Gaining back what is lost: recovering the sense of smell in mild to moderate patients after COVID-19

Lucia Iannuzzi [1], MD

Anna Eugenia Salzo [1], MD

Gioacchino Angarano [2], Full Professor

Vincenzo Ostilio Palmieri [3], Associate Professor

Piero Portincasa [3], Full Professor

Annalisa Saracino [2], Associate Professor

Matteo Gelardi [4], Associate Professor

Michele Dibattista [5], Associate Professor

Nicola Quaranta [1], Full Professor

Iannuzzi L and Salzo AE have equally contributed to this work and should be regarded as joint first authors.

1. ENT Clinic, Department of Biomedical Sciences, Neurosciences and Sense Organs. University of Bari, Bari, Italy

2. Clinic of Infectious Diseases, Department of Biomedical sciences and Human Oncology,University of Bari, Bari, Italy.

3. Clinica Medica "A. Murri", Department of Biomedical sciences and Human Oncology,University of Bari , Bari, Italy

4. ENT Clinic, University of Foggia, Foggia, Italy

5. Department of Biomedical Sciences, Neurosciences and Sense Organs. University of Bari, Bari, Italy

Correspondence to be sent to: Michele Dibattista, Department of Biomedical Sciences, Neurosciences and Sense Organs. University of Bari. Piazza Giulio Cesare n.11, 70125, Bari, Italy. Telephone: 0039 080 5478572. Email: michele.dibattista@uniba.it

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Abstract

The purpose of our cohort study was to quantify olfactory deficits in COVID-19 patients using Sniffin' Sticks and a pre-post design to evaluate olfactory recovery.

30 adult patients with laboratory-confirmed mild to moderate forms of COVID-19 underwent a quantitative olfactory test performed with the Sniffin' Sticks Test (SST, Burghardt®, Wedel, Germany) considering olfactory threshold (T), odor discrimination (D) and odor identification (I). Results were presented as a composite TDI score (range 1–48) used to define functional anosmia (TDI ≤ 16.5), hyposmia (16.5 <TDI< 30.5) or functionally normal ability to smell (TDI ≥ 30.5). Patients also self-evaluated their olfactory function by rating their ability to smell on a visual analogue scale (VAS rating) and answering a validated Italian questionnaire (Hyposmia Rating Scale-HRS). Patients were tested during hospitalization and about two months after symptoms onset.

During the hospitalization the overall TDI score indicated that our cohort had impairments in their olfactory ability (10 % was diagnosed with Anosmia and more than 50 % were hyposmic). Almost all patients showed a significant improvement at around one month following the first test and for all the parts of the SST except for odor identification. None of the subjects at one month was still diagnosed with Anosmia. We also quantified the improvement in the TDI score based on initial diagnosis. Anosmic subjects showed a greater improvement than hyposmic and normosmic subjects.

In conclusion, within a month time window and two months after symptoms' onset, in our cohort of patients we observed a substantial improvement in the olfactory abilities.

Keywords COVID-19, olfactory deficits, olfactory test, Sniffin' Sticks Test- SST.

Introduction

The novel Coronavirus disease 2019 (COVID-19) has grown to be a global public-health emergency since patients were first detected in Wuhan, China, in December 2019. The disease burden of COVID-19 caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS CoV-2) has been increasing continuously with more than 26,763,00 confirmed cases as of September 6, 2020 (1). SARS CoV-2 typically causes common cold symptoms with a wide range of clinical manifestations, but can also cause severe pneumonia, respiratory failure and death (2). Presently, many reports have summarized the clinical features of patients infected with SARS CoV-2, revealing that most patients developed smell and taste alterations, namely ageusia and anosmia (3,4). Total or partial loss of olfactory perception has been proposed as an early marker of SARS-CoV-2 infection (5,6).

Interestingly, chemosensory dysfunction is not associated with nasal symptoms such as rhinorrhea and nasal obstruction (7,8) and may be caused by different and yet unidentified factors. Mechanisms behind olfactory loss could include the "cytokine storm" or the direct damage of the olfactory epithelium expressing host receptors required for efficient SARS-CoV-2 infection in humans (9,10).

Recent investigations about smell and taste dysfunction in COVID-19 patients, even if conducted on large cohorts of subjects, are mostly based on interviews and surveys (11,7, 12,8). Using objective evaluation methods, fewer studies reported a recovery of olfactory abilities after recovery from SARS-CoV-2 infection. Some authors reported, using surveys, an early smell recovery. For example, Lechien et al. (13), in a study on 417 COVID-19 patients, 25% reported smell recovery two weeks after disease resolution, while Lee et al. (14) described complete recovery within three weeks with a median time of improvement of seven days.

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The purpose of our study was to quantify olfactory deficits in COVID-19 patients using Sniffin' Sticks and to study the recovery of olfactory impairments.

Subjects and Methods

Participants

The study included 34 adult patients admitted to the COVID-Hospital of the Policlinico Hospital of Bari, Italy, between April 22 and May 6, 2020. Four patients dropped-out after the first step of examination: three of them did not shown up to the second control, and one needed intensive care. All patients were positive to nasopharyngeal swabs for SARS-CoV-2 molecular tests, and suffered of mild to moderate symptoms (Table 1-2) (2,13). Patients were enrolled on a voluntary basis and signed the informed consent form. All tests were performed with the highest regard for examiner safety with appropriate personal protective equipment. Exclusion criteria included patients with olfactory dysfunctions before the epidemic, patients affected by rhino-sinusal pathologies, previous rhino-sinusal surgery, current or previous use of recreational drugs by inhalation, recent use of nasal topical vasoconstrictors, head injuries, previous head-neck radiotherapy, neurodegenerative pathology, and psychiatric pathology. In addition, non-compliant patients unable to fully understand the aims of the study as well patients needing invasive or non-invasive ventilation were excluded.

During hospitalization, an average of 25 days after COVID-19 diagnosis, we collected an accurate medical history, and performed quantitative olfactory testing using the Sniffin' Sticks Test- SST (Burghardt®, Wedel, Germany) (15,16).

Patient follow-ups (After CoVID-19), using the same test, were carried out a month after the first evaluation, about two months after symptom onset. The study was approved by the

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Ethics Committee of "Azienda Ospedaliero Universitaria Policlinico of Bari", Italy (n.623/2020).

Psychophysical Olfactory Function.

We assessed olfactory function using the SST including olfactory threshold (T), odor discrimination (D), and odor identification (I). The maximum score for each of the three subsections of the SST is 16. Results from the three tests were presented as a composite TDI score (range 1–48) (17) and then used to define functional anosmia (TDI ≤16.5), functional hyposmia (16.5 <TDI < 30.5), or functionally normal ability to smell (TDI ≥30.5) (15) (16). These values were in the 10th percentile for the reference group ((15) updated in (16)).

SSTs were administered first during hospitalization, when patients had been tested positive for SARS-CoV 2 (during COVID-19) and after all but one tested negative using viral swab (after COVID-19).

Subjective evaluation of smell

Rating olfactory abilities on a Visual Analogue Scale

Participants were asked to quantify their olfactory abilities by rating on a Visual Analogue Scale. The examiner asked patients to quantify their olfactory function by putting a mark on a millimetric horizontal line ranging from 0, indicating the perception of a totally impaired olfactory function, to 100, indicative excellent sense of smell. This evaluation was referred to the time of tests (during and after COVID-19) and retrospectively to the onset of symptoms (onset). Results were then calculated by measuring the distance from 0 in mm.

Questionnaire

The Hyposmia Rating Scale (HRS) was developed for assessing olfactory dysfunction in patients with Parkinson's disease. It is a questionnaire based on 6 questions related to the ability to smell different odors (flowers, gas, garbage, perfume, sweat (body odor), cooked food) (18). Each item is measured by a Likert scale from 1 to 5, where the value 1 corresponds to "no difficulty or absence of disturbance" and the value 5 corresponds to "maximum difficulty or maximum intensity disturbance" (HRS range 6-30). The questionnaire was administered as described above.

Statistical analysis

Data analysis was performed with R via Rstudio (19). Jamovi (20) was used for repeated measures ANOVA. Post-hoc comparisons were performed as stated in the figure legends. In our analysis we do not report the effect of age on our results as by correcting for the effect of age we obtained the same significance level as reported in the Results section. In our sample, we did not consider sex as between subjects factor because of our relatively small number of participants (see table 1).

Results

Sniffin' Sticks

We were able to directly asses the olfactory abilities of patients, by performing Sniffin' sticks test, at two time points of the disease, in particular during hospitalization, when patients had tested positive for SARS-CoV-2 (during COVID-19), and after they had tested negative for the viral swab (after COVID-19). Figure 1 shows several parameters that we evaluated and they revealed significant differences between the score during and after COVID-19. First, during the hospitalization the overall TDI score (mean = 27.07, Sd = 7.88 and 95% CI [24.8, 30.6]) indicated that our cohort had impairments in their olfactory ability (Table 3 and Figure

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1, during COVID-19). 10 % of our patients was diagnosed with Anosmia based on their TDI score being lower than 16 (score set by Hummel et al. (15) and updated in 2019 in their normative data (16)) and more than 50 % were hyposmic (Figure 2B). In particular, the olfactory threshold (mean = 5.47, Sd =2.50 and 95% CI [4.5, 6.4]) and Identification (mean = 9.7, Sd =3.97 and 95% CI [8.2, 11.2]) were indicative of this group of patients presenting severe hyposmia.

Interestingly, olfactory threshold, odor discrimination and total score (TDI) were significantly different between During and After COVID-19 while odor identification was not (repeated measures ANOVA F (7,203) = 291.5 p< 0.05 followed by post-hoc Bonferroni. Overall, the TDI score After COVID-19 (mean = 33.67, Sd = 6.52 and 95% CI [31.2, 36.1]) indicated a significant improvement of the olfactory abilities (Figure 1D). The olfactory threshold After COVID-19 (mean= 8.07, Sd = 2.6 and 95% CI [7.1, 9.1]) improved almost 2-fold while less of an improvement was observed in odor discrimination (around 1.2-fold, now mean= 14.20, Sd = 2.27 and 95% CI [13.8, 15.0]) and odor identification (After COVID-19 mean= 11.4, Sd = 3.6 and 95% CI [10.2, 12.6]) . Among our patients all but three had an improvement in their TDI score and, interestingly, one of these subjects reporting the lowest score after one month, was still positive for the virus. None of the subjects at one month was still diagnosed with anosmia. Also, we observed a decrease in patients considered to be hyposmic (Figure 2B, from 53.3% to 26.7% of the total number of patients) and an increased in normosmic patients (Figure 2B, from 36.7% to 73.3%). We also quantified the improvement in the TDI score based on the initial diagnosis. Anosmic subjects showed a greater improvement than hyposmic and normosmic subjects (Figure 2C). Also, the improvement in the group initially considered hyposmic was significantly larger than that of the normosmic (which was not significantly different from 1 p = 0.05).

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VAS and HRS rating

Although we could directly test our participants by using the SSTs, we also asked them to self-rate their olfactory abilities using a Visual Analog scale (VAS rating) and to self-report the abilities to smell common odors as described by the HRS questionnaire (see Methods). By using a VAS rating we could also obtain information about how that subjects self-reported their olfactory abilities at the onset of the disease. Therefore, we had three time points at which to evaluate self-reported rating: onset, during and after respectively. We could observe a progressive increase in ratings (Figure 3A), being significantly lower at the onset (median = 30 with interquantile rage (iqr) = 67.5; respectively $p = 0.00002$ and $p = 0.00001$ after Bonferroni correction) compared to the other two time points. Also, a complete subjective recovery was reported at one month (After COVID-19 median = 100, iqr = 17.5) and being significantly improved compared to the rating During COVID-19 (median = 80, iqr = 30, and $p = 0.0004$ after Bonferroni correction). Also, HRS ratings (During median = 10, iqr = 8.75 and After median = 6, iqr = 3 and $p = 0.0009$) showed a significant decrease in the score indicative of an improvement of the self-rated olfactory abilities (Figure 3B, During median=10, iqr=8.75 and After median=6, iqr=3).

Are these methods reporting a real improvement? We correlated the VAS and HRS questionnaires with the TDI score at different time points (Figure 3C). We found a significant anticorrelation between VAS and HRS ratings during COVID-19 (-0.66, $p<0.05$) and between VAS and HRS after COVID-19 (-0.78, $p<0.05$). Also, a lower but significant correlation between TDI score and VAS during COVID-19 (0.56, $p<0.05$) emerged. TDI score and VAS rating after COVID-19 were correlated (0.59, $p<0.05$).

There was a significant anticorrelation between TDI score and HRS during COVID-19 (-0.50, $p<0.05$) and between TDI score and HRS after COVID-19 (-0.48, $p<0.05$).

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Discussion

During the developing COVID-19 pandemic, it quickly emerged from early anecdotal reports to large scale studies that the sense of smell is severely impaired in affected subjects. Several reports addressed the degree of the impairments by using self-reported surveys that may be unable to precisely characterize the degree of loss in the absence of objective olfactory testing (21,22,23) . It is worth noticing, though, that due to complete lockdown procedures and isolation of patients these could be the only methods to address and quantify the degree of the olfactory loss during that time.

We were able to overcome the problem of the isolation and used a more objective testing method: the Sniffin' Sticks Test (SST). In our clinic we had initially 34 mild to moderate hospitalized patients among which 30 were tested around 20 days after hospitalization and with positive SARS-CoV-2 viral swabs.

We administered the complete battery of tests and found that 10 % of our subjects could be classified as anosmic based on their TDI score (16) and more than half of our participants were hyposmic during COVID-19. During the Covid-19 pandemic it has been reported that the SST is more sensitive in detecting anosmia and hyposmia in comparison to self-reporting or taking a medical history (24) making the SST an appropriate test to use. In our case, we could identify 63 % of participants with reduced olfactory ability.

Lechien et al. (25) also using the SST on 86 patients with COVID-19, found a very similar percentage of participants with olfactory deficits (48% anosmic, 14% hyposmic, 62% total) compared to our study. Other studies used different olfactory tests and found different percentages, in particular highly variable were the proportion of anosmic and hyposmic participants (26,27,28).

By using University of Pennsylvania Identification Tests (UPSIT) Moein et al. (29). reported some degree of smell loss in 96 % of their tested COVID-19 participants. The differences between studies using objective methods could stem from the relative smaller sample size (as in our case) and the different timing of testing during the disease

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progression. Indeed, in our work, we tested at around 20 days since disease onset compared to shorter onset in other studies (26,27,28,29). Despite the delayed timing of our tests, our anosmic participants were around 10 %, similar to the 8 % reported by Le Bon et al. (30) who tested their participants 5 weeks after the onset of olfactory loss and more or less 15 days after their confirmed COVID-19 diagnosis obtained either by RT-PCR on nasopharyngeal swab or serology testing.

Finally, a recent meta-analysis deposited as pre-print reported that studies that used objective methods (i.e Sniffing Sticks) (31,32,27,33,29) to asses olfactory deficits were, in general, more sensitive than those that used subjective methods (i.e. questionnaires) and on average 77 % of COVID-19 patients had been found with olfactory deficits (with a 95 % CI of 61.4-89.2) (34). Again, our data are in line with these results.

Olfactory thresholds during COVID-19 had, as observed also for TDI, a score lower than the cut off value for hyposmia (16). Interestingly, olfactory threshold score was also found to affect the lower overall TDI of a cohort of 72 patients that tested positive (either via viral swab or serological tests) to COVID-19 (30).

It is tempting to speculate that since this part of the test is mainly dependent on the peripheral olfactory system (i.e. olfactory epithelium in the nasal cavity, which is most easily reached by the virus), that it diminished to a larger degree than odor identification and discrimination which are more strongly related to higher cognitive processes (16,35). This might point to a preferential peripheral damage to olfactory perception. This would be consistent with the findings on animal models showing that SARS-CoV-2 through its Spike glycoprotein can bind ACE2 receptors abundantly expressed in the sustentacular cells of the olfactory epithelium and most likely start inflammatory processes, a so called "cytokines storm" that could

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exacerbate the immune response. In the olfactory epithelium, a variety of cytokines are secreted by infiltrating leukocytes and those can affect olfactory receptor neurons and the stem cell niche, this impairing both the odorant responses and the regeneration capability (36,37,10).

Although sustentacular cells are not directly involved in the conversion of a chemical odor signal into an electrical nerve signal by olfactory receptor neurons that is then sent to the brain, they regulate several aspects of the tissue homeostasis, which in turn assures a normal function of olfactory receptor neurons (38). All these events could drive a partial or complete loss of smell. It has been reported recently that a French woman affected by COVID-19, who was tested for olfactory sensitivity and found to be anosmic, presented obstructive bilateral inflammation in the olfactory cleft. Although local tissue inflammation was present, it is not clear whether it affected the integrity of the olfactory epithelium (39).

It has been suggested that the combined assessment of odor detection threshold and odor identification would be the most appropriate test to detect olfactory impairments in Covid-19 patients (30). While in our participants odor discrimination is higher than 10th percentile (cut off value for hyposmia), identification is the other score worth mentioning because of its lower values During COVId-19. It is dependent on semantic memory and involves more difficult cognitive tasks than olfactory threshold and therefore requires intact cognitive skills. So, does this mean that also cognitive skills are impaired in COVID-19 patients? Not necessarily so, as we could consider that these patients, affected by a peripheral olfactory loss, require active relearning of odor identification, which is potentially a more complex and therefore slower process than odor discrimination (35).

Another hypothesis could be that the deficit in odor identification and in particular the lack of a significant improvement could be indicative of more extensive non-

functional pathways due to the potential neuro-invasiveness of the SARS-CoV-2. At our understanding, although neurological symptoms have been reported, this area is subject to an extensive debate (40).

We also used a self-reported questionnaire to gather more information about how participants self-evaluated their sense of smell and also to obtain retrospective data (i.e. VAS at the onset).

Interestingly, we found correlations for VAS ratings at different time points and between VAS and HRS (anticorrelation since the HRS scoring system is reversed, i.e. lower score better olfaction). Still significant, although milder, was the correlations between the TDI scores and the questionnaire ratings, indicating that VAS and HRS with their scoring system based on self-evaluation could be a good proxy to test olfactory abilities.

Improvement of the sense of smell

Questions that up to now do not have clear answers are whether anosmic symptom persist and for how long after a person had a negative viral swab. At around one month from the first test and more or less two months from the onset of COVID-19, we performed a follow-up study with our patients. All but one had been cleared from the viral load and all but three showed an improvement of the TDI score. The higher scores in the follow up study was overall significantly improved from that compared to During COVID-19 except for odor identification. Therefore, although we could not definitely conclude that after two months from the onset of the disease there is a full recovery of the sense of smell, certainly we could state that in this time window there is a substantial improvement in mild to moderate COVID-19 patients. Our results still showed a portion of participants that remained hyposmic (27%) and similar to the 29% found by Le Bon et al. (30). A higher percentage of hyposmic was found by Otte MS et al. (41) after seven weeks after COVID-19 onset. Discrepancies could be due to several factors like different study populations, sample size and duration of initial

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anosmia (30) and days since the onset. Indeed, by applying a machine learning algorithm it has been shown that recovery has the number of days from the onset of the disease as best predictor (6). We observed a larger improvement in those subjects initially diagnosed as anosmic, which was larger compared to hyposmic and normosmic subjects. Indeed, in the follow-up no one was anosmic and all but three (10 %) of our patients improved their scores. Interestingly, the only subject still positive for a viral test was the one with the lowest score. The other two that did not improve belonged to the normosmic group that showed less or non-significant improvement compared to the anosmic and hyposmic group. The overall improvement was also detected by the VAS and HRS questionnaires. Due the opportunity of asking the patients to rate their sense of smell at the onset, we could observe a progressive increase of the ratings during and after COVID-19. Although participants can both underestimate or overestimate olfactory acuity the use of VAS to self-rate olfactory abilities during COVID-19 pandemic has been crucial to detect impairments (e.g. Parma V et al. 2020 (8), Giacomelli et al. 2020 (12) and summarized by Pellegrino et al. 2020 (42)). Also, subjective methods could be used to collect large dataset that are important to create training and testing set of data for machine learning algorithms that could be implemented as diagnostic tools (11) (6).

Strengths and limitations

Our work has both strength and limitations. The use of the SST as an objective test to quantify olfactory impairments is a major strength. Few studies have applied objective measurements (to the best of our knowledge 6 versus 35 and growing studies that used subjective methods). Also, our group was quite homogeneous and balanced for sex (in our case we could not see an effect of sex in our analysis similar to Le Bon et al. 2020 (30) and we had the opportunity to have a pre-post longitudinal design.

Limitations arise mainly from our sample size, smaller than other studies where objective methods were used (25,26,27,31,32,28,29,30,33).

This could limit the generalization of our conclusions and other type of analysis that could reveal more about the relation between COVID-19 symptoms and olfactory deficits. For example, Iravani et al. (33) found that intensity ratings during COVID-19 is dependent on the number of symptoms (i.e. the more symptoms the less intense was the rating of household odors).

Conclusions

By using a psychophysical test to directly asses the olfactory abilities of the patients we found a decreased sense of smell of COVID-19 affected patients. The choice of performing the Sniffin' Sticks test allowed us to not rely exclusively on self-reports and to compare our results with normative datasets available for the general population. In addition, by following-up with our subjects we are beginning to answer the question about gaining back the sense of smell. In our cohort we could show a clear improvement in the olfactory abilities with a negative viral swab within one month time window.

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	Number of patients (%)
Sample Size	30
Age (mean ± sd)	47,47 ± 13
Gender	16 F (53,3%) 14 M (46,7%)
Current smoker Ex-smoker Never a smoker	2 (6,7%) 7 (23,3%) 21 (70%)
Hypertension	7 (23,3%)
Thyroid-related	3 (10%)
Diabetes	2 (6,7%)
Neoplastic diseases	3 (10%)
Previous pulmonary embolism	1 (3,3%)
Fibromyalgia	1 (3,3%)
Polycystic ovary	1 (3,3%)
Allergy	9 (30%)

Table 1: demographic and anamnestic characteristics of patients.

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	Number of patients (%)
Flu like symptoms	30 (100%)
Nasal obstruction	7 (23,3%)
Epistaxis	4 (13,3%)
Nasal discharge	5 (16,7%)
Neurological symptoms	9 (30%)
Headache	*8 (26,7%)
Nausea	*2 (6.7%)
Dizziness	*1 (3.3%)

* Symptoms are not mutually exclusive

Table 2: otolaryngology, flu-like and neurological symptoms.

	Threshold		Discrimination		Identification		TDI	
	During Covid-19	After Covid-19	During Covid-19	After Covid-19	During Covid-19	After Covid-19	During Covid-19	After Covid-19
N	30	30	30	30	30	30	30	30
Mean	5.47	8.07	11.90	14.20	9.70	11.40	27.07	33.67
Sd	2.50	2.70	3.49	2.27	3.97	3.16	7.88	6.52
SE	0.46	0.49	0.64	0.41	0.72	0.58	1.44	1.19
CI	[4.5, 6.4]	[7.1, 9.1]	[10.6, 13.2]	[13.8, 15.0]	[8.2, 11.2]	[10.2, 12.6]	[24.8, 30.6]	[31.2, 36.1]
p	0.003		0.015		0.252		0.001	

Table 3. Sniffin' Sticks score means, standard deviation (Sd), standard error (SE) and 95% confidence interval (CI). p values are from post-hoc Bonferroni comparisons after repeated measures ANOVA F $(7,203)$ = 291.5 $p < 0.05$.
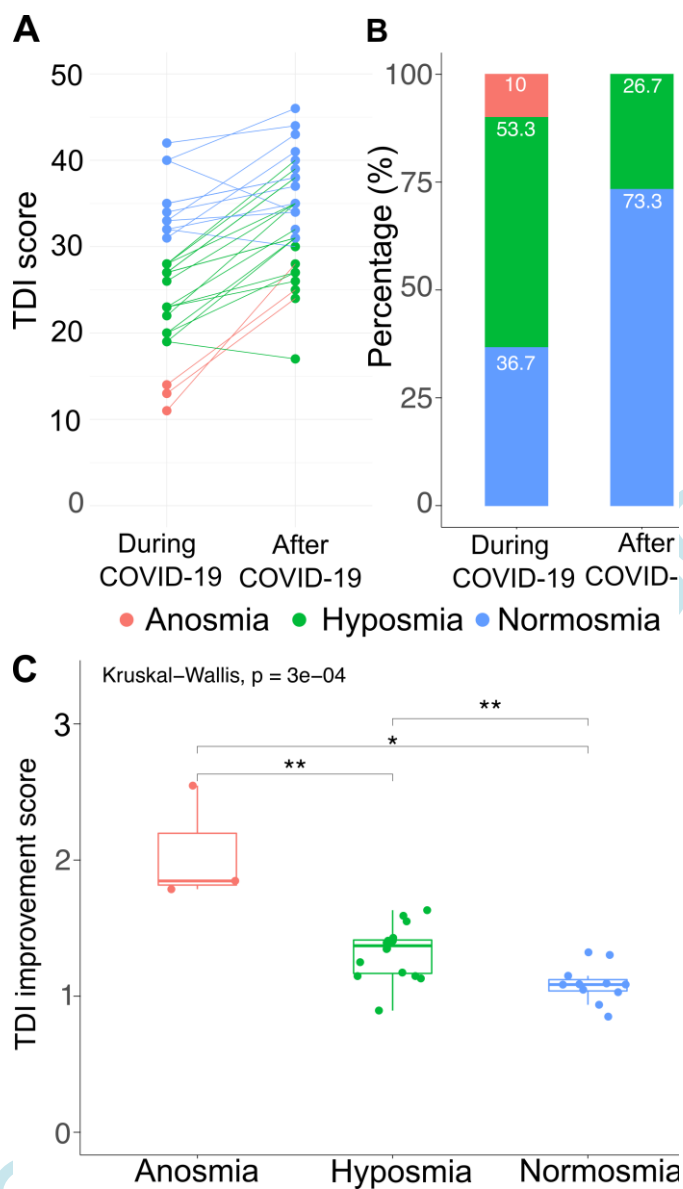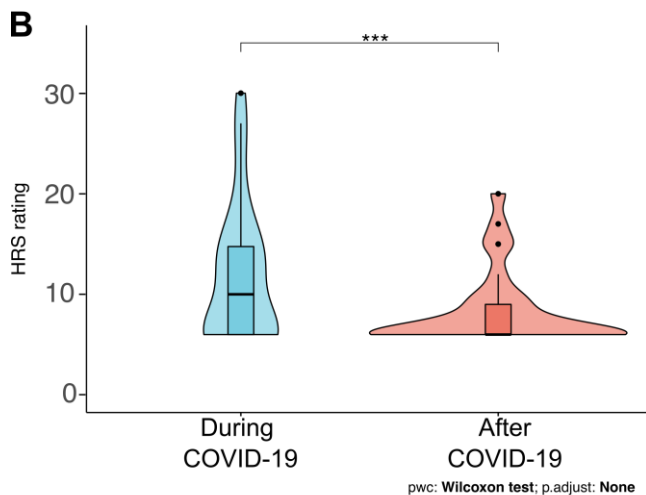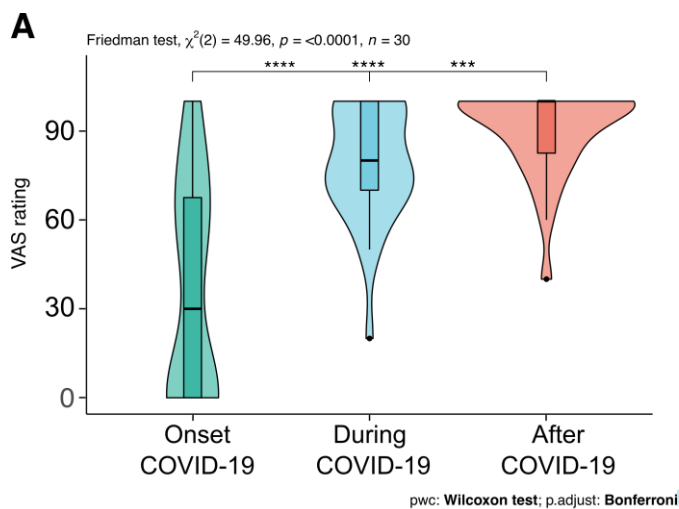
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Figure 1

Figure 2

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Figure 3

A

Friedman test, $\chi^2(2) = 49.96$, $p = {<}0.0001$, $n = 30$

pwc: **Wilcoxon test**; p.adjust: **Bonferroni**

B

pwc: **Wilcoxon test**; p.adjust: **None**

C
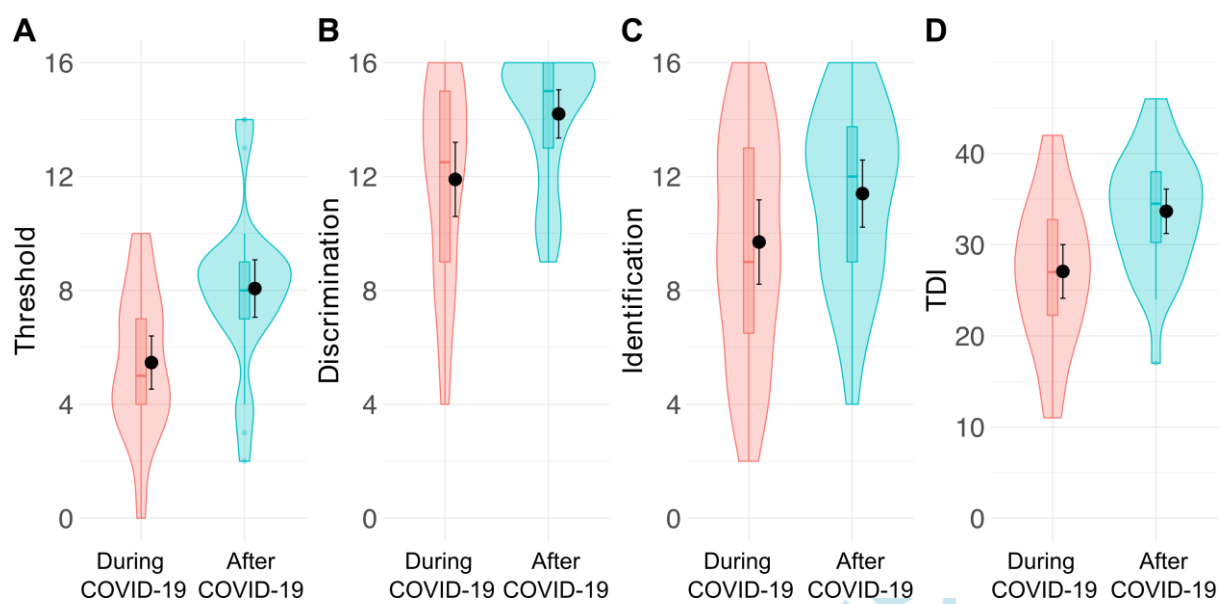
X = non−significant at $p < 0.05$