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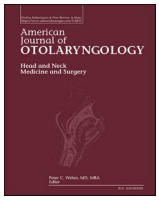

Clinical, virological and immunological evolution of the olfactory and gustatory dysfunction in COVID-19

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ABSTRACT

Purpose: New-onset olfactory and gustatory dysfunction (OGD) represents a well-acknowledged COVID-19 red flag. Nevertheless, its clinical, virological and serological features are still a matter of debate.
Materials and methods: For this cohort study, 170 consecutive subjects with new-onset OGD were consecutively recruited. Otolaryngological examination, OGD subjective grading, nasopharyngeal swabs (NS) for SARS-CoV-2 RNA detection and serum samples (SS) collection for SARS-CoV-2 IgG quantification were conducted at baseline and after one (T1), two (T2) and four weeks (T3).
Results: SARS-CoV-2 infection was confirmed in 79% of patients. Specifically, 43% of positive patients were detected only by SS analysis. The OGD was the only clinical complaint in 10% of cases. Concurrent sinonasal symptoms were reported by 45% of patients. Subjective improvement at T3 was reported by 97% of patients, with 40% recovering completely. Hormonal disorders and RNA detectability in NS were the only variables associated with OGD severity. Recovery rate was higher in case of seasonal influenza vaccination, lower in patients with systemic involvement and severe OGD. Not RNA levels nor IgG titers were correlated with recovery.
Conclusion: Clinical, virological and serological features of COVID-19 related OGD were monitored longitudinally, offering valuable hints for future research on the relationship between host characteristics and chemosensory dysfunctions.

1. Introduction

Coronavirus disease 2019 (COVID-19), caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has been officially recognized as a pandemic by the World Health Organization (WHO), reaching at the time of writing (June 2021) more than 200 countries, with almost 178 million confirmed cases and more than 3 million deaths [1]. Besides nonspecific presenting symptoms, the olfactory and gustatory dysfunction (OGD) soon appeared to be one of the main features at onset, especially in paucisymptomatic cases and early phases of the disease [2–4]. The concomitance of OGD and viral infections is indeed a frequent finding [5], especially in otolaryngology, with OGD typically arising with nasal obstruction and discharge. Nevertheless, OGD in COVID-19 patients is weakly correlated with sinonasal symptoms. Moreover, it typically shows sudden and early onset, being often the only reported symptom [6–9].

The prevalence of SARS-CoV-2 infection in new-onset OGD patients is well acknowledged, although it differs significantly between studies (74–94%) [10–13]. Nevertheless, little is known about its connection with epidemiological variables and comorbidities. Furthermore, only few studies investigated the correlation between viral load on one side and the features of the chemosensitive dysfunction on the other, not taking into consideration serological parameters. Finally, to the best of our knowledge, no cross-sectional or prospective studies have been

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conducted to date employing both serology and molecular assays to better define the prevalence of SARS-CoV-2 infection in new-onset OGD.

The present study was therefore designed in order to longitudinally assess the prevalence of SARS-CoV-2 infection in new-onset OGD patients, basing on molecular and serological quantitative assays. In addition, OGD characteristics such as baseline severity, resolution rate and timing were investigated, correlating specific severity and resolution patterns to relevant clinical, virological and immunological features.

2. Materials and methods

The present observational cohort study was conducted at the Otolaryngology Department of Fondazione IRCCS Policlinico San Matteo (Pavia, Italy), after being approved by the internal review board (reference number: 20200041154). To ensure high quality presentation, the Strengthening the Reporting of Observational studies in Epidemiology guidelines were followed [14]. The study was conducted according to the World Medical Association Declaration of Helsinki.

2.1. Study population

Patients referred to our Department between March and May 2020 for new-onset OGD were consecutively recruited. Written informed consent was obtained from all participants. Inclusion criteria were as follows: age of 18 years or above; new-onset OGD. Exclusion criteria included: preexisting chronic OGD; chronic sinonasal pathologies; nasal decongestant abuse; substance abuse; neuropsychiatric disorders; major head and neck traumas; chemotherapy; radiation of the head and neck region. At the time of enrollment (T0), all participants underwent a baseline interview assessing general demographic and clinical variables (Table 1). A thorough ENT physical examination was conducted for all participants. Endoscopic examination was not performed, to prevent potential aerosolization of viral particles [15,16].

2.2. Molecular and serological testing for SARS-CoV-2

Nasopharyngeal swabs (NS) and serum samples (SS) were prospectively collected from all patients at T0 and after one (T1), two (T2) and four (T3) weeks. Detection and quantification of SARS-CoV-2 RNA were performed on samples collected from the rhinopharynx (FLOQSwabs™, Copan Italia, Brescia, Italy). RNA was extracted from 400 μL of Universal Transport Medium (UTM™) using the QIAsymphony® instrument with the QIAsymphony® DSP Virus/Pathogen Midi Kit (Complex 400 protocol), according to the manufacturer's instructions (QIAGEN, Qiagen, Hilden, Germany). Moreover, specific real-time RT-PCRs targeting RNA-dependent RNA polymerase and E genes were employed to detect the presence of SARS-CoV-2 according to the WHO guidelines[1] and Corman's protocol [17]. Sequential SS were examined using chemiluminescent assay (Liason SARS-CoV-2 S1/S2 IgG, Diasorin, Saluggia, Italy) for quantitative characterization of SARS-CoV-2 anti-S1 and anti-S2 IgG antibodies. Results were given as AU/mL, and a cut-off of 15 AU/mL was set to define positive samples.

Table 1
Clinical and demographic features of the enrolled 170 patients presenting with new-onset olfactory and gustatory dysfunction.

Variable ($N = 170$)	N (%)
Age (median; IQR)	43; 30–51
Gender	
Female	108 (63.5)
Male	62 (36.5)
Healthcare professional	62 (36.5)
Influenza vaccination	21 (12.4)
Smoking	
Never	89 (52.4)
Former smoker	33 (19.4)
Active smoker	48 (28.2)
Allergies	54 (31.8)
Comorbidities	51 (30)
Cardiovascular	16 (8.2)
Asthma	4 (2.4)
Chronic obstructive pulmonary disease	1 (0.6)
Endocrine disorders	21 (12.4)
Autoimmune disorders	11 (6.5)
Obstructive sleep apnea syndrome	4 (2.4)
Other	4 (2.4)

IQR, interquartile range.

2.3. Olfactory and gustatory dysfunction (OGD)

At all times, a dedicated form was administered to investigate OGD features: type (“hyposmia/hypogeusia” versus “anosmia/ageusia”), date and type of onset (“sudden” versus “gradual”), course (“constant” versus “fluctuating”), general signs and symptoms (GSS), nasal symptoms (nasal obstruction, rhinorrhea). Since psychophysical tests were not practicable within COVID-19 emergency state, OGD subjective grading was conducted at all times through dedicated measures. First, a 100-millimiter Visual Analogue Scale (VAS), anchored at each end with verbal descriptors (“no impairment-0” and “extreme impairment-100”), was administered to investigate both gustatory (VAS-G) and olfactory (VAS—O) dysfunctions. The VAS has been already employed for OGD assessment. Moreover, significant correlations have been demonstrated between this scale and the Objective Odor Stick Tests (OOST) [18–21]. Two additional patient-reported outcome (PRO) instruments were also administered: the Hyposmia Rating Scale (HRS) and the Chemosensory Complaint Score (CCS). The HRS was originally developed to grade olfactory dysfunctions in Parkinson's disease [22]. Each of the six items (scent of flowers; unburnt gas; garbage, sewage or other foul smelling materials; scent of perfume; smell of stuffiness or strong body odor; smell of home cooking) is rated on a five-point Likert scale from 1 (“always aware of the smell”) to 5 (“unfamiliar with or never smelt the smell”), with a total score ranging from 6 (no impairment) to 30 (worst impairment). Its clinical utility has been tested in several settings [22–24], demonstrating a strong correlation with the OOST. Taste impairments were graded instead using the CCS [25], originally developed to grade taste alterations in HIV patients and subsequently tested in chronic rhinosinusitis [26], which provides the best approximation of a patient-reported taste metrics. The CCS is an eight-item questionnaire investigating multiple features of taste dysfunctions: change in the sense of taste, change in the way food tastes, presence and quality of a bad taste, effect of medications on taste and changes in quality of four taste subgroups (salt, sweet, sour and bitter). Each of the items is given one point if abnormal. A ninth question deals with the overall severity of the dysfunction; it is given one point in case of a mild or moderate dysfunction or two points for a severe dysfunction. Thus, its total score ranges from 0 (no complaints) to 10 (severe complaints).

Given the context of the present pandemic, no validation studies of these PRO instruments were conducted. However, both the HRS and the CCS were preliminarily translated into Italian by two senior otolaryngologists and one bilingual translator to retain the meaning of the original items.

2.4. Statistical analysis

Qualitative variables were described as absolute frequencies and percentages. Quantitative variables were summarized in terms of median and interquartile range (IQR). Levels of the scales at T0 were compared according to epidemiological and clinical features using Mann-Whitney test or Kruskall-Wallis test (depending on whether the comparison was between two or more independent groups of patients). Scales trends during follow-up were evaluated by linear regression models. Standard errors were estimated with a clustered sandwich estimator in order to allow for intra-group correlation. Similarly, linear

regression models with clustered sandwich standard errors were used to evaluate scales trends during follow-up as RNA copies/mL and IgG titers. Complete recovery free survival was calculated as the time between the enrollment and date of recovery or last follow-up. The crude effect (univariable analysis) of clinical and epidemiological features at T0 on complete recovery free survival was estimated by Cox Proportional Hazard models. Variables with a p-value lower than 0.2 in univariable analysis were candidate to enter in multivariable model. p values < 0.05 were considered statistically significant. All statistical analyses were conducted using the version 16 of the software Stata (StataCorp 2019; Stata Statistical Software: Release 16; College Station, TX: StataCorp LLC).

3. Results

3.1. Study population

Overall, 170 new-onset OGD patients were enrolled: 162 patients completed the follow-up (T3), while 3 and 2 patients were lost at T1 and T2, respectively. The median time from OGD onset to enrollment was 11 days (IQR 6–19). Table 1 summarizes demographic and clinical features of the participants. None of them had pathological findings at the ENT evaluation.

3.2. Molecular and serological testing for SARS-CoV-2

SARS-CoV-2 seroprevalence (SARS-CoV-2 IgG levels) and SARS-CoV-2 RNA copies in NS were evaluated over time (Fig. 1). Globally, SARS-CoV-2 infection was confirmed through NS and/or SS in 134 patients (79%; 95% CI: 72%–85%). SS analysis alone detected only 55 COVID-19 patients (43.3%).

3.3. Olfactory and gustatory dysfunction (OGD)

Clinical features of SARS-CoV-2 patients are depicted in Table 2. The OGD demonstrated sudden onset and constant intensity in most cases. Almost all patients (128; 96%) had a combined perceptual disorder. Seventy-four patients (55%) reported no additional sinonasal symptoms. When associated with nasal obstruction, the OGD occurred at the same time in 19 patients (44%), while it manifested before (median 2 days IQR 2–7) and after (median 5 days IQR 3–8) in 9 (21%) and 15 (35%) patients, respectively. Interestingly, an OGD with or without sinonasal symptoms was the only clinical complaint in 14 cases (11%). With regards to GSS, they occurred simultaneously with the OGD in 27 patients (25%), while they preceded (median 4 days IQR 2–7) or followed (median 5 days IQR 2–8) the OGD in 71 (65%) and in 11 (10%) subjects, respectively. No patients had severe COVID-19 symptoms requiring hospitalization during follow-up.

Table 2
Clinical features of the olfactory and gustatory dysfunction in patients who tested positive for SARS-CoV-2 infection.

Variable ($N = 134$)	N (%)
OGD characteristics	
Sudden onset	123 (91.8)
Fluctuant	15 (11.2)
Olfactory disorder only	6 (4.5)
Taste disorder only	0 (0.0)
Combined perceptual disorder	128 (95.5)
Anosmia and ageusia	100 (74.6)
Anosmia and hypogeusia	15 (11.2)
Hyposmia and ageusia	1 (0.7)
Hyposmia and hypogeusia	12 (9.0)
Other sinonasal signs and symptoms	60 (44.8)
Nasal obstruction	43 (32.1)
Rhinorrhea	44 (32.8)
General signs and symptoms	120 (89.5)
Fever (>37.5 °C/99.5 °F)	89 (66.4)
Headache	73 (54.5)
Asthenia	51 (38.1)
Cough	38 (28.4)
Nausea	23 (17.2)
Myalgia	17 (12.7)
Diarrhea	5 (3.7)
Dyspnea	3 (2.2)
Pharyngodynia	2 (1.5)
Vertigo	1 (0.8)
None	14 (10.5)

OGD, olfactory and gustatory dysfunction.

OGD severity, although fluctuant in 15 patients (11%), showed an overall improving trend. In fact, both the baseline HRS score of 29 (IQR 27–30) and the baseline CCS score of 7 (IQR 5–8) significantly improved to 12 (IQR 6–22) and 0 (IQR 0–3) at T3, respectively ($p < 0.001$). Similarly, baseline VAS-O and VAS-G decreased from 9 (IQR 8–10) and 8 (IQR 5–10) to 2 (IQR 0–5) and 1 (IQR 0–4), respectively ($p < 0.001$). At the univariable analysis, endocrine disorders and positive NS were the only variables significantly correlated with OGD severity (Table 3). Precisely, in case of endocrine disorders or positive NS, patients reported higher subjective impairments. SARS-CoV-2 RNA levels in NS as well as IgG titers showed no significant correlation with OGD severity, not at baseline nor during follow-up (Table 4).

As far as recovery is concerned, 130 patients (97%) reported an improvement of the OGD at T3, but only 53 (40%) recovered completely within 23 days from onset (IQR 18–32). Specifically, higher severity at onset and GSS were associated with a lower probability of complete recovery within T3 (Table 5). A mild association between influenza vaccination and complete OGD recovery was also demonstrated through the multivariable analysis (Table 6). No other relevant associations have

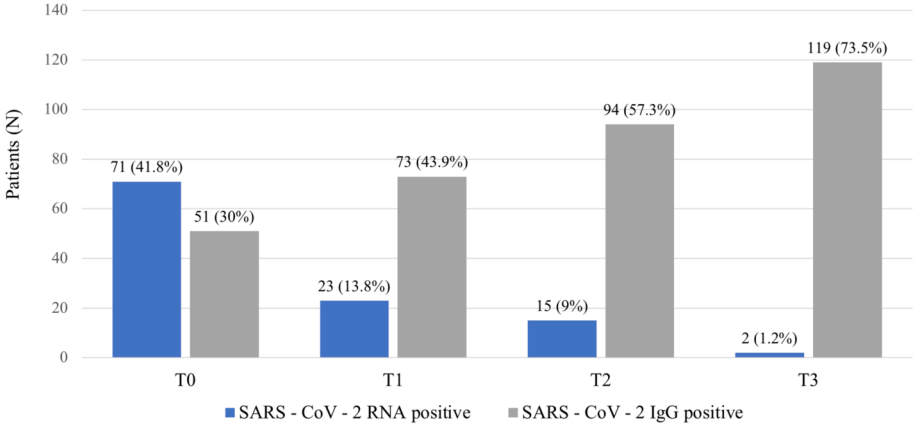

Fig. 1. SARS-CoV-2 seroprevalence (serum IgG positivity) and SARS-CoV-2 RNA detectability (nasopharyngeal swab positivity) at T0, T1, T2 and T3.

Table 3
Association between epidemiological and clinical features and the olfactory and gustatory dysfunction severity at T0.

		VAS-O		VAS-G		HRS		CCS	
Variable		Median (IQR)	p	Median (IQR)	p	Median (IQR)	p	Median (IQR)	p
Sex	Female	0.5 (0–3)	0.769	2 (0–5)	0.789	29 (26–30)	0.987	7 (5–9)	0.345
	Male	1 (0–2)		2 (0–4.5)		29 (27–30)		6.5 (4.5–8)	
Age (years)	<45	0 (0–3)	0.246	2 (0–5)	0.654	30 (26–30)	0.413	7 (5–9)	0.492
	≥45	1 (0–2)		2 (1–4)		29 (27–30)		7 (4–8)	
Smoking	No	1 (0–2)	0.673	2 (0–5)	0.348	29 (26–30)	0.939	7 (5–9)	0.854
	Ex	1 (0–3)		2 (1–4)		28 (27–30)		7 (5–8)	
	Yes	0 (0–2)		1 (0–3)		30 (25–30)		7 (5–8)	
Influenza vaccination	No	1 (0–2)	0.475	2 (0–5)	0.837	29 (26–30)	0.379	7 (5–8)	0.846
	Yes	0 (0–2.5)		2 (0.5–3)		30 (27–30)		7 (5–8.5)	
Allergies	No	1 (0–3)	0.234	2 (0–5)	0.195	29 (26–30)	0.394	7 (5–9)	0.601
	Yes	0 (0–2)		2 (1–4)		30 (27–30)		7 (5–8)	
Hormonal disorders	No	1 (0–3)	0.134	2 (0–5)	**0.047***	29 (26–30)	**0.045***	7 (5–8)	**0.024***
	Yes	0 (0–1)		0.5 (0–2)		30 (28–30)		9 (7–10)	
General symptoms	No	0 (0–2)	0.389	2 (0–4)	0.442	29 (26–30)	0.913	7 (4–9)	0.708
	Yes	1 (0–3)		2 (0–5)		29 (27–30)		7 (5–8)	
Nasal obstruction	No	0 (0–2)	0.146	2 (0–4)	0.428	29 (27–30)	0.235	7 (5–9)	0.464
	Yes	1 (0–3)		2 (0–5)		28 (25–30)		6.5 (5–8)	
Sudden onset	No	2 (1–3)	0.078	2 (1–5)	0.310	28 (23−30)	0.141	5 (3–7)	0.053
	Yes	0 (0–2)		2 (0–5)		29 (27–30)		7 (5–9)	
Concurrent pathologies	No	1 (0–2)	0.920	2 (0–5)	0.920	29 (27–30)	0.959	7 (5–8)	0.626
	Yes	1 (0–2)		2 (1–4)		29 (27–30)		7 (5–9)	
SARS-CoV-2 RNA (NS)	NEG	1 (0–3)	**0.001***	2 (1–5)	**0.019***	28 (25–30)	**0.001***	7 (4–8)	0.189
	POS	0 (0–2)		2 (0–3)		30 (28–30)		7 (5–9)	

VAS-O, Visual Analogue Scale – Olfactory; VAS-G, Visual Analogue Scale – Gustatory; HRS, Hyposmia Rating Scale; CCS, Chemosensory Complaint Score; IQR, interquartile range; NS, nasal swab; NEG, negative; POS, positive; * $p < 0.05$

Table 4
Correlations between log_{10} SARS-CoV-2 RNA copies/mL, SARS-CoV-2 IgG titers and OGD severity at baseline and during follow-up.

	Log_{10} RNA copies/mL		IgG titers	
	T0	Follow-up	T0	Follow-up
VAS-O	R = −0.05, p = 0.690	Coef = 0.11, p = 0.841	R = 0.13, p = 0.337	Coef = −0.02, p = 0.943
VAS-G	R = 0.05, p = 0.657	Coef = 0.76, p = 0.379	R = 0.10, p = 0.435	Coef = 0.08, p = 0.771
HRS	R = 0.07, p = 0.558	Coef = −0.9, p = 0358	R = −0.30, p = 0.051	Coef = −0.11, p = 0.866
CCS	R = −0.01, p = 0.974	Coef = −0.5, p = 0.569	R = −0.14, p = 0.285	Coef = −0.04, p = 0.882

VAS-O, Visual Analogue Scale – Olfactory; VAS-G, Visual Analogue Scale – Gustatory; HRS, Hyposmia Rating Scale; CCS, Chemosensory Complaint Score.

been highlighted. SARS-CoV-2 RNA detectability in NS and SARS-CoV-2 IgG positivity in SS were not associated with OGD recovery rate ($p = 0.523$ and $p = 0.214$, respectively).

4. Discussion

This study aimed to investigate the clinical, virological and immunological features of the COVID-19-related OGD over time. Several studies demonstrated that the OGD is a strong clinical marker of COVID-19, with a positive predictive value of 61% [7], even though its relevant specificity (93–99%) is not matched by an equally high sensitivity (23–43%) [6]. In our investigation, the OGD confirmed its clinical relevance, being the only reported complaint in 10.5% of positive patients. The chemosensory impairment demonstrated fundamental importance for diagnosis even in case of systemic disease, since in 10.1% of patients the OGD anticipated GSS by 5 days (IQR 2–8).

The overall prevalence of SARS-CoV-2 infection, based on NS and SS analyses combined, was 79%. Most studies investigating SARS-CoV-2 prevalence in OGD patients relied on NS results one, like the reports from Lechien (88%) [10], Salmon-Ceron (94%) [11] and Hopkins (74%) [27]. Only two studies so far employs SS testing, specifically a preliminary report from our study group (75%) [9] and a cross-sectional study employing solely qualitative point-of-care serological kits (77.6%) [28]. To the best of our knowledge, this is the first study assessing SARS-CoV-2 prevalence relying on both NS and SS within a controlled clinical setting. Noteworthily, our results highlighted the cruciality of serological testing for COVID-19 diagnosis in OGD patients. In fact, more than 40% of COVID-19 cases were detected only by means of serological essays, implying that NS or SS alone could have underestimated SARS-CoV-2 prevalence. This may be primarily related to the interval between symptoms' onset and laboratory confirmation. Indeed, as we previously observed in a smaller cohort [9], the great majority of patients developed SARS-CoV-2 IgG three weeks after symptoms' onset, while RNA detectability in NS gradually decreased over time (Fig. 1).

As far as clinical features are concerned, literature reveals how COVID-19-related OGD usually presents as a combined disorder,

Table 5
Univariable analysis investigating the influence of epidemiological and clinical variables on the olfactory and gustatory dysfunction (OGD) resolution at T3.

	HR	95%CI	p
Age (years)	0.98	0.96–1.01	0.147
Sex (male vs female)	0.8	0.4–1.4	0.454
Seasonal influenza vaccination (yes vs no)	2.0	1.0–4.0	**0.070***
Smoking			
Ex vs no	1.4	0.7–2.9	0.338
Yes vs no	1.5	0.8–2.9	0.235
General sign and symptoms (yes vs no)	0.5	0.3–0.9	**0.023***
Nasal obstruction (yes vs no)	1.4	0.8–2.5	0.238
Comorbidities (yes vs no)	0.8	0.4–1.6	0.547
Endocrine disorders (yes vs no)	0.9	0.4–2.3	0.866
Allergies (yes vs no)	1.2	0.6–2.1	0.632
Rhinorrhea (yes vs no)	0.8	0.4–1.5	0.463
SARS-CoV-2 IgG titer (AU/mL)	0.9	0.6–1.6	0.801
Log10 RNA (copies/mL)	0.8	0.3–2.0	0.644
VAS-O	1.1	1.0–1.3	0.083
VAS-G	1.1	1.0–1.2	**0.005***
HRS	0.9	0.9–1.0	**0.034***
CCS	0.9	0.8–1.0	**0.003***

HR, Hazard Ratio; CI, Confidence Interval; VAS—O, Visual Analogue Scale – Olfactory; VAS-G, Visual Analogue Scale – Gustatory; HRS, Hyposmia Rating Scale; CCS, Chemosensory Complaint Score; * $p < 0.05$.

Table 6
Multivariable analysis of epidemiological and clinical variables related to OGD resolution at the end of the follow-up period (T3).

	Model 1 (HRS)			Model 2 (CCS)		
	HR	95% CI	p	HR	95% CI	p
Age (years)	0.99	0.97–1.01	0.361	0.99	0.96–1.01	0.232
Seasonal influenza vaccination (yes vs no)	2.33	1.10–4.95	0.027	2.42	1.14–5.14	0.021
General sign and symptoms (yes vs no)	0.54	0.29–1.01	0.053	0.54	0.29–1.00	0.051
HRS	0.93	0.88–0.98	0.009	–	–	–
CCS	–	–	–	0.86	0.79–0.94	0.001

HR, Hazard Ratio; CI, Confidence Interval; HRS, Hyposmia Rating Scale; CCS, Chemosensory Complaint Score.

affecting both smell and taste in most cases [6,7,29–31]. Our results align with published data, with 96% of patients reporting both impairments. In fact, even though retro-nasal olfaction may contribute to flavors perception, emerging evidence depicts direct viral involvement as the primary mechanism underlying COVID-19 gustatory dysfunction [5]. Interestingly, the expression of Angiotensin-Converting Enzyme-2 (ACE-2) – the principal cell receptor for SARS-CoV-2 – has been recently identified in taste organs of murine models [32,33].

With regards to baseline OGD severity, only endocrine disorders, as found by Lechien et al. [38], and RNA detectability in NS were significantly associated with higher impairments. The influence of metabolic disorders on smell and taste is well-acknowledged [34–36], since the olfactory and the endocrine system are intimately linked. Axonal projections to and from the olfactory bulb allow a crosstalk between the olfactory system and the hypothalamus. Moreover, the olfactory mucosa and bulb cells express receptors and peptides involved in metabolic homeostasis [37].

Despite the overall improving trend, only 40% of patients recovered completely from the OGD at T3. Resolution rate appear to be variable in literature, ranging from 13% [39] to 86% [40], mainly depending on follow-up duration and OGD evaluation methodology (quantitative vs qualitative) [41,42]. In our sample, severity at onset was the most relevant variable influencing complete resolution. This evidence is confirmed by other studies investigating the evolution of chemosensory impairments in COVID-19 [43–47]. To the best of our knowledge, the association between OGD and GSS has never been deeply investigated to date. In our study, GSS were associated with lower chances of complete recovery. It might be speculated that systemic involvement could reflect a more severe SARS-CoV-2 infection and, therefore, reduced abilities of the body to cope with the infection. Interestingly, Lovato et al. [48], in their preliminary report on 121 COVID-19 patients, found out that among GSS, the absence of fever was significantly associated with persistent OTD. Authors speculate that, since fever is associated with severe COVID-19, patients with OGD without fever would experience a mild-moderate COVID-19. However, to date no final inferences can be drawn in this matter, and further studies are needed to properly test this hypothesis.

Impressively, a significant association between influenza vaccination and complete recovery at T3 was also verified. Similar trends were also noted before the present pandemic by Flanagan, who highlighted how influenza vaccination rates were significantly lower among OGD patients [49]. Furthermore, this association was also explored on murine models, in which intranasal immunization demonstrated attenuation properties on both viral localization and inflammation of the olfactory bulb [50]. These findings and our results offer interesting hints for future clinical and experimental research, which should be aimed at better understanding the connection between acquired immunity and chemosensory dysfunctions.

Several virological and immunological features of COVID-19 OGD appear noteworthy. First, the only significant virological correlation was found between RNA detectability in NS on one side and OGD baseline severity on the other. Surprisingly, while the presence of SARS-CoV-2 in the nasopharynx was associated with higher perceptual impairments, RNA copies/mL never showed significant correlations with OGD severity. These findings suggest that active SARS-CoV-2 infections might be the only prerequisite for the development of perceptual impairments, not necessarily requiring higher viral loads to reach clinical relevance. The relationship between the viral load and the chemosensory dysfunction is still a matter of debate. Jain et al., comparing the cycle threshold (CT) value on PCR assay in COVID-19 patients with and without OGD, found out that patients with olfactory dysfunction had higher viral load than those without perceptual impairment [51]. However, Vaira et al. and Cho et al. did not find a significant correlation between the CT value and the olfactory function, suggesting that the OGD severity might be related to individual susceptibility rather than viral load [52,53]. Further studies investigating the correlation of viral load and the chemosensory dysfunction COVID-19 related are needed.

With regards to immunological data, no significant associations were found between IgG titers and overall OGD severity or trend. These results appear to be quite unexpected, since higher IgG titers should correspond to more effective responses to the infection and, theoretically, less severe and less lasting symptoms. Nevertheless, also this serological evidence needs further investigation.

Sinonasal symptoms other than the chemosensory impairment itself (i.e., nasal obstruction and nasal discharge) affected less than half of positive OGD patients. However, Naeini and colleagues demonstrated through computed tomography of paranasal sinuses that there were no significant pathological changes in the olfactory clefts and sinonasal mucosa in the 49 anosmic COVID-19 patients analysed [54].

Furthermore, no significant differences in terms of OGD baseline severity, course and resolution rate were found between patients with and without nasal complaints. Notoriously, sinonasal symptoms alone may lead to olfactory dysfunctions, since nasal inflammation and mucosal swelling can prevent olfactory molecules from reaching the olfactory clefts. The persistence of olfactory impairments without major nasal complaints and after the acute phase of COVID-19 strengthens the hypothesis of a direct damage of both the olfactory epithelium and central olfactory pathways. SARS-CoV-2 is a neurotropic virus, able to spread from the peripheral olfactory system to the central nervous system [55–57]. Several reports have demonstrated viral spreading towards the olfactory bulb through the olfactory neuroepithelium [10,58,59]. ACE-2 and TMPRSS2, required for SARS-CoV-2 entry in host cells, were detected in sustentacular and olfactory stem cells in human specimens [60,61]. Central nervous system involvement by SARS-CoV-2 has been proven even radiologically, with MRI evidence of olfactory bulb abnormalities in anosmic COVID-19 patients [62,63]. Moreover, SARS-CoV-2 particles, diffuse infiltration of CD163-positive macrophages and cytotoxic T lymphocytes have been identified in the olfactory bulbs of patients with severe COVID-19 [64]. Moreover, there is emerging evidence regarding the role of SARS-CoV-2 in determining olfactory impairment through blocking the rapid turnover of the olfactory receptors. In fact, according to recent studies, SARS-CoV-2 might attack the nasal serous gland inhibiting the production of the growth factors necessary for stem cells activation and transformation in olfactory receptors [65]. In conclusion, the contribution of different potential pathogeneses to the olfactory impairment of COVID-19 deserves further studies.

This study has several limitations. Firstly, due to the COVID-19 emergency state, the OGD was not evaluated with psychophysical tests or electrophysiological studies, since they were not practicable. These instruments could be potentially employed to screen residual long-lasting OGD after the resolution of the emergency state. Secondly, although a thirty-day follow-up is significantly longer than the average follow-up of previous reports, longer monitoring could have better contributed in evaluating the rate of persistent OGD. Lastly, in reason of

the current emergency, OGD etiologies in SARS-CoV-2 negative patients were not investigated.

5. Conclusion

The present longitudinal study depicts the clinical course of COVID-19-related OGD with regards to its virological and immunological features. Specifically, the combination of SS and NS appeared to be crucial in order to identify a higher rate of positive COVID-19 patients suffering from OGD. A higher OGD severity was reported in case of RNA detectability in NS and concurrent endocrine disorders. Recovery rates were lower in case of higher severity at onset, as well as in case of systemic symptoms. Contrariwise, a higher recovery rate was highlighted for subjects who underwent influenza vaccination, offering hints for future research investigating the relationship between acquired immunity and chemosensory dysfunctions.

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CRediT authorship contribution statement

EM: patient enrollment, patient examination and testing, questionnaire administration, literature revision, drafting of the manuscript; AC: patient enrollment, patient examination and testing, questionnaire administration, literature revision; CR: literature revision, drafting of the manuscript; IC: analysis of blood samples and nasal swabs specimens; VZ, ES: patient enrollment; VVF, CK: data analysis and interpretation; FB, MB: study design, critical revision of the manuscript.

Declaration of competing interest

None.

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