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# Taste and Smell Disorders in COVID-19 Patients: Role of Interleukin-6

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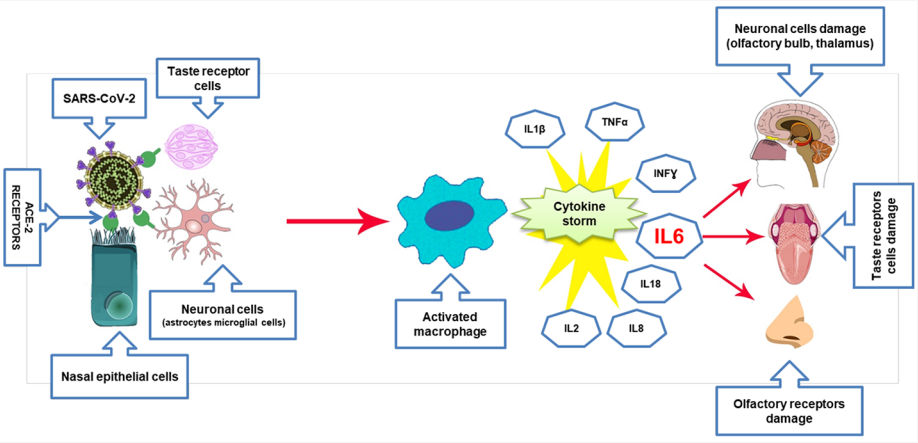


**ABSTRACT:** The rapid recovery of smell and taste functions in COVID-19 patients could be attributed to a decrease in interleukin-6 levels rather than central nervous system ischemic injury or viral damage to neuronal cells. To correlate interleukin-6 levels in COVID-19 patients with olfactory or gustatory dysfunctions and to investigate the role of IL-6 in the onset of these disorders, this observational study investigated 67 COVID-19 patients with taste or smell disorders or both, who did not require intensive care admission, admitted at COVID Hospital of Policlinico of Bari from March to May 2020. Interleukin-6 was assayed in COVID-19 patients with taste or smell disturbances at the time of admission and at the time of swab negativization. At the same time, patients have been given a specific survey to evaluate the severity of taste and smell disturbances. Of 125 patients with smell or taste dysfunctions at onset of disease, 67 fulfilled the inclusion criteria, while 58 were excluded because 35 of them required intensive care admission, 5 were unable to answer, 5 died, 7 had finished chemotherapy recently, and 5 refused to participate. The evaluation of taste and smell disorders was carried out using a survey performed at the time of admission and at the time of swab negativization. Sinonasal outcome test 22 (SNOT-22) was used as a reference for olfactory function assessment, and Taste and Smell Questionnaire Section of the US NHANES 2011−2014 protocol (CDC 2013b) was used as reference for gustatory function assessment. A venous blood sample was taken for each patient to measure IL-6 levels upon entry and at swab negativization. Interleukin-6 levels in COVID-19 patients in relation to olfactory or gustatory disorders were correlated from the time of their admission to the time of swab negativization. Statistically significant correlations were obtained between the decrease of interleukin-6 levels and the improvement of smell ($p$ value < 0.05) and taste ($p$ = 0.047) functions at swab negativization. The acquired results demonstrate the key role of interleukin-6 in the pathogenesis of chemosensitive disorders in COVID-19 patients.

**KEYWORDS:** *SARS-CoV-2, COVID-19, interleukin-6 (IL-6), immune-mediated neurological syndromes, anosmia, dysgeusia*

## INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a viral pandemic that recently emerged from East Asia and quickly spread to the rest of the world, due to the SARS-associated coronavirus 2 (SARS-CoV-2), first defined as 2019-nCoV.

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**Table 1. General Characteristics, Associated Symptoms, and Associated Pathologies of 67 COVID-19 Patients with Smell and Taste Disorders**

| inclusion criteria | exclusion criteria |
|---|---|
| age > 18 years | patients without a laboratory-confirmed diagnosis of COVID-19 infection |
| laboratory-confirmed COVID-19 infection (reverse transcription−polymerase chain reaction, RT-PCR) | patients in the intensive care unit |
| patients clinically able to complete the questionnaire | patients with olfactory or gustatory dysfunctions before the epidemic due to congenital anosmia, the side effects of drugs (in particular chemotherapy), previous surgery or radiotherapy in the oral and nasal cavities, head injury, sinonasal diseases, allergic rhinitis |
| | patients with systemic diseases (iron deficiency, autoimmune diseases) |
| | patients with neurodegenerative disorders (Parkinson's disease, disease Alzheimer's disease, dementia) |
| | patients with major depression |

| General Characteristics | | |
|---|---|---|
| | *n* (percent) | age (years) |
| male | 45 (67.2%) | 65 ± 13.1 |
| female | 22 (32.8%) | 64 ± 15.8 |
| days from COVID-19 symptoms onset | 4 ± 1 | |
| day of duration chemosensitive disorders | 21 ± 7 | |
| **Associated Symptoms** | | |
| fever | 64 (95.5%) | |
| cough | 59 (88%) | |
| asthenia | 49 (73.1%) | |
| headache | 40 (59.7%) | |
| sore throat | 43 (64.2%) | |
| abdominal symptoms | 6 (8.95%) | |
| muscle or joint pains | 61 (91%) | |
| chest pain | 52 (77%) | |
| nausea | 39 (58%) | |
| vomit | 13 (19%) | |
| loss of appetite | 17 (25%) | |
| felt tired | 63 (94%) | |
| altered breathing | 59 (88%) | |
| diarrhea | 10 (14%) | |
| **Associated Pathologies** | | |
| diabetes | 12 (17.9%) | |
| hypertension | 38 (56.7%) | |
| nasal septum deviation | 5 (7.4%) | |
| respiratory insufficiency | 9 (13.4%) | |
| gastresophageal reflux disease | 20 (29.8%) | |
| thyroid diseases | 15 (22.3%) | |

SARS-CoV-2 is a viral strain of the SARS-CoV species related to SARS, belonging to the Coronaviridae family, discovered around the end of 2019.[1,2] Differing from other human coronaviruses, SARS coronaviruses may induce a severe form of pneumonia, potentially lethal.

The target cells of SARS-CoV-2 are those that express the angiotensin-converting enzyme 2 (ACE-2):[3] type II alveolar cells,[3,4] upper and stratified epithelial cells, absorptive enterocytes from ileum and colon,[4] myocardial cells, proximal tubule cells of kidney, bladder urothelial cells,[3] glial cells and neurons,[5] oral tissues cells (especially epithelial cells of the tongue),[6] and nasal epithelial cells, which display the highest expression of ACE-2 receptor in the respiratory tree.[7]

Due to the wide expression of this receptor, the SARS-CoV-2 may induce different clinical pictures, often contemporary and able to determine a complex, pleomorphic scenario.

Severe alteration of taste and smell without rhinorrhea or nasal obstruction can be prodromal symptoms in the early stage of disease.[8,9]

Loss of taste might be linked to bonding between SARS-CoV-2 and receptors for sialic acid, a component of saliva that protects the glycoproteins responsible for the transport of molecules stimulating taste in the taste pores. As a result of this bond, the degradation of taste particles with an alteration of taste is favored.[10−12]

According to others authors, loss of taste can be linked to loss of smell because the brain combines the perceptions of taste from the mouth with what is known as retronasal olfaction.[13,14]

It has been demonstrated that human coronaviruses invade the central nervous system through the olfactory neuroepithelium and spread to the olfactory bulb using a mode of neuron-to-neuron propagation.[15−17]

Furthermore, SARS-CoV-2 can damage the blood−brain barrier, invade the nervous system through the slow cerebral microcirculation, which the facilitates the interaction between the protein S (spike) and the ACE-2 receptors expressed on the capillary endothelium, and interact with the ACE-2 receptors expressed in neuronal cells.[5]

SARS-CoV-2 binds to receptors present on cells of the lower tract respiratory system with interstitial pneumonia that can develop into a severe acute respiratory distress syndrome (ARDS) or can damage other organs (heart, kidney, liver, brain)


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**Table 2. Characteristics of Smell or Taste Disorders in 67 COVID-19 Patients at the First and Second Evaluation (Grading of Disorders)**

| | | grading of disorders | | | | | |
|---|---|---|---|---|---|---|---|
| | no. of patients | none (0) | very mild (1) | mild or light (2) | moderate (3) | severe (4) | bad (5) |
| | | | First Evaluation | | | | |
| olfactory disorders | 44 (65.7%) | 23 (34.3%) | 0 | 4 (9.1%) | 13 (29.6%) | 17 (38.6%) | 10 (22.7%) |
| taste disorders | 17 (25.4%) | 50 (74.6%) | 0 | 3 (17.7%) | 7 (41.1%) | 6 (35.3%) | 1 (5.9%) |
| olfactory and taste disorders | 6 (8.95%) | 61 (91.05%) | 0 | 0 | 0 | 1 (16.7%) | 5 (83.3%) |
| | | | Second Evaluation | | | | |
| olfactory disorders | 22 (32.8%) | 45 (67.2%) | 21 (95.4%) | 1 (4.6%) | 0 | 0 | 0 |
| taste disorders | 10 (14.94%) | 57 (85.1%) | 10 (100%) | 0 | 0 | 0 | 0 |
| olfactory and taste disorders | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

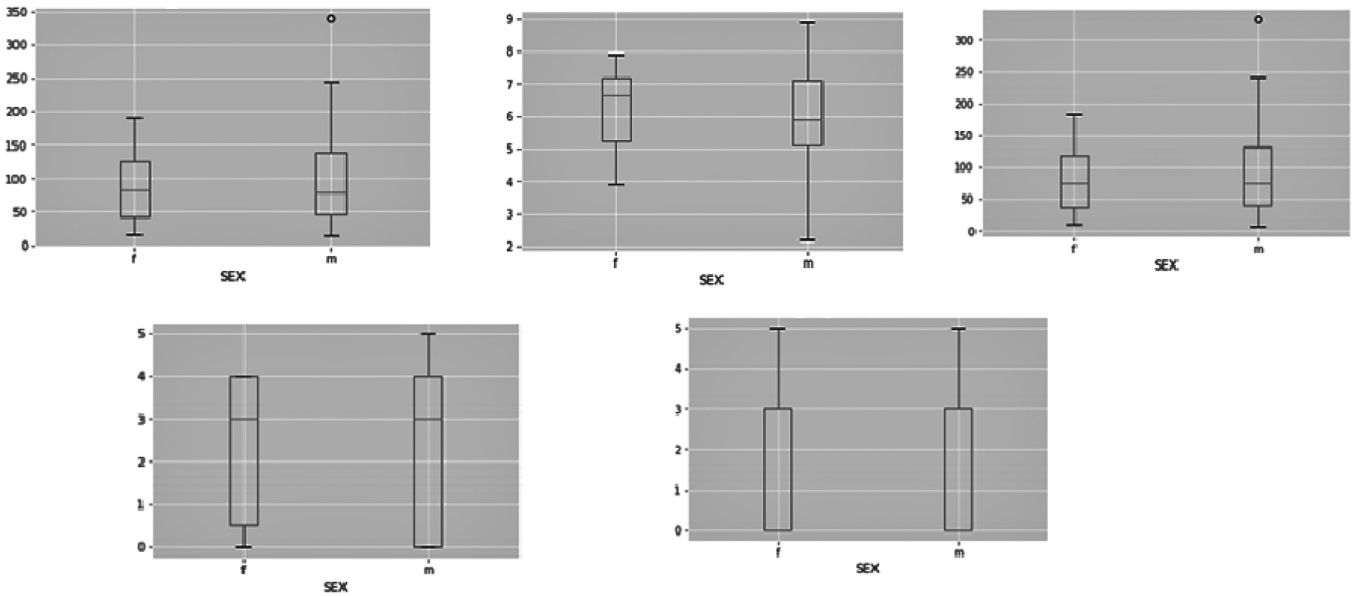


**Figure 1.** (a) Sex distribution of IL-6 levels at the first evaluation; (b) sex distribution of IL-6 levels at the second evaluation; (c) sex distribution of the delta (values at the first evaluation minus values at the second evaluation) value of IL-6 levels; (d) sex distribution of the delta score of smell; (e) sex distribution of the delta score of taste.

with systemic manifestations (ischemias, arrhythmias, encephalitis, seizures, strokes) up to sepsis, septic shock, and patient death.

These clinical manifestations are accompanied by the onset of an inflammatory storm characterized by the release of a wide range of cytokines.[18,19]

A key role in the cytokine storm is played by interleukin-6 (IL-6), which induces a variety of acute-phase proteins (C-reactive protein, serum amyloid A, $\alpha$1-antichymotrypsin, haptoglobin, fibrinogen, and complement components) and activates coagulation cascade with probable onset of disseminated intravascular coagulation. Elevated levels of IL-6 were significantly related to severe clinical manifestations.[20]

Increased IL-6 levels have been found in serum of patients with hyposmia[21] and transient high-level expression of proinflammatory cytokines (TNF-$\alpha$, IL-6) was detected in the olfactory bulb and CNS during different human influenza virus infections (1918 H1N1 influenza virus, 2009 H1N1 influenza virus, and HPAI H5N1 virus)[22−24] Experiments have confirmed that virus-infected microglial cells and astrocytes secrete IL-6[25−27] and primary glial cells cultured in vitro secrete a large number of inflammatory factors, such as IL-6, IL-12, IL-15, and TNF-$\alpha$ after being infected with coronaviruses.[28]

IL-6 could act as an endogenous substance regulating olfactory neuronal activity because it has been shown to regulate neuronal and glial cell activity. In addition, IL-6 can directly inhibit smell function through activating apoptotic pathways using TNF-$\alpha$ or trough neuropoietin (NP), an IL-6 related cytokine that affects signaling through ciliary neurotrophic factor receptor and influences nuclear factor $\kappa$B73 and adenosine triphosphate−ubiquitin-dependent proteolytic pathways.[21]

At present, few studies have investigated the pathophysiology of chemosensitive disorders in patients with laboratory-confirmed COVID-19 mostly attributing it either to CNS ischemic injury or viral damage to neuronal cells. Nevertheless, the rapid recovery of chemosensitive functions would rule out these hypotheses in favor of other mechanisms, such as the reduction of IL-6 levels, that may explain a faster recovery of these functions.

This is the first study that evaluates IL-6 levels in COVID-19 patients with grading of smell and taste disorders. The aim was to monitor and to correlate IL-6 levels in laboratory-confirmed COVID-19 patients with olfactory or gustatory disorders from the time of their admission to the time of swab negativization. The evaluation of olfactory or gustatory disorders was carried


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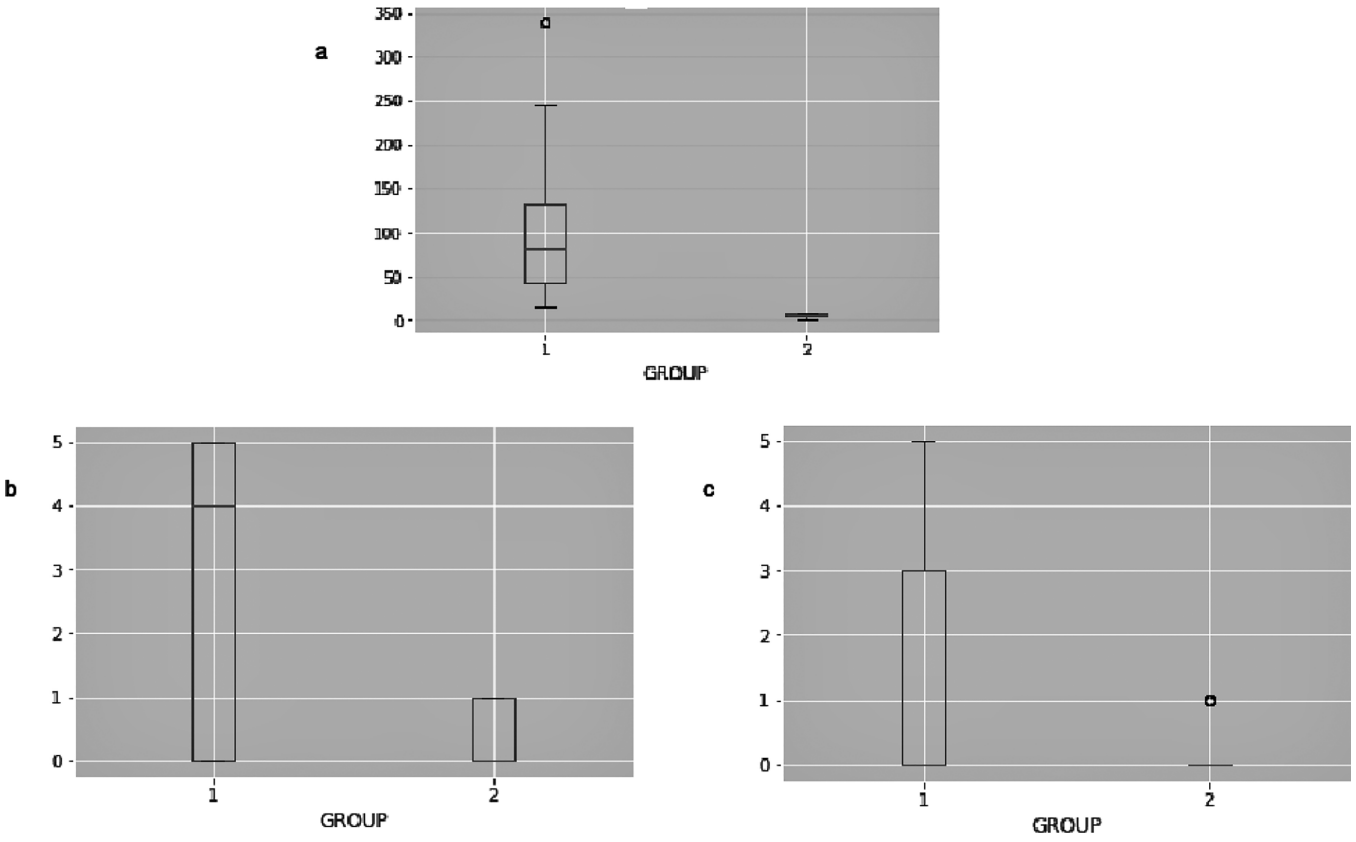


**Figure 2.** (a) IL-6 and (b) smell and (c) taste score distributions in COVID-19 patients at first and second evaluation.

out using a questionnaire performed at the time of admission and at the time of swab negativization.[29,30]

## RESULTS AND DISCUSSION

A total of 67 COVID-19 patients, 45 male (67.2%) (age 65 ± 13.1) and 22 female (32.8%) (age 64 ± 15.8), were admitted at COVID hospital of Policlinico, University of Bari. Table 1 shows the clinical characteristics of the patients and symptoms associated with taste and smell disorders. In all patients, smell or taste disorders occurred before the onset of COVID-19 symptoms (4 ± 1 days), while the duration of the disturbance was 21 ± 7 days.

A total of 44 patients (65.7%) reported isolated olfactory dysfunctions, while 17 patients (25.4%) reported isolated taste disorders, and 6 patients (8.95%) reported combined smell and taste disorders (Table 2). At the time of the second evaluation, 35 patients (52.2%) reported a complete recovery of the chemosensitive functions, while 32 patients (47.8%) reported very mild or mild disorders. In particular, 21 patients (65.6%) showed very mild smell disorder, 1 (3.2%) presented mild smell disorder, and 10 (31.2%) reported very mild taste disorder (Table 2).

At the time of the first examination, complete anosmia was detected in 10 cases (22.7%), severe in 17 patients (38.6%), moderate in 13 patients (29.6%), mild or light in 4 patients (9.1%), very mild in no patients, and absence of disorder in 50 patients (52.3%) (Table 2).

In patients with taste disorders, complete ageusia was detected in 1 case (5.9%), severe in 6 patients (35.3%), moderate in 7 patients (41.1%), mild or light in 3 patients (17.7%), very mild in no patients, and absence of disorder in 50 patients (52.3%) (Table 2).

In patients with associated disorders, 5 (83.3%) had bad form and 1 (16.7%) severe one.

The identified subpopulations were correlated with the levels of IL-6 detected at the time of hospitalization and at swab negativization.

The sex distribution of IL-6 levels in hospitalized patients showed an increase in males (99.6 pg/mL vs 88.5 pg/mL) (Figure 1a), while there were no more differences in the sex distribution at the time of swab negativization (6.3 pg/mL vs 5.8 pg/mL) (Figure 1b).

The distribution of the delta values of IL-6 was mildly higher in females than in males (93.7 pg/mL vs 80.0 pg/mL) (Figure 1c), while the sex distribution of the smell delta score (2044 vs 2045) and of the taste delta score (0.15 vs 0.13) show no significant differences between the two sexes (Figure 1d,e).

The distribution of IL-6 and smell and taste scores in 67 COVID-19 patients was higher at the first evaluation compared to the second one (Figure 2a–c).

The Wilcoxon signed-rank test, used to evaluate the values of IL-6 and the scores related to smell and taste dysfunctions, provided a good indication of the sample. There are statistically significant differences between the values obtained when patients entered the hospital compared to the values obtained at swab negativization ($p < 0.05$) (Table 3a).

To determine a statistically significant correlation between the delta of IL-6 levels and the delta of smell and taste disorders, the Pearson correlation coefficient was calculated.

Significant correlations between IL-6 levels and type of dysfunctions have been found. The olfactory and gustatory dysfunctions had a higher score in patients with higher levels of IL-6. Also, the patients with the association of both disorders had even higher levels of IL-6.


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**Table 3. Values of the (a) Wilcoxon Test and (b) Pearson's Correlation Coefficients between All the Variables of the Dataset Considered**

(a) Wilcoxon Signed-Rank Test

| variable | Wilcoxon test | $p$ value |
|---|---|---|
| IL-6 level (first evaluation) vs IL-6 level (second evaluation) | 2278 | <0.05 |
| score smell dysfunction (first evaluation) vs score smell dysfunction (second evaluation) | 1225 | <0.05 |
| score taste dysfunction (first evaluation) vs score taste dysfunction (second evaluation) | 325 | <0.05 |

(b) Pearson's Linear Correlation Coefficients

| variable | Pearson coefficient ($r$) | 95% confidence intervals | $p$ value |
|---|---|---|---|
| delta IL-6 vs delta score smell | 0.58 | 0.33 to 0.68 | <0.05 |
| delta IL-6 vs delta score taste | 0.24 | 0.003 to 0.45 | 0.047 |
| delta score taste vs delta score smell | −0.38 | −0.567 to −0.15 | <0.05 |

An excellent correlation between the delta of IL-6 levels and the delta score of smell dysfunction has been found ($r = 0.58$, $p < 0.05$).

The delta taste score also showed a statistically significant correlation with the delta of IL-6 levels ($p = 0.047$). These correlations showed that the recovery of chemosensitive functions occurs when IL-6 levels return to normal values (1−7 pg/mL) at the time of swab negativization.

A statistically significant inverse correlation between the delta score taste and the delta score smell has been found ($p < 0.05$) (Table 3b).

Coronaviruses have been identified as a family of viruses that may be associated with anosmia,[31] and SARS-CoV-2 can cause taste and smell dysfunctions without rhinorrhea or nasal obstruction like prodromal symptoms.

Different papers have investigated the occurrence of anosmia/hyposmia and dysgeusia/ageusia in patients with laboratory-confirmed COVID-19, the timing of dysfunction, and associated symptoms, but to our knowledge, no papers have investigated the correlation between severity and timing of these dysfunctions and the levels of IL-6.

In the present study, chemosensitive dysfunctions are more frequent in males (67.2%) and the prevalence of olfactory dysfunction is higher than that of taste dysfunction (65.7% vs 25.4%).[32]

Smell and taste disorders occurred before the onset of COVID-19 symptoms (4 ± 1 days), while the duration of the disturbance was 21 ± 7 days.

The Wilcoxon signed-rank test provided statistically significant differences between (a) levels of IL-6 at the first and at the second assessment ($p < 0.05$), (b) the smell score at the first and at the second assessment ($p < 0.05$), and (c) the taste score at the first and at the second evaluation ($p < 0.05$).

The delta of IL-6 levels correlated with the delta smell score ($p$ value < 0.05) and with the delta taste score ($p = 0.047$), and the delta score taste showed a significant inverse correlation with the delta score smell ($p < 0.05$). These results would demonstrate that smell and taste disorders in COVID-19 patients might be linked to high levels of IL-6.

The exact pathophysiology of taste and smell dysfunctions is not well understood, and only hypotheses can be made based on studies regarding other coronaviruses. Various mechanisms have been hypothesized: (a) a central involvement linked to the ability of the human coronavirus to invade the olfactory bulb and, therefore, to spread to the central nervous system,[31] or (b) linked to the capacity of the virus to enter into cerebral microcirculation and to damage the brain,[5] or (c) a peripheral involvement of the nasal epithelium with direct damage to the olfactory receptor neurons.

Recent reports have shown a high recovery rate of the olfactory function within 1−2 weeks after the onset of dysfunction, a very short time to achieve complete neuronal regeneration.[32−34] Therefore, it has been hypothesized that olfactory disorders are not related to viral damage to neuronal cells but to non-neuronal cells that express ACE-2 receptors such as sustainable cells of the olfactory epithelium, microvillar cells, Bowman's gland cells, horizontal basal cells, and olfactory bulb pericytes.[35] Turski et al. have supposed that the loss of smell is due to the infection of the support cells and vascular pericytes of the olfactory epithelium and bulb with consequent alteration of the function of the olfactory neurons.[35]

The central involvement theory is supported by studies conducted in mouse models that have demonstrated the penetration of SARS-CoV trans-neuronally through the olfactory bulb and the rapid spread of the virus in connected areas of the brain. The authors demonstrated an important presence of SARS-CoV at 60−66 h after infection in the olfactory bulb and subsequently in the regions of the brainstem (midbrain−dorsal raphe), of the thalamus, of the basal ganglia (globus pallidus, ventral and lateral preoptic regions), and of the cortex (piriform and infralimbic cortex), connected to the olfactory bulb. These authors, detecting any signs of inflammatory infiltration, hypothesized that neuronal death occurs due to a cytokine storm, in particular IL-6, produced by neurons under stimulation of the viral N spikes.[36]

The data collected in this study demonstrate a close correlation between the levels of IL-6 and the trend of taste and smell dysfunctions: the reduction of disturbances is accompanied by a progressive reduction of IL-6 levels, which return to normal values when the disturbances disappear.

The improvement of the sensory functions over time would suggest the action of local inflammatory phenomena on the receptors of the olfactory and gustatory cells, rather than permanent cellular damage linked to the action of the virus.

The dysfunctions might be linked to peripheral action of the IL-6 at the level of cell receptors infected by the virus and to central action of IL-6 at the level of intermediate stations of taste and olfactory pathways, especially in the thalamus. In fact, at the thalamic level, both the gustatory path (posteromedial ventral nucleus) and the olfactory path (dorsomedial nucleus, that intervenes in the conscious analysis of odors) converge. These centers are close to the hypothalamus, where the thermoregulatory center, target of IL-6, resides. The transient increase in IL-6 produced by microglial cells and astrocytes in the olfactory bulb and other areas of the central nervous system during this infection could justify the progress of these disorders.

In addition, recent studies have shown that taste cells produce, in response to inflammatory stimuli, high levels of various molecules associated with internal defense responses, including multiple inflammatory cytokines. Excessive production of inflammatory cytokines can cause cell death and inhibit cell renewal in the taste buds, which can contribute to the development of taste disorders associated with various diseases, also due to the paracrine activity of some inflammatory mediators, especially if sepsis occurs.[37−39]


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Due to these references and the data obtained, it could be hypothesized that in the pathogenesis of taste and smell dysfunctions, not fully clarified, a key role is played by IL-6, the main cytokine of the inflammatory cascade, with central and peripheral action.

Several papers suggest that sensory dysfunctions in COVID-19 patients are linked to viral damage to neuronal cells or to a central nervous system ischemic injury. Such mechanisms would result in a longer recovery time for these functions.[40,41]

On the contrary, this study has shown that the recovery times are shorter than those of possible neurological or ischemic damage and that the recovery of these functions is accompanied by the simultaneous swab negativization and by the return to normal of the levels of IL- 6.

To date, COVID-19 remains an important medical and social issue[42] that requires several translational studies to fully understand its molecular bases and to define correct and effective therapy protocols. In fact, we are continuously understanding the main mechanisms of this multifaceted condition, but we do not know its direct and indirect long-term sequelae; also several hypotheses have been proposed about this matter and especially for neurologic,[43,44] nutritional,[45] immunological[46,47] and cardiovascular consequences.[48,49]

## METHODS

This observational study was conducted from March 1 to May 31, 2020, following the provisions of the Declaration of Helsinki. The study was approved by the Ethics committee of the AOU Policlinico Consorziale di Bari (Italy) (No. 6388 COVID19 DOM - protocol number 0034687/12-05-2020) and written informed consent was obtained.

Of 125 patients admitted at COVID hospital of Policlinico, University of Bari, with smell or taste dysfunction or both at onset of disease, 67 (45 men and 22 women) fulfilled the inclusion criteria, while 58 were excluded (35 because of required intensive care admission, 5 who were unable to answer, 5 who died, 7 who had finished chemotherapy recently, and 5 who refused to participate). Inclusion and exclusion criteria are reported in Table 1. Therefore, we mainly included mild to moderate COVID-19 patients, who did not require intensive care admission.

The survey, submitted to each patient, consisted of two parts: the first, for health care professionals, included general questions (age, sex, residence, work activity) and the presence of systemic diseases (hypertension, diabetes, and gastrointestinal and thyroid pathologies) and local diseases (nasal septum deviation); the second, for the patient, investigated taste dysfunctions with 11 questions and smell dysfunctions with 13 questions upon entry and after swab negativization.

The survey examined the onset of associated symptoms concerning the appearance of olfactory and gustatory dysfunctions, timing of the dysfunction (time of onset and duration of the disorder), and the extent of the alteration. Sinonasal outcome test 22 (SNOT-22) was used as a reference for olfactory function assessment.[29] SNOT-22 classified the severity of symptoms as none (0), very mild (1), mild or light (2), moderate (3), severe (4), or bad (5).

Taste and Smell Questionnaire Section (CSQ) of the US NHANES 2011−2014 protocol (CDC 2013b) was used as reference for gustatory function assessment,[30] and the severity of symptoms was classified using the scores none (0), very mild (1), mild or light (2), moderate (3), severe (4), or bad (5). In the presence of the association of both symptoms, severity was classified using the scores none (0), very mild (1), mild or light (2), moderate (3), severe (4), or bad (5).

A venous blood sample was taken for each patient to measure IL-6 levels upon entry and at swab negativization. The sample was collected in 5 mL Vacutainer tubes without anticoagulants. Blood samples were centrifuged (1000 × *g*, 15 min, 4 °C), and the serum was removed and immediately stored at −80 °C until analysis. The IL-6 assay was performed with the chemiluminescence assay using Cobas e801 (Roche Instrumentation).

**Statistical Analysis.** All analyses were performed using R, version 3.53 (R Foundation for Statistical Computing). The variables age, sex, symptom associated, and olfactory and taste disorders are reported in numerals and percentages of the total. The delta score (value at the first evaluation minus value at the second evaluation) has been calculated for the following parameters: IL-6 levels and olfactory and taste dysfunction scores, obtained from the questionnaires administered at the first and the second evaluation. Descriptive statistics for quantitative variables are given as the mean ± SD.

The statistical analysis of differences between the first evaluation and the second evaluation for the parameters IL-6 level and olfactory and taste disease was performed using nonparametric tests (Wilcoxon signed-rank test). In order to evaluate the correlation between the delta IL-6 levels, the delta score of olfactory dysfunction, and the delta score taste dysfunction, Pearson's linear correlation coefficient was applied. For all the tests used, a *p*-value threshold of 5% was adopted.

## CONCLUSION

This study based on clinical evidence and laboratory data highlighted the importance of IL-6 in the pathogenesis of chemosensitive disorders. Further studies would be necessary to investigate any other mechanisms of action of IL-6 both in the appearance of taste or smell disturbances and in the clinical manifestations of COVID-19 patients in order to ensure better clinical and pharmacological management of these patients.

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### Author Contributions

Cazzolla and Lovero had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: Cazzolla, Lovero, Lo Muzio, Santacroce. Acquisition, analysis, or interpretation of data: Cazzolla, Lovero, Lo Muzio, Pozzessere, Procacci, De Ruvo. Drafting of the manuscript: Cazzolla, Lovero, Pepe, Testa, Santacroce. Critical revision of the manuscript for important intellectual content: Cazzolla, Lovero, Lo Muzio, Ciavarella, Crincoli, Di Serio, Schirinzi, Pepe, Santacroce. Statistical analysis: Palmieri, Di Comite. Administrative, technical, or material support: Pozzessere, Procacci, Di Serio, Schirinzi, De Ruvo, Santacroce. Supervision: Lo Muzio, Testa, Ciavarella, Crincoli, Di Serio, Schirinzi, Santacroce.

### Notes

The authors declare no competing financial interest.

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