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Correlations Between Olfactory Psychophysical Scores and SARS-CoV-2 Viral Load in COVID-19 Patients

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Objectives/Hypothesis: The aim of this study was to evaluate the correlations between the severity and duration of olfactory dysfunctions (OD), assessed with psychophysical tests, and the viral load on the rhino-pharyngeal swab determined with a direct method, in patients affected by coronavirus disease 2019 (COVID-19).

Study design: Prospective cohort study.

Methods: Patients underwent psychophysical olfactory assessment with Connecticut Chemosensory Clinical Research Center test and determination of the normalized viral load on nasopharyngeal swab within 10 days of the clinical onset of COVID-19.

Results: Sixty COVID-19 patients were included in this study. On psychophysical testing, 12 patients (20% of the cohort) presented with anosmia, 11 (18.3%) severe hyposmia, 13 (18.3%) moderate hyposmia, and 10 (16.7%) mild hyposmia with an overall prevalence of OD of 76.7%. The overall median olfactory score was 50 (interquartile range [IQR] 30–72.5) with no significant differences between clinical severity subgroups. The median normalized viral load detected in the series was 2.56E+06 viral copies/10^6 copies of human beta-2microglobulin mRNA present in the sample (IQR 3.17E+04–1.58E+07) without any significant correlations with COVID-19 severity. The correlation between viral load and olfactory scores at baseline ($R^2 = 0.0007$; $P = .844$) and 60-day follow-up ($R^2 = 0.0077$; $P = .519$) was weak and not significant.

Conclusions: The presence of OD does not seem to be useful in identifying subjects at risk for being super-spreaders or who is at risk of developing long-term OD. Similarly, the pathogenesis of OD is probably related to individual factors rather than to viral load and activity.

Key Words: COVID-19, viral load, olfactory, anosmia, SARS-CoV-2, coronavirus.

Level of Evidence: 4

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INTRODUCTION

It is now widely established that severe acute respiratory syndrome 2 (SARS-CoV-2) is capable of causing damage to the olfactory pathway and more than 70% of patients complain olfactory dysfunction (OD) during coronavirus disease 2019 (COVID-19).[1–6] The pathogenesis of this disorder has not yet been fully clarified.[7,8] In the first part of the pandemic, many authors attributed the OD to phenomena of neuroinvasion with subsequent apoptosis of the neurons of the olfactory bulb.[9,10] This hypothesis was based on the fact that OD was generally not associated with rhinitis symptoms,[11,12] on some reports of alterations in the bulb on magnetic resonance imaging in COVID-19 patients with anosmia[13] and on the neuroinvasive potential demonstrated by severe acute respiratory syndrome coronavirus 1 in the past.[14] More recently, researchers have focused on the olfactory epithelium as the site of viral damage underlying OD. This second hypothesis is supported by some solid evidence. First, OD appears to be more frequent in mild COVID-19 and regresses within a few weeks in most patients.[15–18] This evidence would be unlikely if OD were caused by an invasion of the central nervous system with neuronal destruction. Second, several authors reported the presence of

olfactory cleft edema on computed tomography in anosmic patients.[19,20] Third, ACE-2 receptors for the virus are highly concentrated on the supporting cell membranes of the olfactory epithelium, whereas they are sparsely present in olfactory neurons.[21,22] Fourth, the first histopathological reports on samples taken from anosmic patients have shown damage to the olfactory mucosa that can range from focal atrophy with inflammatory neuropathy to massive destruction of the epithelium.[23,24]

However, the risk factors for the development of OD in COVID-19 patients have not yet been identified.[1,3,6,11,25] Furthermore, the first studies with long-term follow-up are showing a significant frequency of persistent severe OD, ranging between 5% and 11% of cases.[26–29] This means that COVID-19-related persistent OD could represent a serious public health problem in the coming years. It would be important to identify the risk factors related to the development of persistent OD but none of these have yet been identified.

Based on the most recent pathogenetic theories, an important role could be played by the infecting viral load. Only two studies have previously evaluated the correlations between viral load at the rhino-pharyngeal swab and presence of OD.[30,31] Both of these studies have the merit of having first investigated the possible correlations between viral load and severity of chemosensitive disorders in COVID-19 patients but present some limitations which may have influenced the results obtained. First, the assessment of the olfactory function was not based on psychophysical tests and it has been shown that self-reported olfactory loss alone significantly underestimates the real prevalence of smell disorders in COVID-19 patients.[32,33] Moreover, by reducing the OD to a dichotomous variable, it is not possible to perform a statistical analysis based on the direct correlation between continuous variables, which is certainly more accurate. Second, the viral load is estimated indirectly, through the calculation of the cycle threshold (Ct), which is inversely proportional to the amount of viral nucleic acid in the sample.[34] Ct is in fact defined as the number of reverse transcription polymerase chain reaction (PCR) required to reach a threshold for detection of the viral nucleic acid. However, a growing number of authors are reporting that Ct value is an unreliable index in estimating viral load and therefore its use should be considered with great caution.[35,36]

The aim of this study was therefore to test the hypothesis that the severity and duration of OD, assessed with psychophysical tests, is directly correlated to the viral load on the rhino-pharyngeal swab determined with a direct quantitative method, in patients affected by COVID-19 and admitted to the University Hospital of Sassari. Considering that the studies published so far have conflicting results, we have tried to overcome their limitations in order to provide researchers with further evidence for critical evaluation.

MATERIALS AND METHODS

This cohort observational study was conducted in the COVID departments of the University Hospital of Sassari.

To be enrolled in the study, patients had to meet the following inclusion criteria: adults over 18 years of age, rhino-pharyngeal swab positive for SARS-CoV-2 infection, COVID-19 symptoms present for less than 10 days, patient acceptance for participation in the study. The study exclusion criteria were: uncooperative patients, assisted ventilation, psychiatric or neurological disorders, previous surgery or radiotherapy in the oral and nasal cavities, pre-existing self-reported smell and taste alterations, history of head trauma, allergic rhinitis, chronic rhinosinusitis. A control group with subjects screened for SARS-CoV-2 infection, with no history of previous infection, and whose nasopharyngeal swab was negative was included with the aim of comparing the prevalence of smell loss in those without COVID-19.

The study was conducted in accordance with the ethical standards of the institutional research committee (approval no. PG/2021/5471) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Some clinical and epidemiological information was collected for all patients: age, gender, and COVID-19 symptoms with the COVID-19 symptom index (CSI).[37] The CSI evaluates the severity of 24 common symptoms of COVID-19 with a score ranging from 0 (no problem) to 4 (very serious problem). The CSI also rates self-reported olfactory and gustatory loss as normal (0), partial (1), and total loss (2). The overall score thus obtained can therefore vary from 0 (no symptoms) to 100 (very serious symptoms). All patients were followed up clinically until the nasopharyngeal swab was negative. The overall clinical severity of COVID-19 was classified according to Tian et al.[38] in mild, moderate, severe, and critical.

Psychophysical olfactory evaluation was performed with the Connecticut Chemosensory Clinical Research Center test (CCCRC). The CCCRC is a validated, widely used and easy to perform psychophysical test. The methodology, the scoring system and its application in COVID-19 patients have been extensively described in previous studies.[39–42] The CCCRC includes the assessment of the olfactory threshold using solutions with increasing concentration of N-butyl acid and an identification task for common odorants. Threshold testing was performed presenting solutions of N-bunatol in deionized waters, decreasing concentration in 8 steps. The strongest butanol concentration was 4% in 60 mL of deionized water (bottle 0). Each other bottle (from 1 to 8) contained a subsequent 1:3 N-butanol dilution. Two identical squeezable bottles were presented to the patient: one containing the N-butanol solution, starting from the major dilution, and the other filled with deionized water. The patient was then asked to close one nostril and squeeze the bottle immediately below the other, reporting which of the two bottles smelled most. The threshold was identified when the subject gave the correct answer 4 times. In case of error, the next most concentrated solution was given to the patient. The threshold was quantified for each of the two nostrils with a score from 0 to 8 corresponding to less concentrated bottle that the patient was able to correctly detect. The average between values of the two nostrils expressed the overall score. For the identification test, common odorants, familiar to the Italian population, were placed inside 180 mL opaque jars covered with gauze. One at a time, the samples were presented to the patient in the same way as the threshold test. Therefore, the patient was asked to identify the odorant on a list containing the 10 test items and 10 distractors. The overall identification score was obtained from the average of the two nostrils.

The composite olfactory score, thus obtained from the sum of the threshold and identification scores, allows to clinically classify the olfactory function in five categories: normal (scores 90 and 100), mild (scores 70 and 80), moderate (scores 50 and 60), or severe hyposmia (scores 20, 30, and 40) and anosmia (scores 0 and 10). The CCCRC test was performed by the same blinded researcher at the time of the enrollment and 60 days after.

Immediately after the olfactory test, a rhino-pharyngeal swab was performed by the same experienced head and neck surgeon who belongs to the Swab team of the University Hospital of Sassari. Rhino-pharyngeal swabs resuspended in 3 mL universal transport medium (Copan Diagnostics Inc., Brescia, Italy) were used for SARS-CoV-2 molecular detection. Nucleic acid extraction was performed from a 100 μL starting sample volume by using GeneAll® RibospinTM vRD II kit (GeneAll Biotechnologies Co., Seoul, South Korea) and an elution volume of 30 μL.

Molecular detection and quantification of SARS-CoV-2 was performed by means of QUANTICOR™ CE-IVD kit (Hiantis S.r.l, Milan, Italy). The assay allows multiplex real-time reverse transcription polymerase chain amplification and quantification (qPCR) of N1 and N3 viral gene targets as well as of the endogenous human beta-2microglobulin (B2M) mRNA. A total volume of 15 μL amplification mix is used for each reaction, composed of 10 μL master mix and 5 μL nucleic acid extract. Real Time PCR was performed using Biorad CFX96 (Bio-Rad Laboratories Inc., Milan, Italy), as indicated in the manufacturer instruction for use. Quantification of viral RNA and human mRNA was determined by the use of independent standard curves. Normalized viral loads were expressed by the ratio of viral RNA copies to B2M mRNA copies/reaction. A companion software developed by Lutech SpA (Lutech SpA, Milan, Italy) was used for analysis of qPCR results.[43,44]

The statistical analysis was performed with SPSS 26.0 (IBM, Armonk, NY). Categorical variables are reported in numerals and percentages of the total. Descriptive statistics for quantitative variables are given as the mean ± standard deviation or median (interquartile range [IQR]). The Kruskal-Wallis test was performed to evaluate the statistical significance of differences in olfactory scores and viral load between clinical severity groups. The correlation between olfactory scores and viral load was assessed with the Pearson's correlation coefficient. The correlations between the severity of COVID-19 symptoms assessed by CSI and viral load was studied by the ANOVA test. The level of statistical significance was set at $P < .05$ with a 95% confidence interval.

RESULTS

Following the inclusion and exclusion criteria, 60 COVID-19 patients were enrolled in this study. Table I summarizes the epidemiological and clinical characteristics of the patients (Table I). Twenty-four patients (40%) developed a mild form of COVID-19, the disease was moderate in 22 (36.7%) and severe in 14 cases (23.3%). At baseline, 12 patients (20% of the cohort) presented with anosmia, 11 (18.3%) severe hyposmia, 13 (18.3%) moderate hyposmia, and 10 (16.7%) mild hyposmia with an overall prevalence of OD of 76.7%. The overall median olfactory score was 50 (IQR 30–72.5) with no significant differences between clinical severity subgroups (Table II). Thirty subjects were included in the control group: 15 men and 15 women with a mean age of 57.2 ± 16.7 years. The two groups did not show significant differences in gender distribution ($P = .171$) and age ($P = .254$). Compared with the study population, in the control group only 5 patients (16.7%) had OD on the CCCRC test including mild hyposmia in 4 cases and moderate in one. The overall median CCCRC score in the control group was 100 (IQR 90–100) with significant difference from the study group ($P < .001$).

TABLE I.
General and Clinical Features of the Study Population.

Gender	
Male	39 (65%) [95% CI 51.6–76.9%]
Female	21 (35%) [95% CI 23.1–48.4%]
Age (yr), mean ± SD	64.4 ± 13 [95% CI 61.1–67.7]
Days from COVID-19 symptoms onset, mean ± SD	7 ± 3.2 [95% CI 6.2–7.8]
Clinical stage, No of patients (%)	
Mild	24 (40%) [95% CI 27.6–53.5%]
Moderate	22 (36.7%) [95% CI 24.6–50.1%]
Severe	14 (23.3%) [95% CI 13.4–36%]
Critical	0 (0%) [97.5% CI 0–4.7%]
Baseline olfactory function assessment (n = 60), No of patients (%)	
Normal	14 (23.3%) [95% CI 13.4–36%]
Mild hyposmia	10 (16.7%) [95% CI 8.3–28.5%]
Moderate hyposmia	13 (21.7%) [95% CI 12.1–34.2%]
Severe hyposmia	11 (18.3%) [95% CI 9.5–30.4%]
Anosmia	12 (20%) [95% CI 10.8–32.3%]
Baseline CCCRC score, median [IQR]	
Threshold (range 0–50)	20 [10–30]
Identification (range 0–50)	35 [20–50]
Overall (range 0–100)	50 [30–72.5]
60-day olfactory function assessment (n = 56), No. of patients (%)	
Normal	36 (64.3%) [95% CI 50.4–76.6%]
Mild hyposmia	7 (12.5%) [95% CI 5.2–24.1%]
Moderate hyposmia	6 (10.7%) [95% CI 4–21.9%]
Severe hyposmia	4 (7.1%) [95% CI 2–17.3%]
Anosmia	3 (5.4%) [95% CI 1.1–14.9%]
60-day CCCRC score	
Threshold (range 0–50)	40 [30–50]
Identification (range 0–50)	50 [40–50]
Overall (range 0–100)	90 [70–100]
Viral load (RNA copies/mL), median (IQR)	2.56E+06 (3.17E+04–1.58E+07)

B2M = beta-2-microglobulin; CCCRC = Connecticut Chemosensory Clinical Research test; CI = confidence interval; IQR = interquartile range; RNA = ribonucleic acid; SD = standard deviation.

The median viral load detected in the series was 2.56E+06 RNA copies/10^6 copies of B2M mRNA (IQR 3.17E+04–1.58E+07) without any significant correlations with COVID-19 severity (Table II).

The correlation between viral load and olfactory scores was weak ($R^2 = 0.0007$) and not significant ($P = .844$) (Fig. 1).

Fifty-six patients (93.3% of the cohort) completed the 60-day follow-up. At this observation time, 36 patients (64.3%) presented with normal olfactory function while 20 (35.7%) had persistent OD on psychophysical test, including mild (7 cases, 12.5%), moderate (6 cases, 10.7%), and severe hyposmia (4 cases, 7.1%) and anosmia (3 cases, 5.4%). The overall median CCCRC score was 90 (IQR 70–100). The correlation between the CCCRC score at 60 days and the viral load of the swab at the

TABLE II.
Olfactory Scores and Viral Load According to Clinical Severity of COVID-19.

COVID-19 Severity[32]	Mild (n = 24)	Moderate (n = 22)	Severe (n = 14)
CCCRC score Median (IQR)	50 (30–70)	50 (15–70)	70 (50–97.5)
Kruskal-Wallis test		$P = .247$	
Viral Load (RNA copies/10^6 copies of B2M mRNA) Median (IQR)	2.21E+06 (6.32E+04–1.58E+07)	4.72E+06 (4.03E+04–9.15E+06)	7.67E+05 (1.74E+04–2.68E+07)
Kruskal-Wallis test		$P = .748$	

B2M = beta-2-microglobulin; CCCRC = Connecticut Chemosensory Clinical Research Center test; IQR = interquartile range; RNA = ribonucleic acid.

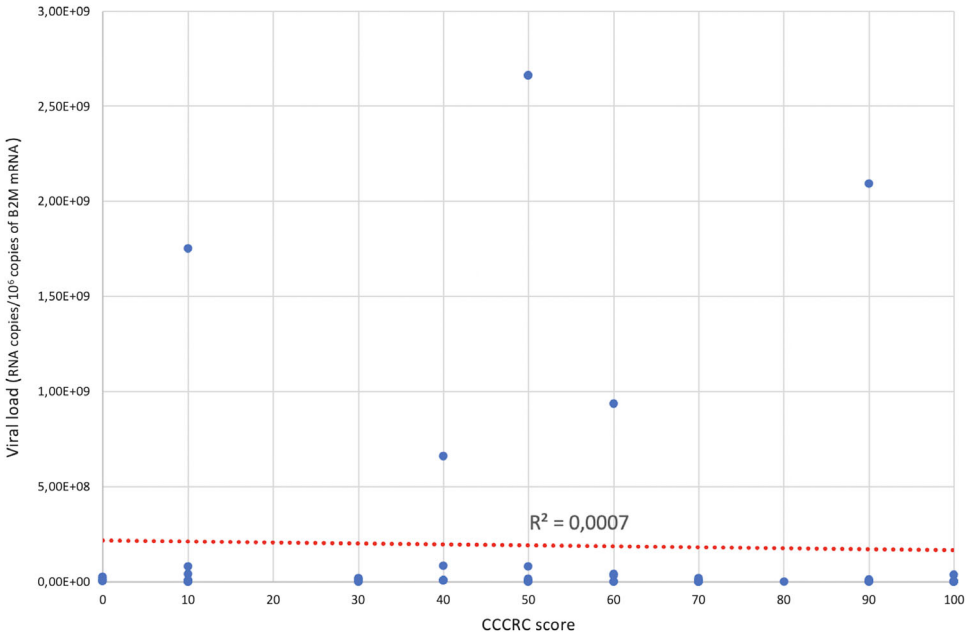

Fig. 1. Correlation analysis between viral load and Connecticut Chemosensory Clinical Research Center test (CCCRC) scores at baseline. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

onset of infection was not statistically significant ($R^2 = 0.0077$; $P = .519$) (Fig. 2).

There was no significant correlation between viral load and severity of COVID-19 symptoms either at baseline or at the end of the observation period, including other symptoms of nasal inflammation such as nasal obstruction (baseline $P = .387$, 60 days $P = .894$), rhinorrhea (baseline $P = .462$, 60 days $P = .553$), and nasal burning (baseline $P = .323$, 60 days $P = .679$) neither with the severity ($P = .158$) and recovery of gustatory dysfunction ($P = .378$) (Table III).

DISCUSSION

Only two studies have previously evaluated the correlation between OD and viral load in COVID-19 patients[30,31] with conflicting results. Cho et al.[30] evaluated the correlations between the viral Ct determined on the rhino-pharyngeal swab and self-reported olfactory loss in 85 COVID-19 patients, without finding significant correlations. Conversely, in a study with the same methodological setting by Jain et al.,[31] a statistically significant correlation between self-reported chemosensory loss and lower Ct values (i.e., a higher viral load) was reported.

The study of the correlations between OD and viral load has value both from an epidemiological and a pathogenetic point of view. If this symptom was actually associated with a higher viral load, it would be useful in identifying super-spreaders so as to isolate them, blocking the contagion chain.[45,46] From a pathogenetic point of view, the correlation between viral load and the severity of the OD could provide indications on the extent to which the latter is related to individual rather than viral factors. However, the studies published so far,[30,31] who were the first to evaluate possible correlations between viral load and the severity of the olfactory disorder, present some limitations which may have influenced the results obtained. First, the only self-reported olfactory loss was considered and no psychophysical evaluation of the olfactory function was carried out. In fact, it is now well known that considering the self-reported olfactory loss alone leads to a significant underestimation of the frequency of OD compared with psychophysical tests.[32,33] Second, viral load was never directly determined but the

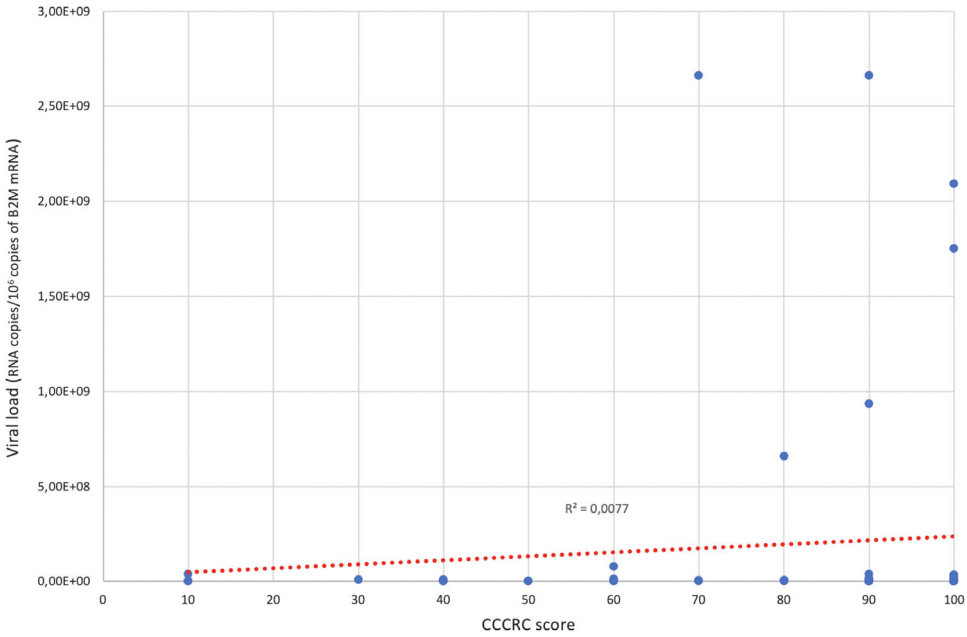

Fig. 2. Correlation analysis between viral load and Connecticut Chemosensory Clinical Research Center test (CCCRC) scores at 60 days. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

TABLE III.
Correlations Between CSI and Viral Load.

CSI Symptoms[36]	Baseline Score, Mean ± SD	ANOVA *P*-value	60-day Score, Mean ± SD	ANOVA *P*-value
Fever	2.3 ± 1.3	NS	0.05 ± 0.2	NS
Asthenia	2.6 ± 1.3	NS	0.4 ± 0.8	NS
Cough	2.3 ± 1.2	NS	0.2 ± 0.5	NS
Chest pain	1.5 ± 0.6	NS	0.06 ± 0.3	NS
Appetite loss	2.2 ± 1.1	NS	0.1 ± 0.4	NS
Joint pain	2.7 ± 1.3	NS	0.2 ± 0.5	NS
Muscle pain	2.6 ± 1.3	NS	0.1 ± 0.4	NS
Headache	2.4 ± 1.2	NS	0.2 ± 0.4	NS
Diarrhea	1.4 ± 0.9	NS	0.04 ± 0.2	NS
Abdominal pain	1.2 ± 0.7	NS	0.02 ± 0.1	NS
Nausea	1.9 ± 1.3	NS	0.06 ± 0.2	NS
Conjunctivitis	1 ± 1.1	NS	0.04 ± 0.3	NS
Urticaria	0.9 ± 0.7	NS	0	NS
Sticky throat mucus	1.1 ± 1.1	NS	0.12 ± 0.4	NS
Nasal obstruction	1.3 ± 0.9	NS	0.2 ± 0.5	NS
Rhinorrhea	1.4 ± 1	NS	0.11 ± 0.3	NS
Nasal burning	1.3 ± 1.2	NS	0.3 ± 0.6	NS
Throat pain	0.9 ± 1	NS	0.06 ± 0.3	NS
Ear pain	0.6 ± 0.8	NS	0.02 ± 0.1	NS
Face pain	0.6 ± 0.9	NS	0	NS
Swallowing difficulties	1 ± 1	NS	0.06 ± 0.3	NS
Voice issues	0.9 ± 0.7	NS	0.08 ± 0.3	NS
Mouth burning	1.1 ± 1	NS	0.2 ± 0.6	NS
Smell loss (score 0–2)	0.8 ± 0.8	NS	0.4 ± 0.6	NS
Taste loss (score 0–2)	0.7 ± 0.8	NS	0.2 ± 0.5	NS
Overall score	38.8 ± 20.6	NS	4.6 ± 11.1	NS

CSI = COVID-19 symptom index; NS = not significant.

authors considered viral Ct as its indirect estimate. However, a growing number of authors recommend not using Ct as a direct estimate of viral load in SARS-CoV-2 infection because it can introduce errors that cannot be overlooked.[35,36,47] The most important is that, unlike quantitative tests such as the one used in this study, the determination of Ct in diagnostic PCR reactions does not allow normalization to be performed using samples' human endogenous targets, normally present on the nasopharyngeal mucosa so as to reduce the bias introduced by an operator-dependent procedure such as rhinopharyngeal swab.[36]

In this study, patients were evaluated within the first 10 days of symptom onset, when OD should not yet have begun to recover[18,48] detecting an OD prevalence of 76.7%. This prevalence is similar to that previously reported by other authors.[1–5,11,15,16,18,39,41,42] This early period also coincides with the peak of the viral load, which generally decreases starting from the 10th day after clinical onset.[49] Olfactory scores did not show significant differences depending on the severity of COVID-19. This finding is in line with what was reported in other series of the first wave[3,6,15,18,39,42] but conflicts with other studies that attribute to ODs a protective value for the development of severe forms of disease.[17,50] Likewise, viral load showed no correlation with COVID-19 severity. The prognostic value of viral load is still debated, although some authors have found a correlation between higher viral load and severe COVID-19,[51] many others have not found significant differences between symptomatic and asymptomatic patients.[52,53] The results of our study support the latter thesis, it is very likely that the clinical severity of COVID-19 is predominantly related to the degree of pulmonary involvement and systemic inflammation and not to the viral load in the nasopharynx.

Finally, the correlation between viral load and olfactory scores at baseline and 60-day control was weak and not significant leading to two main conclusions. According to Cho et al.[30] and in contrast with Jain et al.,[31] viral load does not appear to have any correlation with the presence, severity, or duration of OD which is likely related to individual factors rather than viral load and activity. SARS-CoV-2 infection can impair olfactory function at multiple anatomical levels and through a variety and combination of pathophysiological mechanisms that are not mutually exclusive. Unfortunately, these factors and mechanisms have not yet been identified. This also suggests that strategies such as nasal irrigation with iodine solutions, which have been proposed as methods to reduce viral load, are unlikely to have protective effect on olfactory function.

This study has some limitations. The number of patients included in the study is still limited and the monocentric setting could have prevented the detection of any differences between different regions. Some of the control group may have falsely tested negative for COVID-19 infection. Compared with the study by Cho et al.[30] (85 COVID-19 patients) and Jain et al.[31] (200 COVID-19 patients), the number of patients included is lower and the risk of a type 2 statistical error cannot be excluded. However, the use of psychophysical tests to assess smell reduces the risk of reporting error. Moreover, the direct viral load assay allows to normalize the viral load based on the sample levels of beta-2-microglobulin, a protein normally present on the mucosal surface of the nasopharynx, reducing the risks of error introduced by inadequate execution of the nasopharyngeal swab.

CONCLUSION

We did not identify any correlation between the viral load and the severity of olfactory loss, measured with psychophysical scores. The results of our study further build on those reported by Cho et al.[30] as viral load does allow neither prediction of the severity of OD nor the risk of developing long-lasting OD. The pathogenesis of OD may be related to the local inflammatory response rather than to the viral load, and treatments aimed to reduce viral load are therefore unlikely to be able to modulate the natural history of COVID-19-related OD.

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