≤9	2.0 (0.2-7.0)	15.0 (8.6-23.5)	P =0.0015
TDI≤30.5 OR TSS≤9	18.0 (11.0-26.9)	58.0 (47.7-67.8)	P <0.0001

TDI, Threshold, Discrimination, Identification; TSS, Taste Strip Score.