Smell loss associated with SARS-CoV-2 is not clinically different from other viruses: a multicenter cohort study*

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Abstract

Background: Although the COVID-19 pandemic has increased the prevalence of cases with olfactory loss, other respiratory viruses can also cause this condition. We aimed to compare the prevalence of acute SARS-CoV-2 infection and other respiratory viruses in patients with sudden smell loss, and to assess the impact of SARS-CoV-2 viral load and co-infection on olfactory symptoms.

Methods: Patients with sudden smell loss were recruited in a multicenter prospective cohort study in 15 hospitals in Brazil. Clinical

questionnaire, Connecticut Chemosensory Clinical Research Center (CCCRC) olfactory test and nasopharyngeal swab to perform a PCR-based respiratory viral panel were collected at first visit (day 0) and 30 and 60 days after recruitment.

Results: 188 of 213 patients presented positive test result for SARS-CoV-2, among which 65 were co-infected with other respiratory viruses (e.g., rhinovirus, enterovirus, and parainfluenza). 25 had negative test results for SARS-CoV-2. Patients in both SARS-CoV-2 and non-SARS-CoV-2 groups had objective anosmia (<2 points according to the psychophysical olfactory CCCRC) at day 0, with no significant difference between them. Both groups had significant smell scores improvement after 30 and 60 days, with no difference between them. Co-infection with other respiratory viruses, and SARS-CoV-2 viral load did not impact olfactory scores.

Conclusion: Patients with sudden smell loss associated with SARS-CoV-2 and other respiratory viruses had similar presentation, with most participants initiating with anosmia, and total or near total recovery after 60 days. SARS-CoV-2 viral load and co-infections with other respiratory viruses were not associated with poorer olfactory outcomes.

Key words: smell, olfaction disorders, smell loss, virus diseases, SARS-CoV-2, viral load

Introduction

The high prevalence of sudden smell loss associated with SARS-CoV-2 infection has raised awareness to the importance of this symptom. In the first months of COVID-19 pandemic, facing high rates of contagion with abundant severe cases and reduced access to diagnostic tests, health care workers worldwide used the presence of sudden olfactory loss as basis to recommend social isolation due to its high specificity [1].

Viral infections are considered the leading causes of sudden smell loss, even before the COVID-19 pandemic [2–5]. Although its pathophysiology is still not fully understood, it is known that rhinovirus, enterovirus, influenza, and parainfluenza are capable of causing persistent anosmia [2,6]. Thus, it is important to determine the prevalence of the different respiratory viruses that can cause sudden olfactory loss and to understand whether SARS-CoV-2 differs from other respiratory viruses in clinical features and prognosis. Additionally, the understanding of factors influencing prognosis in sudden smell loss, such as the presence of co-infection or viral loads, could help in orientation and proper management of such patients.

Some studies have compared the characteristics of sudden olfactory losses caused by SARS-CoV-2 and other viruses, concluding that the severity was higher in COVID-19 [5,7–9]. However, those studies did not evaluate the olfactory evolution of these groups prospectively, and only one [5] used a validated psychophysical method to accurately measure olfactory ability.

To close some of these gaps, this study was done to compare sudden smell losses associated with SARS-CoV-2 and other respiratory viruses, regarding their main characteristics and progression in 60 days, in addition to the impact of co-infections and viral load on olfactory symptoms.

Materials and methods

This is a multicenter, prospective, observational cohort study, which analyzed the olfactory evolution of participants with sudden anosmia over a period of 60 days, comparing those with SARS-CoV-2 with those infected with other respiratory viruses, or without a defined viral etiology. This study followed the recommendations of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines [10].

Patients

Recruitment took place between June 2020 and June 2021 in 15 health care centers located in 10 cities in Brazil: Ribeirão Preto/SP, São Paulo/SP, Brasília/DF, Londrina/PR, Belo Horizonte/MG, Porto Alegre/RS, Campinas/SP, Goiânia/GO, Rio de Janeiro/RJ, and Curitiba/PR. The study was approved by the Research Ethics Committee of all participating centers (CAAE: 31058620.4.1001.5440), and all patients signed an informed consent form before enrollment. In all centers, patients suspected of COVID-19 were assessed in specific areas separated from other patients to prevent virus transmission. All examiners used personal protective equipment (PPE), which included an N95 mask, eye protection, gloves, cap, and gown.

The inclusion criteria comprised age between 18 and 60 years, main complain of sudden olfactory loss of 10 days or less of duration, and confirmation of anosmia/hyposmia in a psychophysical test. Exclusion criteria were history of previous olfactory alteration prior to sudden worsening, associated nasal diseases such as chronic rhinosinusitis or sinonasal tumors, and neurodegenerative diseases.

All participants completed a sociodemographic and clinical questionnaire upon enrolment and were instructed to assess their ability to smell and taste, and to record whether or not they presented sensation of nasal obstruction, or felt a burning sensation prior to the episode of sudden anosmia through a visual analog scale (VAS) ranging from 0 to 10, with 0 being the worst possible sensation and 10, the best. They were also asked about

their known history of SARS-CoV-2 infection, the presence of symptoms of flu-like illness, and other characteristics related to the alteration in the ability to smell, such as distortion and fluctuation. The patients underwent the Connecticut Chemosensory Clinical Research Center (CCCRC) [11] olfactory test to confirm anosmia/hyposmia, defined by a score lower than 6, followed by nasopharyngeal swab collection.

Participants were followed up for 2 months, evaluated at the day of inclusion (day 0) and at the following 30±7 and 60±7 days window periods. Clinical questionnaire about smell and taste condition, the CCCRC olfactory test, and nasopharyngeal swab were performed in all visits.

Olfactory testing

The CCCRC test, validated for Brazilian Portuguese [11], assesses both olfactory threshold and identification ability, in addition to evaluate the nasal trigeminal perception. It is inexpensive and easy to apply. The final result consists of the composite score obtained by the mean score in the two parts of the test (threshold and identification). The scores varied from 0 to 7 and were categorized as: normosmia (6.0 to 7.0); mild hyposmia (5.0 to 5.75); moderate hyposmia (4.0 to 4.75); severe hyposmia (2.0 to 3.75), and anosmia (0 to 1.75). The trigeminal evaluation was done by assessing the individual's ability to detect and identify the substance menthol.

The nasopharyngeal secretion samples were obtained by gently rotating the swab for 10 seconds after reaching the nasopharyngeal region. After sample collection, the tip of the swab was placed in a sterile 15 mL flask containing 3.0 mL of viral transport medium (VTM)[12,13]. The collected material was stored at -80°C until it was sent to the Virology Research Center of the Ribeirão Preto Medical School - University of São Paulo (FMRP-USP) for analysis. Samples were transported on dry ice.

RT-PCR

The nucleic acids were extracted using the AllPrep® DNA/RNA/miRNA Universal Kit (Qiagen). Approximately 1.0 µg of RNA was added to random hexamer primers and the Multiscribe™ Reverse Transcriptase enzyme (Applied Biosystems) to obtain complementary DNA (cDNA). SARS-CoV-2 genome was detected with qRT-PCR targeting the E and N2 genes of the viral genome, following the protocols established by the Charité Research Organization and the American CDC [12,13]. For the quantification of SARS-COV-2 genome, a standard curve was prepared with serial decimal dilutions of a plasmid in which the amplicon of the N gene was cloned. Additionally, all samples were tested by qRT-PCR for the seasonal respiratory viruses: respiratory syncytial virus (HRSV), metapneumovirus (HMPV), enterovirus (HEV), rhinovirus (RV), endemic coronaviruses (HCoV), parainfluenza (HPIV), influenza (FLU), human bocavirus (HBoV) and adenovirus (AdV). The endogenous RNase-P gene was amplified as an internal control. Specific primers and probes, and amplification protocols for the respiratory viruses and housekeeping genes have been previously published [13] (Supplementary Table 1). The reactions were conducted with 3.0 µL of cDNA, 10 µM of the forward and reverse primers, 5 µM of the probe, 0.15 µL of ROX, and 7.5 µL of TaqMan™ Master Mix (Sigma-Aldrich, St. Louis, MO, USA) (Supplementary Table 3). All RT-PCR assays were performed on a StepOnePlus™ Real-Time PCR thermal cycler (Applied Biosystems, Foster City, CA, USA) (Supplementary Table 1 and 2). The qRT-PCR tests were processed collectively at the end of the sample collection, and the patients received the results only at the conclusion of the study, after several months. When samples tested positive by qRT-PCR for RNAse-P and negative for SARS-CoV-2; or negative for RNAse-P at the virology laboratory, the enrollment center was contacted to confirm whether a SARS-CoV-2 rapid test or RT-PCR assay had been conducted locally during the first patient's visit. Patients who had positive test for SARS-CoV-2 at the initial visit were included in the SARS-CoV-2 group. However, these individuals were not included in the analysis of viral load and co-infection due to their lack of a positive result in the tests conducted according to our study protocol. Patients were excluded if their samples tested negative for RNAse-P by qRT-PCR (considered as degraded during transport/storage) and did not have any local qRT-PCR result at the enrollment center.

Statistical analysis

The evaluation of olfactory evolution by the VAS and the CCCRC test, and the comparison between groups, was carried out using a linear mixed-effects model, and the values are described in mean (standard deviation). Clinical improvement was defined as an improvement in the category of the CCCRC, as there is no established minimal clinical important difference for this test. The evolution of number of patients with severe olfactory loss (CCCRC score < 4) and the comparison between groups were assessed with McNemar's test and Fisher's exact test, respectively. The association between demographic and clinical characteristics, and olfactory evolution was assessed with Fisher's exact test. Spearman's test was used to assess the correlation between olfactory ability and viral load. CCCRC score was reparametrized from a 0 to 7 to a 0 to 10 scale and its agreement to VAS was assessed with the Bland-Altman plot. In all analyses, we set the significance level at 5%. Statistical analysis was performed using the SPSS software, version 17.01 (SPSS Inc., USA).

Results

A total of 230 individuals were recruited, with 188 participants in the SARS-CoV-2 group (81%) and 25 in the non-SARS-CoV-2 group (19%); 17 were excluded due to the quality of the collected sample (Figure 1). Among the patients in the SARS-CoV-2 group, 164 were diagnosed using the research protocol's qRT-

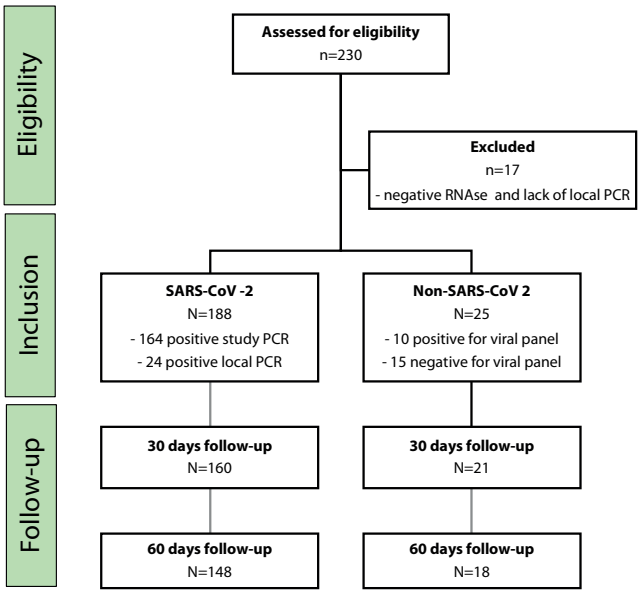

Figure 1. Study population flow chart.

Figure 1. Study population flow chart.

PCR, confirming the COVID-19 infection. Other 24 patients had a negative test when all the samples were analyzed, but had been diagnosed with COVID-19 through local qRT-PCR tests conducted at the enrollment center. During follow-up period, 32 patients did not attend the day 30 visit, representing a loss of 15%, and 15 did not attend the day 60 visit, totaling a loss of 22% at the end of 60 days.

Both SARS-CoV-2 and non-SARS-CoV-2 groups had a similar mean age, with a pre-dominance of adults in the third and fourth decades of life (Table 1); in both groups, there was a pre-dominance of females. As for the characteristics of the olfactory disorder, hyposmia/anosmia was the most frequent symptoms in both groups, followed by parosmia, phantosmia, and fluctuating olfactory perception. All patients perceived a worsening in all specific nasal, olfactory, and taste-related symptoms during the episode of sudden anosmia. Apart from the olfactory complaint, the most reported symptoms in both groups were changes in taste perception, followed by headache.

Of the 188 participants in the SARS-CoV-2 group, 164 were diagnosed by RT-PCR according to the research protocol, and their nasal swabs were analyzed for the presence of other respiratory viruses. Among them, 123 (75%) participants had single infection with SARS-CoV-2, while the others 41 (25%) presented co-infection, mainly with rhinovirus and enterovirus (Table 2). Of the 25 participants in the non-SARS-CoV-2 group, 10 (40%) presented other respiratory virus infection.

During the 60-day follow-up period, a progressive improvement in olfactory function was observed in both groups, according to both VAS and CCCRC test (Table 3, Figures 3 and 4).

When the VAS was analyzed, both groups of patients reported a normal mean score of "ability to smell" prior to the acute episode

Characteristics	SARS-CoV-2 n=188	Non-SARS-CoV-2 n=25
Mean Age (SD)	36.8 (10.8)	38 (11.1)
Gender no (%)		
Female	125 (66.5)	16 (64)
Male	62 (33)	9 (36)
Olfactory symptoms no (%)		
Anosmia/hyposmia	169 (89.9)	19 (76.0)
Parosmia	20 (10.6)	7 (28.0)
Phantosmia	9 (4.8)	4 (16.0)
Fluctuation	23 (12.2)	6 (24.0)
Nasal symptoms VAS mean (SD)		
Ability to smell		
Before	9.44 (1.07)	9.16 (1.25)
Baseline	1.31 (1.82)	2.36 (2.38)
Nasal obstruction		
Before	7.60 (3.09)	7.44 (3.18)
Baseline	5.98 (2.91)	5.76 (2.86)
Ability to taste		
Before	9.72 (0.71)	9.72 (0.46)
Baseline	2.65 (2.87)	3.56 (3.24)
"Burning" sensation		
Before	9.59 (1.16)	9.68 (0.69)
Baseline	3.87 (3.42)	4.32 (3.60)
Other symptoms no (%)		
Dysgeusia	162 (86.2)	19 (76)
Headache	144 (76.6)	19 (76.0)
Fatigue	120 (63.8)	15 (60.0)
Muscle pain	114 (60.6)	13 (52.0)

of olfactory loss, with significant worsening at day 0. The intensity of acute olfactory loss in the SARS-CoV-2 group [mean (SD), 1.31 (1.82)] was significantly greater than in the non-SARS-CoV-2 group [2.36 (2.38)] [difference between means: 1.04 (95%CI: 0.29 to 1.79); p-value=0.006]. In both groups, the olfactory ability significantly improved at day 30 and continued to improve, yet less, at day 60. The difference between the VAS scores recorded at day 0 and day 30 in the SARS-CoV-2 group was 6.26 (95%CI, 5.92 to 6.59, p<0.001), while in the non-SARS-CoV-2 group, it was 5.69 (95%CI, 4.77 to 6.62, p<0.001). Meanwhile, the difference between the VAS scores at day 0 and day 60 in the SARS-CoV-2 group was 7.18 (95%CI, 6.84 to 7.52, p<0.001), whereas in the non-SARS-CoV-2 group was 6.07 (95%CI, 5.10 to 7.04, p<0.001). No significant difference was observed between groups when comparing the evolution of the VAS scores at day 0, and day 30 or day 60 (Table 3, Figure 2).

The evolution of olfactory capacity evaluated by the CCCRC

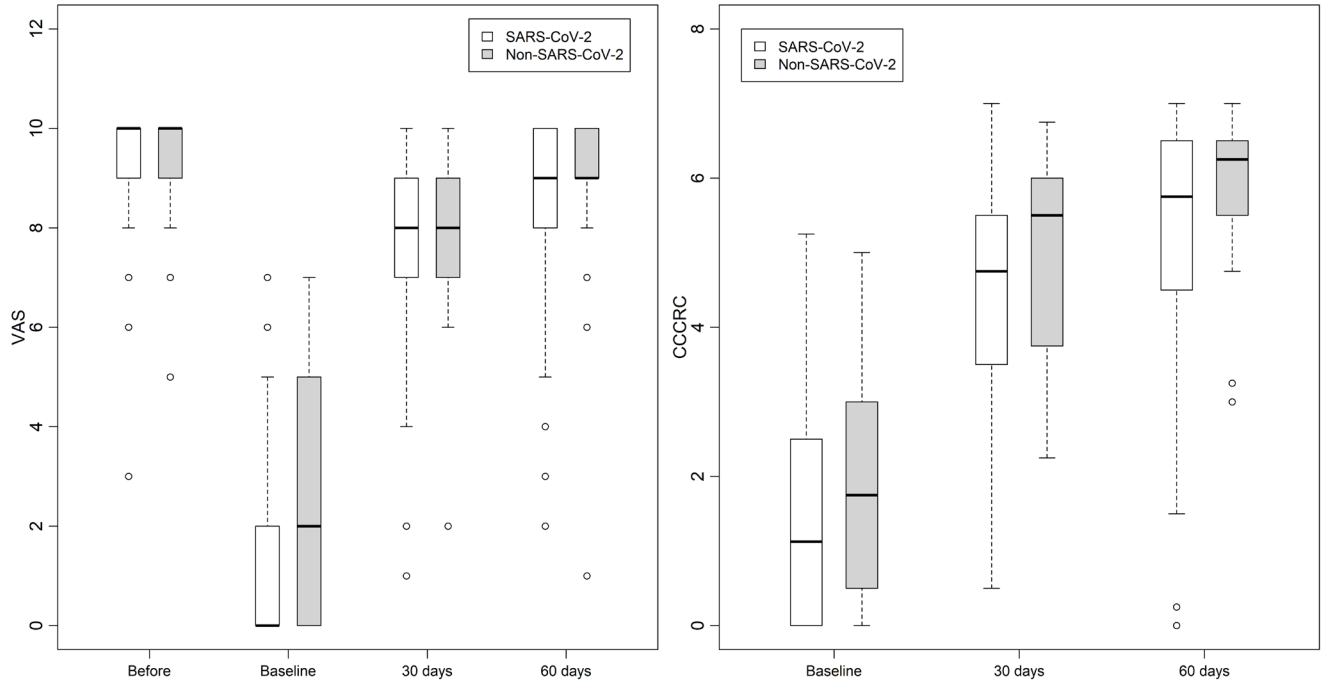

Figure 2. Change in smell function determined by the VAS and the CCCRC in SARS-CoV-2 and non- SARS-CoV-2 groups.

Table 2. Prevalence and association of respiratory viruses in patients with acute smell loss.

Virus	SARS-Cov-2 n=164 (%)	Non-SARS-CoV-2 n=10(%)
Isolated Sars-Cov-2	123 (75)	-
Rhinovirus	11 (6,7)	2 (20)
Enterovirus	7 (4,2)	4 (40)
Parainfluenza 1	4 (2,4)	1 (10)
Bocavirus	3 (1,8)	2 (20)
Metapneumovirus	3 (1,8)	0
Adenovirus	2 (1,2)	0
Adenovirus + Bocavirus	2 (1,2)	0
Rhinovirus + Respiratory syncytial virus	2 (1,2)	0
Rhinovirus + Enterovirus	1 (0,6)	1 (10)
Adenovirus + Enterovirus	1 (0,6)	0
Influenza	1 (0,6)	0
Parainfluenza 1	1 (0,6)	0
Rhinovirus +Adenovirus	1 (0,6)	0
Rhinovirus + Metapneumovirus	1 (0,6)	0
Respiratory syncytial virus	1 (0,6)	0

test showed a similar pattern to the VAS. At day 0, both SARS-CoV-2 and non-SARS-CoV-2 individuals had a mean score of less than two, corresponding to anosmia, with no significant difference between them. The difference between the CCCRC scores at day 0 and day 30 in the SARS-CoV-2 group was 3.10 (95%CI, 2.83 to 3.37, p<0.001), while in the non-SARS-CoV-2 group was 3.01 (95%CI, 2.32 to 3.69, p<0.001). Meanwhile, the difference between the CCCRC scores at day 0 and day 60 in the SARS-CoV-2 group was 3.96 (95%CI, 3.69 to 4.24, p<0.001), whereas in the non-SARS-CoV-2 group was 3.93 (95%CI, 3.20 to 4.66, p<0.001). No significant difference was observed between groups when comparing the evolution of the CCCRC scores at day 0, and day 30 or day 60 (Table 3, Figure 2).

The percentage of patients with severe hyposmia or anosmia, according to the CCCRC score, progressively decreased over time and was similar in the SARS-CoV-2-positive and negative groups (Figure 3). At day 0, respectively 89% and 84% of the participants from the SARS-CoV-2-positive and negative groups exhibited severe impairment of olfactory function (score less than 4). At day 30, these numbers decreased to 37% and 28% (p<0.01 and p<0.01), and at day 60, to 15% and 11% (p<0.01 and p<0.01), respectively. No significant difference was observed in the percentage of severe cases between groups in any visit (day 0, p=0.32; day 30, p=0.48; day 60, p=1.00).

The agreement between VAS and CCCRC was considered weak in all three visits, due to the 95% CI wide range in the Bland-Altman plot. Also, the day 30 and 60 plot suggests that the CCCRC presents scores on average 1 point lower than the VAS (Supplementary Figure 1).

When comparing the olfactory evolution of the participants with single SARS-CoV-2 infection to those with SARS-CoV-2 infection associated with other respiratory viruses, no differen-

Table 3. Change in smell function determined by the VAS and the CCCRC diagnosed with SARS-CoV-2 or non-SARS-CoV-2 infection.

	SARS-CoV-2				Non- SARS-CoV-2			
	Before	Baseline	30 days	60 days	Before	Baseline	30 days	60 days
n	188	188	160	148	25	25	21	18
VAS, mean (SD)	9.44 (1.07)	1.31 (1.82)	7.56 (2.22)	8.47 (1.90)	9.16 (1.25)	2.36 (2.38)	8.10 (1.81)	8.56 (2.18)
CCCRC, mean (SD)	-	1.46 (1.48)	4.49 (1.53)	5.39 (1.47)	-	1.90 (1.62)	4.94 (1.34)	5.83 (1.15)

ces were observed between groups in both the VAS and CCCRC scores (Supplementary Figure 2).

We also evaluated whether sex, ethnicity, comorbidities, smoking, and the intensity of olfactory loss or the first symptom presented upon inclusion in the study could influence the prognosis of olfactory recovery. For this purpose, a covariance analysis was performed. Among these factors, only smoking demonstrated a statistically significant association with worse olfactory recovery in both groups, with a relative risk of 2.76 (95% CI, 1.13 to 6.76). It is noteworthy that no correlation was observed between the viral load of SARS-CoV-2 at the enrollment visit and a worse prognosis in olfactory recovery at the visit after 60 days, both in the VAS and the CCCRC test (Supplementary Figure 3).

Finally, the trigeminal reflex to the burning sensation when inhaling menthol was also compared between groups. Trigeminal dysfunction at day 0 was observed in both groups, affecting 54.3% of the patients in the SARS-CoV-2 group and 36% in the non-SARS-CoV-2 group. At day 60, recovery was near complete in all patients in the study (Supplementary Figure 4). No significant difference was observed between groups regarding this parameter.

Discussion

To the best of our knowledge, this is the first study that evaluated prospectively the recovery of olfactory function in patients with sudden olfactory loss associated with the SARS-CoV-2 virus compared to other viral infections. Contrasting with previous cross-sectional and retrospective studies [2–5], the loss of smell in patients with SARS-CoV-2 infection has similar characteristics to other viral etiologies. At the enrollment visit, both groups were classified as anosmia based on the CCCRC results, with no statistical difference between groups. When VAS was considered, the significantly lower score of the SARS-CoV-2 group was not considered clinically relevant, as this difference was just over 1 point, and both means were below 3 points.

In addition, after 60 days, both groups showed a significant recovery in olfaction, with no difference between groups, suggesting a favorable outcome for most patients regardless of the etiology. However, 11% of the non-SARS-CoV-2 and 15% of the SARS-CoV-2 patients persisted with severe hyposmia or anosmia according to the CCCRC scores, a prevalence compatible with previous studies [9,14,15]. According to a meta-analysis, the frequency tends to decrease even more and 4% of the SARS-CoV-2 patients persist with smell loss after 180 days [15]. Although this seems to be a relatively small percentage, considering the high prevalence of post-viral smell loss after the COVID-19 pandemic, there is a large number of patients with serious impact on quality of life, especially those with anosmia and parosmia, and it is critical that the medical community recognizes it and provides adequate monitoring and guidance. Moreover, there is an urgent need to develop clinical trials due to the currently limited therapeutic alternatives [16]. It is important to note that at the time of the study, there was no consensus regarding the best treatment evidence for sudden loss of smell, and it was not the objective of this study to evaluate therapeutic responses. Each participating center had the autonomy to treat their patients based on their respective hospital guidelines and protocols.

Besides the SARS-CoV-2, the respiratory viruses most frequently detected in patients with sudden anosmia were rhinovirus and enterovirus, alone or in association with other viruses. This is in agreement with other published studies [4]. However, this is the first study to report the follow-up of a cohort of non-SARS-CoV-2 acute smell loss patients. After 60 days, 11.1% had persistent severe hyposmia and none of them presented anosmia.

In the non-SARS-CoV-2 group, we detected at least one respiratory virus in 10 of the 25 patients. Due to this restricted number, it was not possible to analyze the specific clinical features of each respiratory virus. Of note, we did not detect any respiratory virus in 15 patients, indicating that the etiology of smell loss in these patients, viral or not, was indetermined.

Remarkably, patients infected by SARS-CoV-2 in association with other respiratory viruses did not have worse olfactory loss, nor worse prognosis when compared to patients infected by SARS-CoV-2 alone.

The viral load of SARS-CoV-2 at enrollment was not associated with a worse olfactory prognosis, which agrees with previous reports [17,18]. Smoking, which is known to be associated with olfactory disorders [19], was associated with a poor prognosis for recovery in both groups. However, it was not possible to conclude that smoking impaired the healing of the olfactory pathway after the viral infection, or that these patients already had a previous unnoticed olfactory dysfunction.

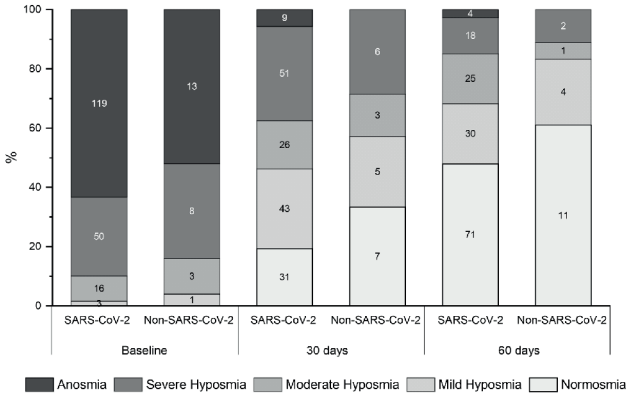

Figure 3. Change in the distribution of subjects per CCCRC olfactory category in SARS-CoV-2 and non- SARS-CoV-2 groups.

The main limitations of this study were the loss to follow-up of more than 20% of the patients, which was possibly associated with the recovery of olfactory ability and a loss of interest in returning for subsequent visits; and the exclusion of 7% of the patients due to sample degradation during storage and transport. Furthermore, our study did not investigate the impact of specific strains of SARS-CoV-2 on olfactory function, nor did it explore the influence of vaccination against SARS-CoV-2. It is worth mentioning that the vaccination program was initiated in the last 3 months of recruitment. However, based on our clinical observations and in agreement with the findings of the study, it appears that the clinical presentation and severity of post-viral olfactory loss are similar among patients with infection by the main respiratory viruses, including different strains of SARS-CoV-2. We have also noted that, following the predominance of Omicron strain and the impact of vaccination, there has been a significant decrease in the prevalence of olfactory loss. Nevertheless, we would like to emphasize that this perception is anecdotal and was not assessed in the present study.

Finally, this multicenter study represents the largest cohort in Latin America to evaluate sudden olfactory loss. The participation of research centers from 10 different cities across 3 different regions of Brazil, in addition to enhancing patient recruitment, increases the ability to generalize the results, characterizing the profile of post-viral olfactory loss in the country. This was also the first cohort to use a validated psychophysical method, such as the CCCRC test, to compare COVID-19 anosmia to other non-SARS-CoV-2 post-viral smell loss. As reported in the literature, there is no agreement between the results of these tests and patient perception, as assessed by the VAS, indicating that patients with deficits in the test are not always able to perceive and report such loss [17,18,20]. Thus, this study demonstrates more accurately the actual olfactory status of the patient than if they were submitted to a questionnaire alone.

Conclusion

The characteristics of the olfactory loss associated with SARS-CoV-2 and the other viruses were similar, with most participants' clinical presentation initiating with anosmia and with full or almost full recovery in both groups after 60 days. The viral load of SARS-CoV-2 and co-infection with other respiratory viruses were not associated with a worse olfactory presentation at diagnosis or a worse outcome.

Authorship contribution

MMM, FCV, RBM, ET, EA and WAL contributed to study design, data collection, interpretation of results and drafting. MZF, AAM, LES, TML, MVS, SRM, DMC and DCA contributed to data analysis or interpretation. All other authors were involved in data collection. All authors participated in scientific discussions and approved the final manuscript.

Conflict of interest

There is no conflict of interest.

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This manuscript contains online supplementary material

SUPPLEMENTARY MATERIAL

Results

5. Surgical treatment of post-radiation nasopharyngeal necrosis

5.1 Indications

1) Necrosis of the nasopharyngeal mucosa and soft tissue without obvious skull base osteonecrosis. Tumor recurrence should be excluded (Level of evidence quality: Moderate; Consensus: High; Category: IIA; GRADE of recommendation: Moderate).
2) Localized skull base osteonecrosis at an extent not exceeding the greater wings of the sphenoid bone. No involvement of the internal carotid artery (Level of evidence quality: Moderate; Consensus: High; Category: IIA; GRADE of recommendation: Moderate).
3) Necrosis involving the carotid sheath or petrosal internal carotid artery, and thinning or deformation of the internal carotid artery, potentially accompanied by pseudoaneurysm (BOT is recommended before operation): a) for BOT-negative patients, internal carotid artery embolization pretreatment is recommended; b) for BOT-positive patients, internal carotid artery bypass grafting or stent implantation should be considered to avoid arterial rupture and massive bleeding) (Level of evidence quality: Moderate; Consensus: High; Category: IIA; GRADE of recommendation: Moderate).
4) Extensive skull base necrosis with internal carotid artery involvement with or without intracranial involvement. (BOT is again recommended, as stated in point 3. Collaboration with neurosurgeons is needed for the intracranial lesions and skull base reconstruction) (Level of evidence quality: Low; Consensus: Moderate; Category: IIB; GRADE of recommendation: Low).

5.2 Surgical methods

1) Endoscopy-guided debridement of radiation-related nasopharyngeal necrosis: This refers to the endoscopy-guided complete removal of the necrotic tissue of the nasopharynx, using a biting forceps, suction cutter or plasma knife, until the underlying healthy tissue is exposed. Bone tissue affected by osteonecrosis of the skull base can be removed using a high-speed electric micro-drill until healthy bone is exposed. The removed tissues should be sent for postoperative pathohistological examination.
2) Reconstruction using a vascularized posterior nasal septal-floor mucoperiosteum pedicled flap: The mucosal flap is usually selected from the involved side of the lesion and rotates backward to cover the nasopharyngeal wound. For patients with oropharyngeal necrosis, the ipsilateral inferior turbinate mucosa can be taken together with a septal-floor mucoperiosteum flap to achieve adequate wound coverage. If the necrotic area is too large, bilateral mucoperiosteal flaps or a temporalis muscle flap may be considered.

6. Surgical treatment of post-radiation rhinosinusitis

6.1 Indications

1) Inflammatory thickening of the mucosa of the sinuses caused by radiation therapy, with symptoms of sinusitis, persistent nasal congestion and excessive nasal discharge. The therapeutic effect is not satisfactory after at least 12 weeks of standardized drug treatment and nasal lavage [1,2] (Level of evidence quality: High; Consensus: High; Category: IA; GRADE of recommendation: High).
2) Facial pain or pressure due to radiation induced-abnormalities affecting the drainage of ostiomeatal complex [1,2]. (Level of evidence quality: High; Consensus: High; Category: IA; GRADE of recommendation: High).
3) Complications affecting the cranium, orbit, etc. caused by nasosinusitis [1,2] (Level of evidence quality: High; Consensus: High; Category: IA; GRADE of recommendation: High).

6.2 Surgical methods and other combined treatments

1) Removal of inflammatory secretions and crusts of the mucosa in the nasal cavity and sinuses.
2) Maxillary sinus opening
3) Ethmoid sinus opening
4) Sphenoid sinus opening
5) Frontal sinus opening surgery
6) Other auxiliary methods: Catheter-guided balloon dilatation of paranasal sinuses: This technology can alleviate sinusitis by expanding the natural sinus orifice and promoting ventilation and drainage of nasal sinuses, but it is not suitable for ethmoid sinus surgery.

7. Surgical treatment of radiation-related otitis media with effusion

7.1 Indications

1) Patients with aural fullness lasting more than 3 months with hearing loss. Tympanic effusion or eardrum perforation can be seen by otoendoscopy. The presence of middle ear effusion or suspected granulation visible on imaging examination (Level of evidence quality: High; Consensus: High; Category: IA; GRADE of recommendation: High) [3,4].
2) The presence of eustachian tube dysfunction (ETS result ≤ 5 points) (Level of evidence quality: High; Consensus: High; Category: IA; GRADE of recommendation: High) [3,4].

7.2 Surgical methods and other combined treatments

1) Tympanocentesis with drainage, tympanic injection of triamcinolone acetonide and ambroxol hydrochloride, combined with

the insertion of a ventilation tube.
2) Mastoid surgery, including mastoidotomy, radical mastoidectomy and modified radical mastoidectomy [4].
3) Tympanoplasty, including myringoplasty, ossicular chain reconstruction, exploratory tympanotomy, tympanic cavity reconstruction, or similar strategies [4].

8. Surgical treatment of radiation-related encephalopathy

8.1 Indications

1) For patients who fail medical treatment with symptoms and signs that may indicate progressive deterioration of neurocognitive function and raised intracranial pressure (Level of evidence quality: Low; Consensus: Moderate; Category: IIB; GRADE of recommendation: Low).
2) For patients with recurrent seizures or intracranial hypertension caused by progressive encephalopathy, especially in the presence of a midline shift on imaging examination (Level of evidence quality: Low; Consensus: Moderate; Category: IIB; GRADE of recommendation: Low).
3) For patients with intracranial hemorrhage, brain abscess, or a cystic lesion with a noticeable space-occupying effect, given that conservative treatments are often ineffective in these situations (Level of evidence quality: Low; Consensus: High; Category: IIA; GRADE of recommendation: High).
4) Patients with a cerebral hernia (Level of evidence quality: Low; Consensus: Low; Category: III GRADE of recommendation: Not recommended).

8.2 Surgical methods

1) Patients with mild radiation brain injury are usually first given a conservative treatment. If they fail to respond to the first-line treatment, surgical removal of necrotic brain tissue can be considered.
2) Decompressive craniotomy and debridement of necrotic brain tissue are the treatment of choice for severe brain injury.
3) Surgical methods for the treatment of radiation-induced temporal necrosis include debridement of temporal lobe lesions via the pterional approach and debridement of temporal lobe lesions via a temporal horseshoe incision.

9. Surgical treatment of radiation-related epistaxis

9.1 Indications

1) Identified location of arterial or venous hemorrhage in nasal cavity and nasopharynx (Endoscopic hemostasis is recommended) (Level of evidence quality: Moderate; Consensus: High; Category: IIA; GRADE of recommendation: High).
2) Poor control of hemorrhage after nasal packing (Anterior and posterior nostril packing is recommended) (Level of evidence quality: Low; Consensus: High; Category: IIA; GRADE of recommendation: High).
3) Patients with poor control of hemorrhage after nasal packing as well as anterior and posterior nostril packing or nasopharyngeal massive hemorrhage (Endovascular embolization is recommended) (Level of evidence quality: Low; Consensus: High; Category: IIA; GRADE of recommendation: High).

9.2 Surgical methods and other combined treatments

1) Endoscopic hemostasis: This approach refers to nasal packing hemostasis, high-frequency electrocoagulation hemostasis or microwave-assisted coagulation hemostasis under endoscopic observation.
2) Anterior and posterior nostril packing
3) Internal carotid artery embolization: This approach consists of three steps: General occlusion test, intensive blood pressure reduction and permanent internal carotid artery embolization.
4) Internal carotid artery stenting

Discussion

Post-radiation nasopharyngeal necrosis

The prognosis of conservative treatment alone for nasopharyngeal necrosis is generally unfavorable [5-7]. Endoscopic debridement (which can be repeated if necessary) is the mainstay of treatment for post-radiation nasopharyngeal necrosis, although its long-term efficacy remains uncertain. Most studies suggest that repeated endoscopic debridement could alleviate headaches and foul nasal odor to a variable degree in all patients. Nevertheless, the nasopharyngeal mucosa could be fully epithelized in only 25% of the cases, with 13.4-28.6% of patients achieving an apparent cure for this condition [7,8]. Another study found that the repair rate of endoscopic debridement could reach 63.2 and 50.9% in patients with mild and moderate necrosis, respectively. However, the therapeutic effect in patients with severe necrosis was poor, and the repair rate was only 17.0% [9]. Incomplete debridement and difficult wound epithelization were the main patterns of treatment failure. A vascularized mucosal flap is an effective method to improve wound healing. Many studies have shown that the addition of a vascularized mucosal flap can significantly improve the condition of nasopharyngeal necrosis, with success rates reaching 72.3-87.5% [10-12]. Therefore, endoscopy-guided debridement combined with a vascularized mucosal flap may represent the ideal treatment for nasopharyngeal necrosis. Nevertheless, large-scale phase III clinical trials, preferably with QOL assessment, are recommended to assess its efficacy. Notably, many studies found that the exposure of the internal carotid artery is an independent adverse prognostic factor of nasopharyngeal necrosis. Therefore, pre/perioperative treatments of the internal carotid artery (such as BOT and internal carotid artery embolization) are recommended [5-13].

Post-radiation rhinosinusitis

Endoscopic sinus surgery also shows a certain benefit for rhinosinusitis in patients who received radiotherapy for head

and neck squamous cell carcinoma such as nasopharyngeal carcinoma. After the operation, the symptoms of nasal congestion or discharge were significantly improved, and the signs of rhinosinusitis in CT were alleviated [14]. In addition, the ultrastructure of the sinonasal mucosa was normalized, so that the clearance function of cilia and the mucus blanket was significantly improved [15]. A retrospective cohort study included nasopharyngeal carcinoma patients with a history of radiation therapy or chemoradiotherapy at the Stanford sinus center from 2006 to 2015. In patients with rhinosinusitis after radiotherapy, the SNOT-22 score was significantly improved 6 to 12 months after endoscopic sinus surgery compared with the control group [16]. It should be noted that radiotherapy can cause severe acute inflammation of the mucous epithelium, and surgery should be considered only after the acute response to radiotherapy has subsided (e.g., half a year after radiotherapy), and if the symptoms of sinusitis were not relieved by conservative treatment. Endoscopic sinus surgery is only one part of holistic treatment for radiation-induced sinusitis. The underlying cause of sinonasal mucosal inflammation cannot be removed or changed by surgery. Only continuous surgical cavity nursing and comprehensive drug treatment can promote the gradual morphological and functional recovery of the sinonasal mucosa.

Radiation-related otitis media with effusion

Ventilation tube insertion after radiotherapy can significantly improve the patients' hearing while reducing symptoms such as tinnitus, ear tightness and headache. The efficacy of grommet insertion is higher than 80% [3]. A randomized controlled trial confirmed myringotomy and ventilation tube insertion can significantly improve the air conducted pure tone hearing threshold and air-bone gap compared with the observation group without treatment, and the hearing of patients can be significantly improved [17]. However, the optimal time of myringotomy and ventilation tube insertion after radiotherapy in patients with nasopharyngeal carcinoma needs to be further explored. Consistent care is required after tube insertion to avoid purulent otitis media or residual tympanic membrane perforation. Nasopharynx cleaning combined with myringotomy, drainage, ventilation tube insertion and tympanic drug injection was found to be 24% more effective than myringotomy and ventilation tube insertion alone, while also significantly reducing the complications after treatment [18].

In recurrent, persistent secretory otitis media that does not respond to treatment, mastoidectomy can improve the ventilation and drainage of the mastoid space, tympanic sinus, tympanum and eustachian tube, reduce the recurrence of secretory otitis media, and improve the average air conducted pure tone hearing threshold after operation [19]. Tympanoplasty has a satisfactory therapeutic effect in patients with non-cholesteatomatous chronic otitis media, in which it can reduce the negative pressure acting on the tympanum and improve hearing [20]. However, there is still insufficient research on the effects of mastoidoplasty and tympanoplasty, with even fewer reports focusing on secretory otitis media after radiotherapy. Therefore, whether to adopt this kind of surgery should be carefully considered based on the specific situation of each patient.

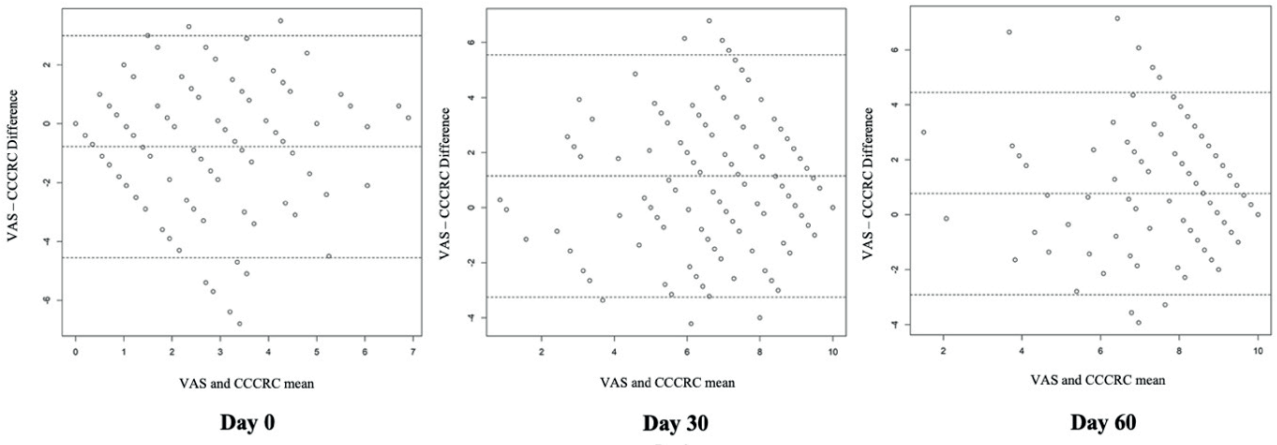
Radiation-related encephalopathy

Patients with radiation-induced brain injury who are asymptomatic (i.e., diagnosed based on radiographic changes without symptoms) or mildly symptomatic should be first managed pharmacologically (including glucocorticoids, bevacizumab, and other symptomatic treatments) [21,22]. However, in patients who do not respond to drugs and show symptoms and signs of raised intracranial pressure, surgical treatment is indicated to alleviate the pressure effect. The purpose of surgery is mainly the debridement of the injured brain tissue. The post-surgical complication rate is less than 19%, and is mainly related to surgical wounds or chest infections. The recurrence rate of postoperative radiation brain injury is approximately 6.3% [23]. Furthermore, 33% of patients who initially present with a unilateral temporal lobe lesion may eventually develop bilateral lesions. There are case reports of sequential operations for bilateral lesions or unilateral surgery alone for the more severe side [24].

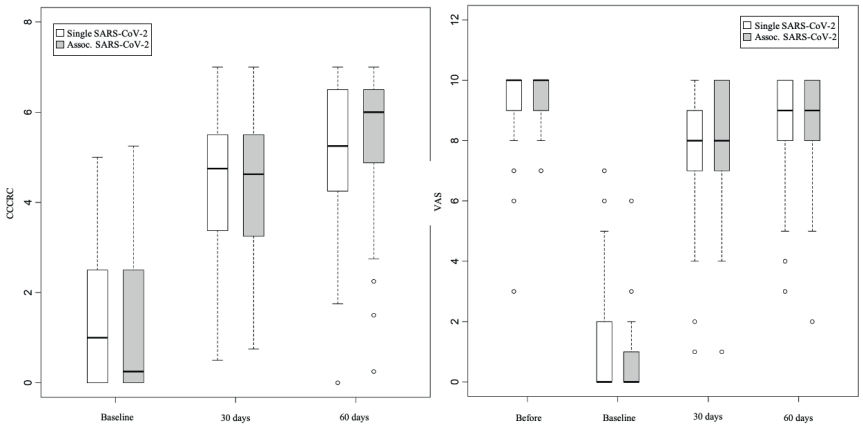
Radiation-related epistaxis

The efficacy of treatments for epistaxis after radiotherapy for nasopharyngeal carcinoma mainly depends on the hemostatic effect, but no related large-scale clinical trial has been reported to date. There are only a few retrospective studies, which mainly focused on massive nasopharyngeal hemorrhage. A retrospective study included 59 patients with massive nasopharyngeal hemorrhage after radiotherapy for NPC, all of whom underwent nasal packing. Among them, 50 were treated with interventional embolization and 3 received a stent with a tectorial membrane in the internal carotid artery. Among the 53 patients who underwent interventional therapy, 46 cases (86.8%) achieved effective hemostasis [25]. In another retrospective study including 32 patients, 24 were treated with anterior and posterior nostril packing to stop bleeding in the nasal cavity and nasopharynx, while 7 were treated with interventional embolization of the external carotid artery because hemorrhage was still difficult to control after nostril packing. Of the 32 patients, 25 (78.13%) were rescued eventually [26]. In addition, other retrospective studies have shown that internal or external carotid artery embolization is a further option for nasopharyngeal necrotic hemorrhage if the hemostatic effect of anterior and posterior nostril packing is poor [27-29].

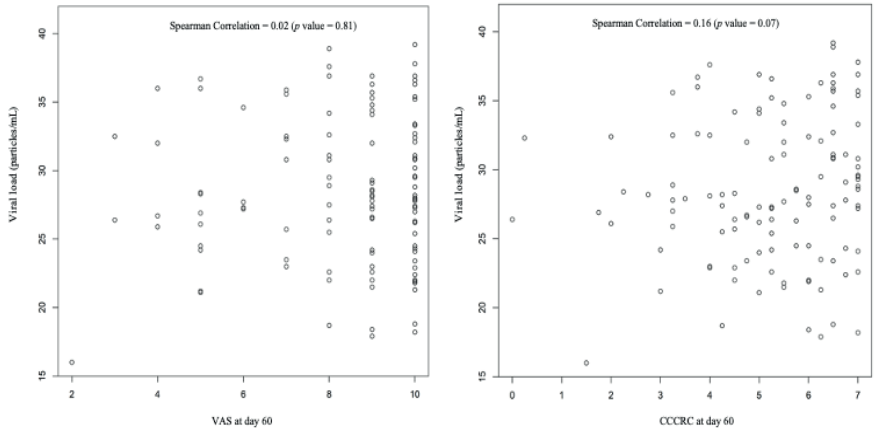
SUPPLEMENTARY MATERIAL



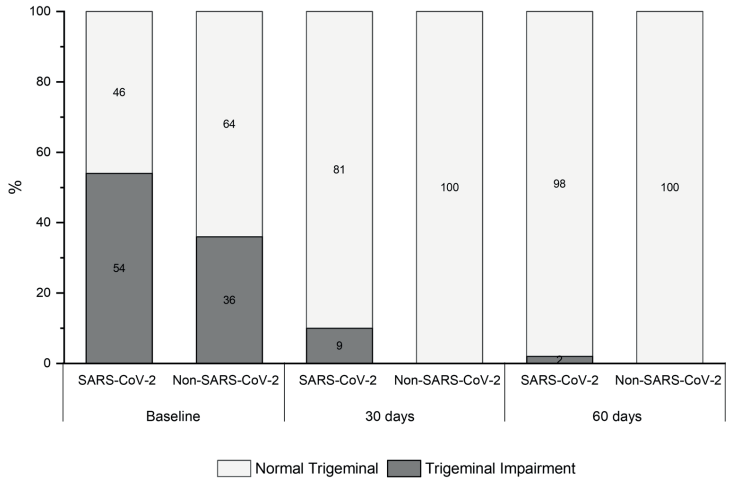
Supplementary Figure 1. Plot of differences between methods VAS and CCCRC vs. the mean of the two measurements. The bias is presented by the center line. The lower and upper lines represent the limits of agreement (95%).



Supplementary Figure 2. Box-plot of the change in smell function determined by the CCCRC and the VAS in single SARS-CoV-2 infection and SARS-CoV-2 associated with other viroses.



Supplementary Figure 3. The scatterplot shows the relationship between SARS-CoV-2 viral load at day 0 and smell function at day 60 by the CCCRC and the VAS. Spearman's correlation measures the strength and direction of association between the variables.



Supplementary Figure 4. Percentage of participants with trigeminal impairment, by group, by visit.

Supplementary Table 1. Sets of primers and probes for SARS-CoV-2 detection by RT-PCR.

Gene	Oligos	Sequence	Reference
	Foward	5′-ACAGGTACGTTAATAGTTAATAGCGT-3′	
E	Reverse	5′-ATATTGCAGCAGTACGCACACA-3′	Corman et al., 2020
	Probe	5′-Fam- ACACTAGCCATCCTTACTGCGCTTCG-BHQ1-3′	
	Foward	5′-TTA CAA ACA TTG GCC GCA AA-3′	
N2	Reverse	5′-GCG CGA CAT TCC GAA GAA-3′	US_CDC-2019
	Probe	5′-Fam-ACAATTTGCCCCCAGCGCTTCAG-BHQ1-3′	
	Foward	5′-CCCAGCCATGTACGTTGCTA-3′	
β-actin	Reverse	5′-TCACCGGAGTCCATCACGAT-3′	Proenca-Modena et al., 2012
	Probe	5′-Fam-ACGCCTCTGGCCGTACCACTGG-Tamra-3′	

Supplementary Table 2. SARS-CoV-2 sequence oligonucleotides.

Oligonucleotide	RT-nPCR phase	Orientation	Nucleotide sequence (5′ –3′)
PC2S2a	First	+	TTATGGGTTGGGATTATC
PC2AS1a	First	-	TCRTCASWSAGWATCATCA
PC2AS1b	First	-	TCRTCAGARAGWATCATCA
PC2AS1c	First	-	TCGTCRGASARKATCATCA
PCSa	Second	+	CTTATGGGTTGGGATTATCCTAARTGTGA
PCSb	Second	+	CTTATGGGTTGGGATTATCCYAAATGTGA
PCNAs	Second	-	CACACAACASCWTCRTCAGAKAGWATCATCA
PCseqS	Sequence	+	CTTATGGGTTGGGATTATCC
PCseqAs	Sequence	-	TCRTCAGAKAGWATCATCA

Supplementary Table 3. Sets of primers and probes for other respiratory virus detection by RT-PCR.

Virus and endogenous control	Primer	Sequence	Target
HRSV	RSVA-F	GCTCTTAGCAAAGTCAAGTTGAATGA	N
	RSVA-R	TGCTCCGTTGGATGGTGTATT	N
	RSVA-PB	Fam-ACACTCAACAAAGATCAACTTCTGTCATCCAGC-Tamra	N
	RSVB-F	GATGGCTCTTAGCAAAGTCAAGTTAA	N
	RSVB-R	TGTCAATATTATCTCCTGTACTACGTTGAA	N
	RSVB-PB	Joe-TGATACATTAAATAAGGATCAGCTGCTGTCATCCA-Tamra	N
HMPV	HMPV-F	GTGATGCACTCAAGAGATACCC	N
	HMPV-R	CATTGTTTGACCGGCCCCATAA	N
	HMPV-probe	Fam-CTTTGCCATACTTCAATGAACAAC-Tamra	N
HEV	HEV-F	GCGGAACCGACTACTTTGGG	5´UTR
	HEV-R	CTCAATTGTCACCATAAGCAGCC	5´UTR
	HEV-probe	Fam-TCCGTGTTTCCTTTTATTCTTATA-MGB	5´UTR
RV	RV-F	ACMGTGTYCTAGCCTGCGTGG C	5´UTR
	RV-R	GAAACACGGACACCCAAAGTAGT	5´UTR
	RV-probe	Fam-TCCTCCGGCCCCTGAAT-BHQ1	5´UTR
HCoV	HCoV-F3	TGGCGGGTGGGATAATATGT	Pol
	HCoV-ocF	CCTTATTAAAGATGTTGACAATCCTGTAC	Pol
	HCoV-R3	GAGGGCATAGCTCTATCACACTTAGG	Pol
	HCoV-ocR	AATACGTAGTAGGTTTGGCATAGCAC	Pol
	HCoV-P2	Fam-ATAGTCCCATCCCATCAA-Tamra	Pol
	HCoV-Poc	Fam-CACACTTAGGATAGTCCCA-Tamra	Pol
HPIV	Para1-F	CATTATCAATTGGTGATGG	HN
	Para1-R	CTTAAATTCAGATATGTATCCTG	HN
	Para1-probe	Fam-CTTAATCACTCAAGGATGTGCAGATATA-Tamra	HN
	Para3-F	CTCGAGGTTGTCAGGATATAG	HN
	Para3-R	CTTGAGGTTGTCAGGATATT	HN
	Para3-probe	Fam-AATAACTGTAAACTCAGACTTGGTACCTGACTT-Tamra	HN
FLU	InfA-F	GACCRATCCTGTCACCTCTGAC	M
	InfA-R	AGGGCATTYTGGACAAAKCGTCTA	M
	InfA-probe	Fam-TGCAGTCCTCGCTCACTGGGCACG-BHQ1	M
	InfB-F	AAATACGGTGGATTAAATAAAAGCAA	HA
	InfB-R	CCAGCAATAGCTCCGAAGAAA	HA
	InfB-probe	Vic-CACCCATATTGGGCAATTTCCTATGGC-Tamra	HA
HAdV	HAdV-F	GCCACGGTGGGGTTTCTAAACTT	Hexon
	HAdV-R	GCCCCAGTGGTCTTACATGCACAT	Hexon
	HAdV-probe	Fam-TGCACCAGACCCGGGCTCAGGTACTCCGA-Tamra	Hexon
HBoV	HBoV-F	GCACAGCCACGTGACGAA	NP1
	HBoV-R	TGGACTCCCTTTTCTTTTGTAGGA	NP1
	HBoV-probe	Fam-TGAGCTCAGGGAATATGAAAGACAAGCATCG-Tamra	NP1
β-Actin	β-Actin-F	AGATTTGGACCTGCGAGCG	β-Actin
	β-Actin-R	GAGCGGCTGTCTCCACAAGT	β-Actin
	β-Actin-PB	Fam-TTCTGACCTGAAGGCTCTGCGCG-BHQ1	β-Actin