Scent & Sensibility

A journey towards recovery in COVID-19 induced olfactory disorders

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The mind that opens to a new idea never returns to its original size
- Albert Einstein

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General Introduction

Introduction

We all recognize those moments: a single scent can bring you back to a distant memory, illustrating vivid pictures of people, places, and nostalgic feelings. From an evolutionary standpoint, olfaction is considered as the oldest and most primitive of the sensory modalities. Olfaction takes up the largest part of mammalian genome, comprising ~3% of all genes [1]. The sense of smell is the first working sensory modality. Even a fetus has the ability to detect and discriminate flavors in the womb. The sense of smell allows newborns to bond with their mothers within hours after birth [2,3]. Despite these fundamental roles, our sense of smell tends to be undervalued and overshadowed by more conspicuous senses. However, smell plays a crucial role in our quality of life and safety. The olfactory function is essential for feeding behavior, social interactions, danger, and psychological conditions [4,5]. Interestingly, in the clinical field as well as in research there was limited attention to this sense [6]. The year 2020 brought an unexpected shift. In March 2020 the WHO declared COVID-19 to be a global pandemic with amongst others, olfactory loss as one of the most common symptoms of the virus [7]. Suddenly, a vast number of individuals were confronted with the consequences of olfactory dysfunction. Healthcare workers were unprepared to face this burden and to provide adequate guidance to these patients [8,9]. This thesis offers a comprehensive insight into the diagnosis, treatment, epidemiology, and progression of smell loss induced by COVID-19, shedding light on this valuable sense that has garnered heightened interest in recent times.

Chemical senses

Smell, taste, and trigeminal sensations form together the chemical senses. The perception of flavor is a combination of the sense of taste and smell, along with trigeminal sensations. The aroma of food plays a crucial role in enhancing and differentiating flavors. When you eat or drink, volatile compounds interact with our olfactory system, adding complexity and nuance to the overall flavor experience. Our taste system is organized through taste receptor cell clusters, forming taste buds on the tongue who can perceive five primary tastes: sweet, sour, bitter, salty and umami. These clusters transmit signals from the tongue to the brain via gustatory sensory neurons of three cranial nerves; the facial nerve, glossopharyngeal nerve and the vagal nerve (CN VII, CN IX, CN X) [10]. Trigeminal sensations are perceived by the cranial trigeminal nerve (CN V), triggered by physical factors and chemicals, leading to feelings of touch, temperature, and pain perceptions [11–13].

Olfactory system

The nose consists of two cavities separated by the septum. Within these cavities, air flows through the nasopharynx before continuing to the lungs for gas exchange. The nose contains three conchae on each side, that guide the airflow with their odorant molecules towards

the olfactory epithelium, at the roof of the nasal cavity. The olfactory epithelium consists of different cell types, responsible for the detection of odors and for their transmission to brain parts where odors are interpreted as smell [14].

Odorant molecules are detected by the cilia of the olfactory sensory neurons (OSNs), also called the olfactory receptor neurons (ORNs), located in the olfactory epithelium. These neurons convert these chemical stimuli into electrical signals and transmit them via the olfactory nerve to the olfactory bulb and then to the olfactory cortex in the brain. The orthonasal pathway involves volatile molecules from the external environment entering through the nose and reaching the olfactory sensory neurons (OSN). The retronasal pathway can be explained by the release of molecules in food during the mastication in the oral cavity, which reach the cilia of the OSN through the oropharynx (Figure 1). This is why flavor perception is so heavily influenced by olfaction [14–18].

The olfactory nerve is the shortest and first cranial nerve (CN I), and along with the optic nerve, does not emerge from the brainstem, but arises from the cerebrum. The olfactory nerve passes from its receptors in the nasal mucosa to the olfactory epithelium directly to the brain, entering the skull through the cribriform plate of the ethmoid bone [14,19–21].

Before odors can be detected, odorant molecules must first dissolve in the mucus secreted by the Bowman's glands in the olfactory epithelium. The axons from the OSNs form the olfactory nerve, and in the olfactory bulb, these axons synapse with mitral cells within structures called glomeruli. From the glomeruli, olfactory information reaches through the olfactory tract to the olfactory cortex. This primary olfactory cortex engages with various cortical and limbic structures, enabling the integration of scent with memory and emotions [14].

Sustentacular cells in the olfactory epithelium serve as structural support for OSNs [19,20], characterized by their microvilli layer that observes the environment. These cells form tight junctions with olfactory sensory neurons, creating a barrier that protects them from harmful substances in the mucus while providing metabolic support. Sustentacular cells are crucial for olfaction, as they stabilize and repair the olfactory epithelium and produce the proteins for the cilia of the OSNs on which odors bind [21].

A unique phenomenon of smell is the ability to regenerate, in contrast with other senses. The olfactory system maintains a dynamic balance between apoptosis (cell death) and neurogenesis (the generation of new neurons). The regeneration of olfaction relies on the basal cells in the olfactory epithelium, which can form new stem cells from which new olfactory cells can develop [22–26].

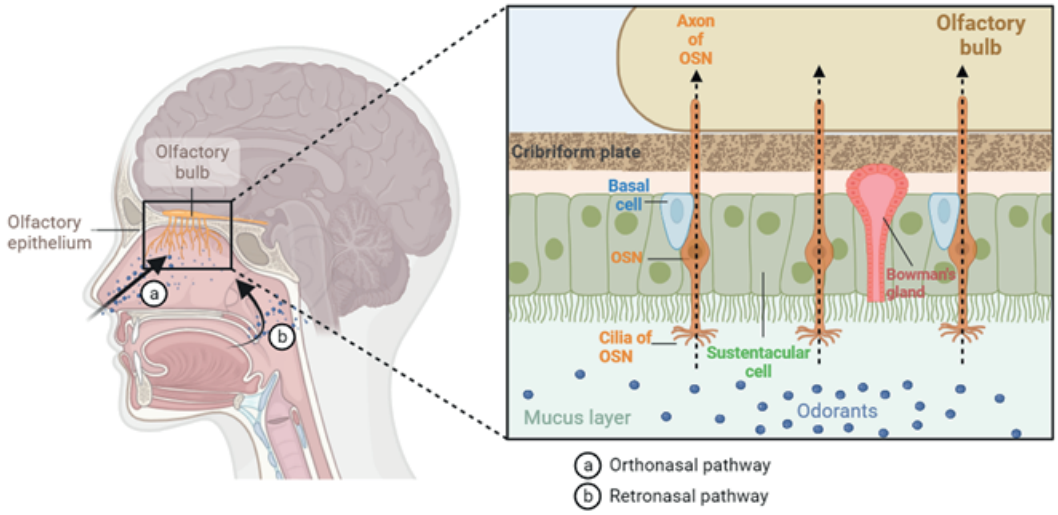

Figure 1. The ortho and retronasal pathway and the olfactory epithelium.

How to smell?

The chemical senses are the only senses that allow us to experience the world around us by perceiving chemicals. All odorants are tiny molecules with different structures, and somehow these different structures are experienced as scents [18,27]. Guanine nucleotide protein (G protein) coupled receptors (GPCRs) in human cell membranes are connected to pathways that transfers external signals such as molecules, peptides or neurotransmitters [28]. GPCRs are the biggest group of proteins in the human genome [29,30]. More than half of all GPCRs are specialized to interact with odor molecules, and they are called odorant-receptors (ORs). In 2004, Buck and Axel won the Nobel prize for Psychology and Medicine, for their research on ORs [27]. Each olfactory neuron expresses a single type of protein receptor. When an odor binds to a receptor protein, a series of steps occur to generate a receptor potential, converting chemical information into electrical signals that the brain can interpret [14,27]. It remains unclear how these odorants are recognized by these ORs. Humans possess around 400 different receptors, which are used in combination, allowing one odorant to bind to several receptors and vice versa (Figure 2) [31] We used to believe that humans could perceive around 10,000 different odors, with 500 of them detectable at low concentrations. However, recent research indicates that this number is significantly higher, possibly exceeding one trillion [32].

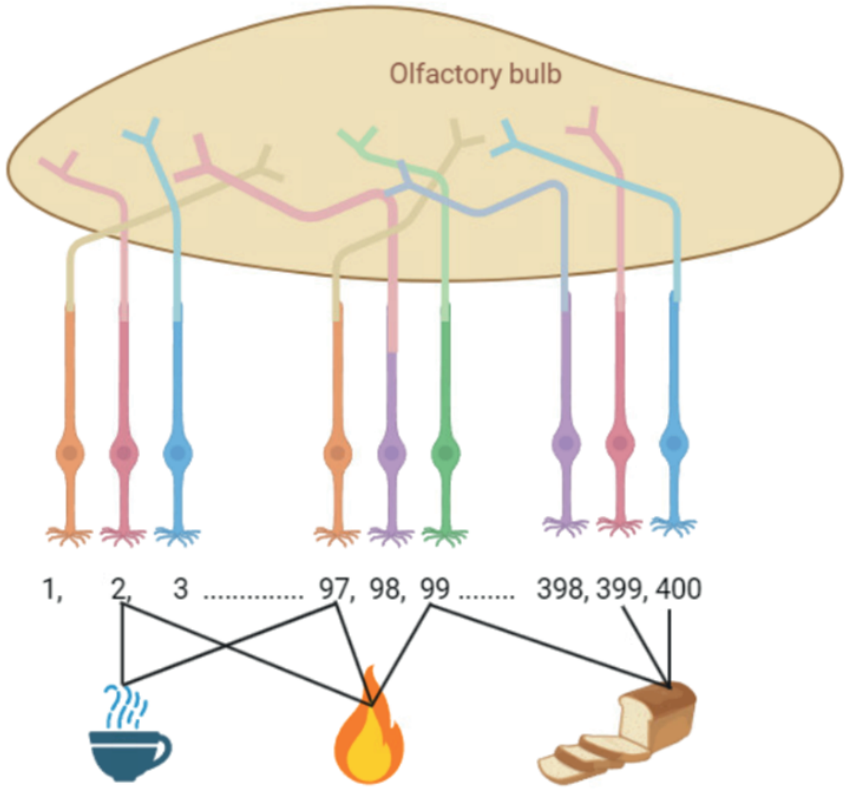

Figure 2. Binding of different odor molecules to OSNs.

Odors, emotion and memory

Memories triggered by the senses, especially through smell and taste, can be incredibly powerful and influential, an occurrence commonly referred to as the Proust Effect [33]. The olfactory cortex is a part of the paleocortex, from an evolutionary point of view the oldest part of the brain, triggering emotions and accounting for memory. The axons of mitral cells form the olfactory tract, projects directly to the olfactory cortex without passing through the thalamus. This is why scents quickly evoke memories and emotions [14,34]. The primary olfactory cortex is located on the base of the frontal lobe and on the inferior surface of the temporal lobe. These regions further project to other areas of the brain (Figure 3). The piriform cortex connects to the thalamus, hippocampus, hypothalamic nuclei, and amygdala, which influence olfactory-guided memory [14,34–36]. The connections between the piriform cortex, hypothalamus, and amygdala influence visceral, appetitive, and sexual behaviors, as well as emotional reactions to odors [10,37].

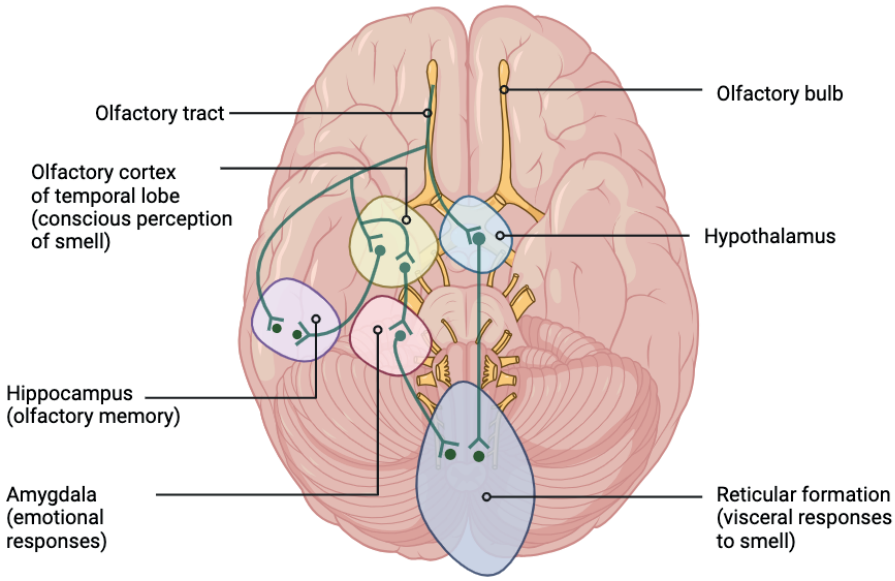

Figure 3. Olfactory pathway in the brain.

Consequences of olfactory dysfunction

Olfactory dysfunction can be categorized in anosmia (total loss of smell), hyposmia (partial loss of smell), phantosmia (olfaction without a source) or parosmia (altered sense of smell).

Impaired olfactory function significantly impacts the daily lives of individuals. The sense of smell plays a crucial role in one's eating habits by influencing flavor perception or appetite. Olfactory disorders can alter eating habits, causing weight changes, but can also reduce enjoyment of food and social events [38–42]. Patients with parosmia, suffering from distorted smell and taste perception, often experience a notable disturbance in their daily lives, as they encounter unfamiliar or unpleasant flavors [43,44]. Impairment in olfaction also affects the ability to detect dangers like smoke, gas, or spoiled food and it can bring difficulties in maintaining personal hygiene [45].

Life consists of multiple experiences decorated by odors that evoke memories and emotion, therefore smell greatly influence overall well-being [38,40]. People describe their loss as if they miss a dimension in their life [46–49]. The sense of smell triggers memories and emotions, and its decrease can lead to challenges in social interaction or in psychological issues, such as depression or isolation [50].

Causes of olfactory dysfunction

Ordinarily, odors find their way to the olfactory epithelium in the nose. However, in conditions like a normal cold, the olfactory mucosa becomes swollen due to inflammation,

preventing odors from reaching the olfactory epithelium. This can also be the case in allergies, rhino(sinusitis) or nasal polyps. Once the swelling subsides, the sense of smell returns as the nerves remain undamaged. This is called conductive olfactory dysfunction [51]. In sensorineural olfactory loss there is impairment of the sense of smell caused by issues in the OSNs, their receptors, or their central projections [52]. This can be the case in neurodegenerative diseases, like Parkinson's or Alzheimer's disease [53–55], but the most prevalent cause is aging [56,57]. Other causes are head trauma [58], smoking [59], viral infections such as COVID-19, malignancies or their treatments such as radiotherapy or chemotherapy, and sometimes the cause remains unknown (idiopathic) [52,54].

Pathophysiology of COVID-19 induced smell loss

Interestingly, nasal obstruction and a swollen olfactory epithelium do not account for the olfactory dysfunction seen in COVID-19 [44,60,61]. The Angiotensin Converting Enzyme (ACE2) receptor, found on the membranes of various human cells, plays, apart from other factors, a crucial role in facilitating the entry of SARS-CoV-2 via the S-protein of the virus [62] (Figure 4). The olfactory epithelium showed to have higher signal intensities of ACE2 receptors, than the respiratory epithelium, suggesting that SARS CoV-2 is prone to affect the olfactory epithelium and thus the smell function [60].

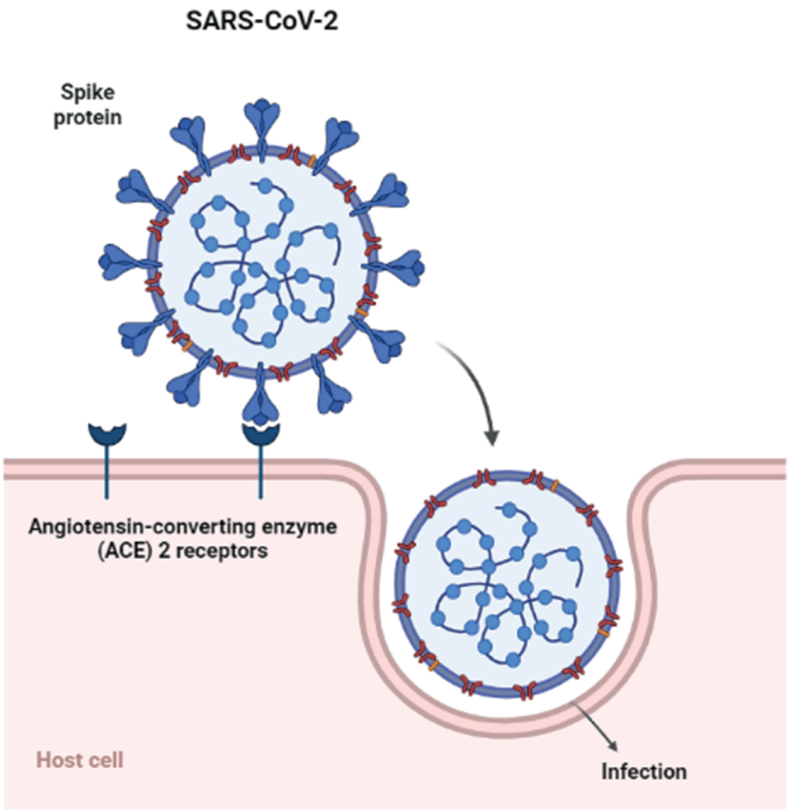

Figure 4. Entry of the SARS-CoV-2 virus via the Spike protein.

An immune response triggers the activation of lymphocytes and macrophages in the olfactory epithelium, leading to the release of cytokines and damage in the area. This inflammatory response is also seen in certain brain areas and in the olfactory bulb [63,64]. However, their role in olfactory disorders is still unclear [65]. ACE2-receptors are thought to have a higher expression in the olfactory epithelium in comparison to the respiratory epithelium and especially in the non-neuronal cells, mainly the sustentacular cells (Figure 5). These cells play a crucial role in assisting OSNs with odor processing and aiding the transduction cascade of the olfactory epithelium. As a result, damage to the sustentacular cells has an indirect impact on the OSNs and the olfactory epithelium, ultimately in olfactory disorders [61,66].

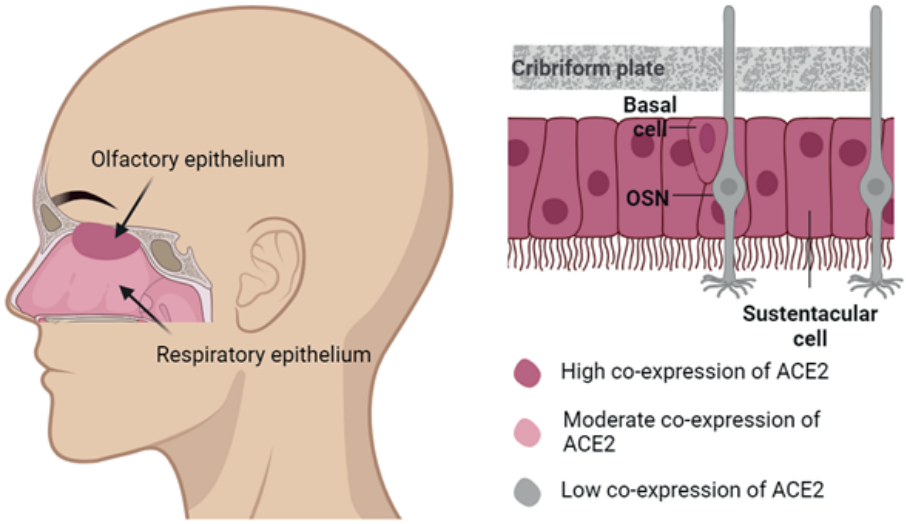

Figure 5. ACE2 expression in the nasal cavity and olfactory epithelium.

Prevalence of olfactory disorders

The COVID-19 pandemic has exposed the world to a large number of patients with smell loss. In 40-50% of COVID-19 cases smell and taste disorders occur [67,68]. Although most patients with COVID-19-related smell or taste loss recover within days or weeks, 5% of patients suffer from persisting symptoms [69,70]. Based on evolving insights, new SARS-CoV-2 variants, tend to have a lower occurrence of smell loss [71,72] and cases recover more quickly compared to the early stages of the pandemic [73]. However, prevalence still remains around 30% of cases [72]. In 2023, worldwide more than 700 million cases of confirmed COVID-19 have been reported, suggesting up to 350 million cases with smell or taste loss of which 17.5 million patients with symptoms longer than 6 months [74,75]. In COVID-19 cases, smell and taste disorders are among the most common symptoms experienced, which shed light to the importance of these senses [76]. Changes or (partly) loss in the sense of smell or taste were added to the clinical screening profile for COVID-19 and to the list of official persisting symptoms after COVID-19 by the Center for Disease Control and Prevention and the World Health Organization [7]. The prevalence of non-COVID-19 related smell disorders is estimated between 1% and 3% [57,77,78], primarily caused by sinonasal issues, post-infectious, or post-traumatic conditions, aging, and neurodegenerative diseases [44,57,76,77,79–81]. Around one-third of the general 65-year-old population experiences impaired smell function, with more than one-fourth experiencing impaired taste function [56]. However, research on (COVID-19-induced) smell disorders faces challenges due to heterogeneity in studies stemming from variations in populations, reporting systems, healthcare setups, diagnostic and measurement methods of olfaction [82]. The prevalence of smell disorders in both COVID-19 and non-COVID-19

related cases is expected to rise due to changing demographics and heightened environmental influences [83,84]. Healthcare systems need to be prepared to offer assistance to these patients, as it has substantial influence on their health and well-being [79] and because they frequently report feelings of isolation when their symptoms are not recognized by medical professionals [8].

Clinical assessment of olfactory disorders

Clinical assessment of olfactory function is crucial, not only for clinical diagnosis and understanding the underlying mechanisms of olfactory dysfunction in COVID-19 patients, but it also enables healthcare professionals to provide patients with objective measurements of symptom severity, and guidance throughout their recovery. Preferably, olfaction should also be assessed through psychophysical tests, as self-reported olfaction has limited correlation with objective measures [85–90]. However employing validated questionnaires for remote or time-saving assessment could provide as an alternative.

The most utilized psychophysical test in Europe is the well-established and validated Sniffin' Sticks Test® (SST) [91,92]. This test involves pen-like devices filled with odorants to assess the lowest concentration of a scent that can be discerned, the ability to identify odors and the ability to discriminate odors [93]. The SST, offers a comprehensive evaluation of olfactory function, but it is time-consuming (30-60 minutes) and demands patient concentration [89,94]. To address the need for a quicker olfactory test in clinical routine, the SST-12, a screening version of SST, has been developed [95]. It assesses the identification of 12 odors and takes just 5 minutes. The SST-12 serves as a diagnostic tool for screening olfaction in causes unrelated to COVID-19 [95–97]. In this thesis we will explore its diagnostic accuracy in detecting smell loss when induced by COVID-19, as the pathophysiology of smell loss varies between COVID-19 and other causes [98,99].

Intervention and prognosis

COVID-19 induced smell loss emerged as a novel disease, and as such, there was no established effective treatment. Given that persistent loss of smell following COVID-19 is believed to result from an inflammatory response, corticosteroids have been considered as a potential treatment option [100]. Some studies have examined the use of corticosteroids in nasal sprays, but no significant benefits were observed [101–103]. In a few small studies, patients who received a short oral prednisolone treatment reported an improvement in their sense of smell [104,105]. However, these studies carry a low level of evidence due to their limited sample size, short follow-up periods, and non-blinded study designs. The uncertainty surrounding the available evidence has led to a lack of consensus on treatment approaches. Therefore, the debate continues regarding whether or not to prescribe steroids for COVID-19-induced loss of smell [106].

For other post-viral sensorineural olfactory losses (e.g., caused by influenza or herpes),

olfactory training has been a suggested effective treatment [107–113]. During olfactory training, a patient consciously smells a set of four familiar odors twice a day for a period of 3 months. It has been shown to promote the regeneration of the number and activity of ORNs through repetitive odor exposure and has therefore been proposed as a potential treatment option for COVID-19 induced olfactory disorders as well [114,115]. If olfactory training truly speed up and increase the extent of smell recovery in COVID-19 induced smell disorders is still under debate [46,111,116–119].

The challenge of treating olfactory disorders persists, given the limited availability of evidence-based solutions [44,120,121]. These limited treatment options available are in contrast with the severity of symptoms experienced by patients. Counseling these individuals is challenging, primarily due to the insufficient knowledge regarding the disease prognosis. This is because of a notable scarcity of studies incorporating longitudinal data and the use of objective measurements [122,123]. Therefore, it is crucial to gain more insight in the clinical course and treatment options with well conducted study designs [8].

Aim and outline of the thesis

In this introduction, we underscored the high incidence of olfactory disorders in COVID-19 cases and their substantial impact on patients' quality of life. Interestingly, there is little known about its clinical assessment, treatment options and clinical course. This thesis focusses on the following research questions:

I) Is it possible to detect olfactory loss caused by COVID-19, using a screening olfactory test?

II) Is there an effective treatment for COVID-19 induced olfactory disorders?

III) What is the incidence, severity, and course of COVID-19 induced olfactory disorders, and how do they compare to olfactory disorders unrelated to COVID-19?

In clinical practice the golden standard for psychophysical measurement of olfaction is time-consuming. Therefore, we aimed in **Chapter 2** to assess whether a screenings test could offer comparable diagnostic accuracy to the golden standard, thus potentially shortening the assessment time in clinical settings.

Considering the potential role of inflammation as the main cause of COVID-19-induced smell loss, we sought the effect of prednisolone for COVID-19 induced smell disorders. To obtain the highest level of evidence, we conducted a Randomized Controlled Trial (RCT) to answer this research question. In this thesis we provide the protocol in **Chapter 3** and its outcomes in **Chapter 4**.

The limited treatment options for COVID-19 induced smell loss forced us to conduct a case-control study in order to evaluate the effect of olfactory training, as shown in in **Chapter 5**. We aimed to investigate the clinical course of smell loss by objectively assessing olfaction one year after COVID-19 in **Chapter 6**. Additionally, we compared the incidence,

course and severity of COVID-19 induced smell loss versus non-COVID-19 related causes for smell loss in **Chapter 7** by performing a longitudinal cohort study.

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Part I

Diagnostics

2

Diagnostic accuracy of the screenings Sniffin' Sticks Test (SST-12) in COVID-19 induced olfactory disorders.

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Abstract

Objective olfactory function can be assessed using validated olfactory tests like the Sniffin' Sticks Test (SST). However, their extensive nature makes them less suitable for clinical practice. To address this, shorter olfactory tests like the screenings Sniffin' Sticks Test (SST-12) can be used for screening purposes and reduce testing time. The SST-12 serves as a diagnostic tool for screening olfaction in cases unrelated to COVID-19. However, these screening tests are uncertain regarding their accuracy in detecting olfactory dysfunction in patients with COVID-19 as the plausible cause. We aim to determine the diagnostic accuracy of the SST-12 in adults with post-COVID-19 olfactory dysfunction.

We performed a diagnostic accuracy study with data from 113 consecutive COVID-19 diagnosed patients who experienced objectified smell loss ever since. At approximately 6 months after their diagnosis, all participants underwent the SST (reference standard). A part of the SST is the SST-12 (index test). Diagnostic accuracy of the SST-12 is measured as negative predictive value (NPV), positive predictive value (PPV), sensitivity, and specificity. The SST-12 detected smell loss in 85 patients among 91 patients with smell loss and ruled out smell loss in 15 patients among the 22 patients without smell loss based on the reference standard. Making sensitivity 93.4% (CI 0.87-0.97), and specificity 68.2% (CI 0.48-0.85). Out of the 92 patients with a positive test result on SST-12, 85 patients had indeed smell loss (PPV 92.4% CI 0.86-0.97), and out of the 21 patients with a negative test result, 15 patients had no smell loss, regarding the reference standard (NPV 71.4% CI 0.50-0.88).

The findings suggest that the SST-12 holds promise as a useful tool for identifying individuals with smell loss, also in individuals with COVID-19 as cause, but it is important to have a good understanding of the interpretation of the results of the SST-12 when considering its implementation in clinical practice.

Introduction

Olfactory dysfunction has emerged as a common symptom in COVID-19. Persistent symptoms can result in a decline of quality of life, affecting nutritional, physical well-being and cognitive functioning [1–4]. Thus, assessing olfactory function accurately and efficiently is essential. Not only for clinical diagnosis and understanding the underlying mechanisms of olfactory dysfunction in COVID-19 patients, but it also enables healthcare professionals to provide patients with objective measurements of symptom severity, and guidance throughout their recovery.

Merely 10% of ENT-surgeons utilizes psychophysical tests for the evaluation of olfaction [5]. Most ENT-surgeons utilize subjective questionnaires, but this approach often yields inconsistent results and potentially leads to underestimation of the extent of the problem [5]. The most utilized psychophysical test in Europe is the validated Sniffin' Sticks Test® (SST) [5,6]. The SST provides a comprehensive evaluation of olfactory function, including the ability to identify specific odors, discriminate between different odors, and detect odor thresholds. These type of extended tests are the gold standard for diagnosing olfactory disorders [5,7]. However, the time-consuming nature of the SST (around 30 to 60 minutes) [8,9] and the need for sustained concentration from patients make it less suitable for routine clinical practice [10].

In response to the need for more time-efficient olfactory tests, a screening version of the SST, known as the SST-12, has been developed [11]. The SST-12 serves as a diagnostic tool for screening olfaction in causes unrelated to COVID-19. As only 12 scents have to be identified, the test can be done in 5 minutes [12]. The SST-12 focuses solely on the identification subdomain, providing a quick assessment of an individual's ability to identify twelve odors [13–15]. As the pathophysiology of smell loss varies between COVID-19 and other causes, particularly impacting the threshold domain [16,17], we explored the SST-12's ability to also detect smell loss in patients with COVID-19 as the plausible cause [13].

Methods

Patients and procedures

In order to assess the diagnostic accuracy of the SST-12, we included 113 consecutive patients. The cohort of participants included in this study originated from the COCOS trial [18]. This was a randomized controlled trial determining the possible benefit of an oral prednisolone treatment (10 days 40mg) on the olfactory function in patients with COVID-19 induced smell disorders. The institutional Review Board of the University Medical Center Utrecht approved the research protocol (21-635/G-D, October 2021). We obtained from all patients written informed consent in order to participate. Patients were recruited via the Dutch media and via the National patients association, between November 2021 and January 2022. Patients approached us by email and consecutive eligible patients were planned for inclusion by telephone. Inclusion criteria were adult patients with good understanding of the Dutch language, with a PCR confirmed COVID-19 diagnosis within 12 weeks before first measurement, and at least 4 weeks of smell loss since their diagnosis. Patients visit the outpatient clinic for Ear, Nose and Throat at baseline for the assessment of the SST and thereby objectify their smell loss. They were excluded when we objectified no smell loss at baseline with SST (TDI score >30.5), or when we found other causes for smell loss objectified by a nasendoscopy, such as nasal polyps or (rhino)sinusitis (Figure 1), or pre-existing smell loss before the COVID-19 diagnosis. These aforementioned criteria make loss of smell due to COVID-19 the most plausible cause. All patients signed informed consent in order to participate. They all performed the SST again at approximately 6 months after their diagnosis (Figure 1) [19]. Results showed no difference in olfactory function between patients who received prednisolone and those who received placebo [20]. More information about the inclusion- and exclusion criteria and the study procedures of the RCT are described elsewhere [19,20]. In our analysis, we utilized the results of the SST during the visit approximately 6 months after diagnosis. The reason behind selecting this specific time point is that predictive values are influenced by the prevalence of the disease [21]. By choosing the second visit, we aimed to achieve a well-balanced representation of smell loss prevalence [22,23].

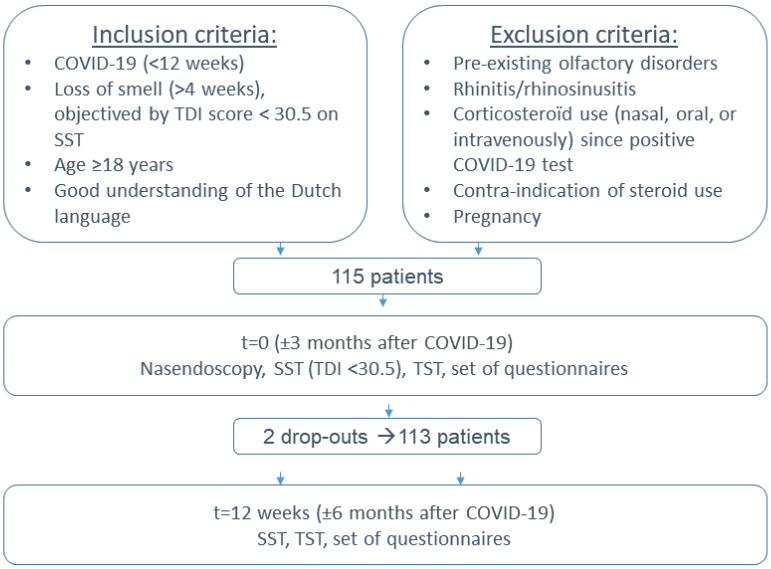

Figure 1. Procedures and participant flow-chart. TDI score; Threshold-Discrimination-Identification score; SST; Sniffin' Sticks Test; TST; Taste Strip Test.

Index Tests and reference standard

The reference standard is the extended version of the Sniffin' Sticks test® (SST) [24]. The Index test is the SST-12. These Sniffin' Sticks are manufactured by Burghart, a company with medical certification (ISO 13485). This certification ensures that both the odorants and their solvent used in the test are safe for health, providing confidence in the test's reliability. We used for both tests the translated version. The SST consists of three subdomains, one of which is the identification test. In the identification subdomain of the SST, patients are presented with 16 different odors and are required to identify the corresponding scent from a set of four options. Out of these 16 odors, 12 are included in the SST-12. So, the moment of assessing the reference standard and index test was at exactly the same moment, ensuring that there was no access to outcome information from one test when assessing the other.

Statistical analysis

In the sample size calculation, we assumed that more than 50% of the participants would experience a loss of smell. By setting a diagnostic test power of 0.95, a delta of 0.1, a statistical power of 0.8, and a significance level of 0.05, a total of 68 patients was required. All analyses were done in IBM SPSS Statistics 27.0. The reference standard (SST) was used as recommended in the development paper [25], where normosmia is defined as a TDI score >30.5 [26]. Thresholds for the SST-12 were defined as a normosmia (SST-12 ≥ 11). a

hyposmia (10 > SST-12 > 6), or an anosmia (SST-12 ≤ 6) [13] In this analysis, we used only the threshold values for hyposmia and normosmia to determine whether a patient possesses a normal sense of smell. We constructed a 2 by 2 table to determine the accuracy and calculated the negative predictive value, positive predictive value, sensitivity and specificity with their confidence intervals. We reported the results according to the STARD guidelines [27].

Outcome measurements

Negative predictive value (NPV)

The NPV is the probability of not having the condition when the test result is negative. This can be calculated by dividing the number of true negative results (TN) by the total number of negative test results. The NPV is important when the aim is to avoid missing cases of smell loss, although there is a change of false-positive (FP) cases [21]. In our study this is an important outcome, in order to provide guidance to affected patients and because no harmful follow-up diagnostics or treatments are available for false-positive cases. Moreover, the test targets only individuals who self-report smell loss suspicion, minimizing unnecessary concern which can be the case in random screening.

Positive predictive value (PPV)

The PPV is the probability of having the condition when the test result is positive. This can be calculated by dividing the number of true positive results (TP) by the total number of positive test results. The PPV is particularly relevant when the follow-up diagnostic or treatment procedures may have potential harm, costs or other forms of impact [21].

Sensitivity

Sensitivity is the measure of a test's accuracy in medical diagnostics. It represents the percentage of true positive test results (TP) among all diseased individuals. The higher the sensitivity, the greater the likelihood that someone who truly has the disease will receive a positive test result, which is useful when ruling out a disease is desirable. With high sensitivity, there will be fewer false-negative (FN) test results. The sensitivity is calculated using the following formula (TP/(TP+FN) [21].

Specificity

Specificity is the measure of true negative test results (TN) among non-diseased individuals. The higher the specificity, the greater the likelihood that someone who does not have the disease will receive a negative test result, which is useful when confirm a disease is desirable. With high specificity, there will be fewer false-positive (FP) test results. The specificity is calculated using the following formula (TN/(TN+FP)) [21].

Results

Table 1 describes the characteristics and outcome measurements at the moment of performing the SST. Median time in days between conformed COVID-19 and the utilized tests for this analysis is 140 (IQR 128-154). Median TDI score on SST was 27.5 (IQR 23.63-30.0).

	N=113
Age, years	50 (40.5-57)
Sex	
Female	72 (63.7)
Male	41 (36.3)
Time between confirmed COVID-19 test and test performing, days	140 (128-154)
Sniffin' Stick Test (SST)	
TDI score	27.5 (23.63-30.0)
Threshold	4.5 (3.3– 5.6)
Discrimination	11.0 (10.0-13.0)
Identification	11.0 (10.0-13.0)

Table 1. Characteristics and outcome measurements at the moment of performing the SST. Data are presented as median (IQR) or n (%).

Table 2 presents the outcomes of the 2 by 2 table analysis. Sensitivity was 0.934 (CI 0.87-0.97). Among the 91 individuals with smell loss regarding to the reference standard, 85 (93.4%) participants were detected with a positive test result on the SST-12. Specificity was 0.682 (CI 0.48-0.85). Among the 22 individuals without smell loss, 15 (68.2%) participants were ruled out to have smell loss. The PPV of the SST-12 was calculated as 0.924 (CI 0.86-0.97). Among the 92 individuals with a positive test result, 85 (92.4%) participants did have smell loss based on the reference standard. The NPV of the SST-12 was calculated as 0.714 (CI 0.50-0.88). Among the 21 individuals with a negative test result, 15 (71.4%) participants did not have smell loss based on the reference standard. (Table 2)

	Reference standard		
	Smell loss	No smell loss	Total
Index test			
SST-12 positive	85	7	92
SST-12 negative	6	15	21
Total	91	22	113

Table 2. Cross-tabulation.

Discussion

The aim of this study was to identify the diagnostic accuracy of the SST-12 for COVID-19 induced loss of smell. We found a high PPV 92.4% (CI 0.86-0.97) and sensitivity 93.4% (CI 0.87-0.97), and an acceptable NPV 71.4% (CI 0.50-0.88) and specificity 68.2% (CI 0.48-0.85). These findings were achieved by a comprehensive cohort design which included a large sample size of consecutive patients enrolled within a concise period. All patients had a confirmed COVID-19 diagnosis by PCR in the same timeframe since performing the SST. Besides we used a predetermined threshold, which contributes in the validity and generatability of the results. To the best of our knowledge, Vandersteen et al. performed the only study that included patients with post COVID-19 olfactory dysfunction to investigate the diagnostic accuracy of the SST-12, but their small sample size and wide confidence intervals raise uncertainty about the applicability of their results [12]. Hummel et al investigated the diagnostic accuracy of the SST-12 and of the Q-sticks (a three-odor test), but in non-COVID-19 related cases [13,28]. Our results, however are comparable to theirs.

It is important to note that as we followed the typical sequence for all subdomains, from threshold to discrimination and identification, some patients may have experienced decreased concentration during the final identification test. In the SST-12, patients only perform the identification part. While this may yield different results compared to the extended SST's identification section, as all patients underwent this procedure, we do not expect it to significantly impact our findings.

The high sensitivity in our study indicates that among patients with smell loss, a high number of patients will indeed receive a positive test result, resulting in few false negatives. If the SST-12 had been used in this study, six patients (6.6%) would have received a false-negative test result. The high sensitivity value suggests that the SST-12 is effective in correctly identifying individuals with smell loss and minimizing false-negative test outcomes. However, in the study of Sorokowska et al. there was a high number of false-negatives, but this contradiction in comparison with our study can be found in the fact that they used the Q-sticks, and because they did not use the SST as reference standard [6].

We found a moderate specificity, indicating that the SST-12 is amendable for obtaining false positives. In this study, if the SST-12 had been used instead of the SST, seven patients (32%) would have received a false-positive test result. Though, the interpretation and usefulness of any diagnostic test is dependent on the setting in which it is used. Most smell tests will be used in clinical settings, in patients in which more objective knowledge about their ability to smell can be crucial for provide guidance throughout their recovery trajectory. The consequences of the high sensitivity in combination with the relatively low specificity is a possibility of detecting a relative high number of false positives. There are however no harmful or expensive follow-up diagnostic tools or unnecessary treatment options for smell loss when patients test false-positive.

The moderate NPV we found, suggests that there is a possibility of missing the diagnosis. This could be attributed to the fact that the SST-12 only assesses the identification ability, while in COVID-19 patients the threshold domain seems most affected [16,17]. The reason for this is the fact that of the olfactory threshold assessment primarily relies on the peripheral olfactory system, specifically the olfactory epithelium, which is the part most accessible to the SARS-CoV-2, while the identification and discrimination components are more closely associated with higher-level cognitive processes [29,30]. These patients may perform well on the SST-12, but still have COVID-19-induced loss of smell, which can only be assessed by the SST. Since COVID-19 induced smell disorders can have a significant impact on individual's quality of life, it is advisable to combine the SST-12 with other clinical information to make an accurate diagnosis. In cases where the disease is suspected, even if the SST-12 is negative, additional testing may be necessary. Alternatively, healthcare workers can still provide guidance and support to help patients manage their complaints. The high PPV we found in combination with the high sensitivity makes the SST-12 especially helpful for identifying smell loss.

Considering the diagnostic accuracy of the SST-12, it has the potential to aid in early detection and in monitoring disease progression. In general practice the SST-12 will mostly be used for counseling, and to follow the trajectory of the smell function since patients cannot objectify the vague improvement of their smell themselves. Understanding the limitations and potential false results of the test is relevant for managing patient expectations and ensuring appropriate counseling.

Conclusion

Our findings suggest that the SST-12 holds promise as a screenings tool in identifying smell loss, also in patients with COVID-19 as the most plausible cause.

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Part II

Treatment & Therapy

3

COCOS-trial: Corticosteroids for COVID-19 induced loss of Smell. Protocol for a single-centered, double-blind, randomized, placebo-controlled trial.

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Abstract

Introduction

Hyposmia and anosmia are common in COVID-19. Most patients regain normal smell within 4 weeks, but severe loss of smell persists roughly in 20% after two months and may last up to a year or longer. These persistent smell disorders greatly influence daily life. It is hypothesized that COVID-19 induces inflammation around the olfactory nerve and in the olfactory pathway, leading to smell disorders. Corticosteroids might reduce this local inflammatory response and improve smell.

Methods and analysis

We will conduct a single-centered, randomized, placebo-controlled trial to determine the efficacy of a short-high dose treatment of oral prednisolone for persistent loss of smell after COVID-19 in the early phase. We will include 116 patients with persistent (>4 weeks) loss of smell within 12 weeks of COVID-19 diagnosis, based on a positive PCR/antigen test. One group receives 40mg of prednisolone for ten days and the other group receives matching placebo treatment. In addition, all patients will perform smell training for 12 weeks. The primary outcome is objective olfactory function measured by means of Sniffin' Sticks Test (SST). Secondary outcomes are objective gustatory function by means of Taste Strip Test (TST) and subjective taste and smell ability, trigeminal sensations, quality of life, and nasal symptoms, measured by 3 questionnaires. These outcomes will be measured at inclusion before treatment and 12 weeks later.

Ethics and dissemination

The institutional Review Board of the University Medical Center Utrecht approved the research protocol (21-635/G-D, October 2021).

Strengths and limitations

- This is a double-blinded randomized controlled trial with large sample size to allow for a comparison between prednisolone and olfactory training or only olfactory training in patients with smell loss after COVID-19.
- We use objective measurements for the primary outcome and for one secondary outcome; smell- and taste function.
- Multiple outcome measurements besides objective smell- and taste function will be assessed, such as quality of life and nasal symptoms.
- To consider the effect in the long term, an extra follow-up measurement after 6-12 months could be considered.
- The Questionnaire of Olfactory Disorders had not been validated in Dutch patients with smell loss after COVID-19.

Background

Partial or complete loss of smell ability, respectively hyposmia and anosmia, are common early features in COVID-19 [1,2], which occurs in about 2 in every 3 patients [3,4]. Though the vast majority of patients recovers within 4 weeks, severe loss of smell persists roughly in one of five patients after two months [5]. Reduced ability to smell, hyposmia, persists in 10-46% after 6 months [6-8] and can last up to a year or longer with 7-9% being functionally anosmatic [9,10]. Beyond smell loss, patients also report taste disorders and smell alterations, starting after a period of absent smell [11,12]. In parosmia (a distorted sense of smell), odors are perceived different than usual, or phantosmia, odors can be perceived without odor source. Persistent olfactory disorders are associated with a significant reduction in patients' quality of life, including increased depressive symptoms and nutritional issues [13].

There is no definitive answer yet to the pathophysiology of olfaction disorders during and after COVID-19. In common cold viruses, the loss of smell is typically due to swelling of the nasal mucosa. However, swelling of the nasal mucosa is not observed in SARS-CoV-2 infections [1,14,15]. It is hypothesized that the SARS-CoV-2 causes loss of smell by entering the supporting neural cells in the olfactory epithelium through the ACE2 receptor [16]. In response, a rapid autoimmune response activates lymphocytes and macrophages, and causes release of cytokines. This auto-immune response can differ greatly between patients and may explain the variation in long-term olfactory disorders [14,17]. This inflammatory response during COVID-19 is also seen in certain brain areas, as the olfactory pathway [18,19].

There is no scientifically proven treatment for post COVID hyposmia or anosmia yet. In other post-viral loss of smell, involving the olfactory bulb, smell therapy is to date the only proven beneficial treatment to improve olfactory function [20,21]. During smell therapy, a patient sniffs every day a set of known odors over a period of three months. Consistent training will speed up and increase the extent of smell recovery [20, 21]. Smell therapy is now advised to all patients with persistent loss of smell after COVID-19 [22], however, effects seem limiting on its own.

In diseases with nerve function loss due to inflammatory response, such as sudden sensorineural hearing loss or Bell's palsy, a short course of high-dose oral corticosteroids is prescribed [23,24]. In early stages of these diseases, the auto-inflammatory effects are reversible. Oral steroids have recently been given to patients who suffer from anosmia post SARS-CoV-2 infection with promising effects: two randomized controlled trials included patients with persistent anosmia one month after COVID-19, showed that higher number of patients regained function after corticosteroid treatment, compared to the control group

[25-27]. Despite the limited number of cases included, short follow-up, and the non-blinded trial design, clinical effects seem promising.

If treatment with prednisolone in combination with smell therapy is efficient, a long-term disability can be prevented. Therefore, we propose to investigate the efficacy of oral corticosteroid treatment in combination with smell therapy in a, single-centered, double-blinded, placebo-controlled randomized trial.

Methods and analysis

Study design and setting

The study is a single-centered, randomized, placebo-controlled clinical trial performed at the Otorhinolaryngology department of the University Medical Center Utrecht (UMCU) in the Netherlands. Patients will be randomly assigned to one of the two groups: 10 days prednisolone or placebo. In addition, all patients will perform smell training for 12 weeks. At first visit, before start of therapy, patients olfactory and gustatory function will be tested by the Sniffin' Sticks Test (SST) and Taste Strips Test (TST). Subjective measurements consist of questionnaires. After ten days treatment, all patients are called by the investigator to check treatment compliance and if they had any side effects. After 12 weeks, evaluation will take place by means of SST, TST and related questionnaires (Figure 1).

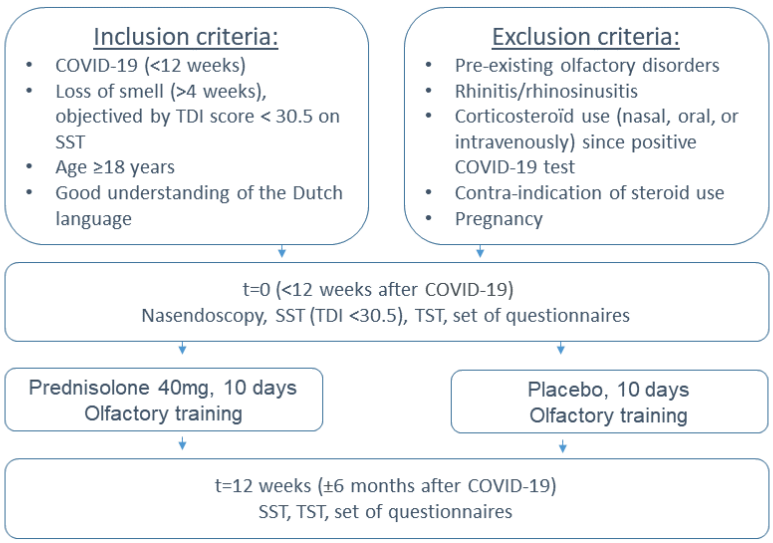

Figure 1. Study design. TDI score; Threshold-Discrimination-Identification score; SST; Sniffin' Sticks; TST; Test Taste Strip Test.

Patient involvement

The national patients association was involved in the conduct of the study, applying for the funding, and in recruiting patients. Patients who participate in this trial, who prefer, will be informed of the results.

Study Objectives

The primary objective of this study is to determine the efficacy of a short high-dose treatment of oral prednisolone for persistent loss of smell after COVID-19. This will be measured with objective Sniffin' Stick Tests. Secondary objectives are to investigate the efficacy of prednisolone on objective gustatory function measured with the objective Taste Strip Tests and on subjective olfactory, gustatory and trigeminal function, impact of smell/taste changes on quality of life, and nasal symptoms by additional questionnaires.

Study population

116 patients will be included (>18 years old) with persistent (>4 weeks) smell loss within 12 weeks after COVID-19 diagnosis based on a positive test (PCR or antigen). For recruitment we will collaborate with public health services, otorhinolaryngology clinics, and the local patient organisation. Inclusions are expected to take a maximum of 18 months, depending mainly on infection numbers in the Netherlands. Inclusions started at the 16th of November until the 10th of February. Follow-up outcomes were measured between the 2th of February and the 10th of May. Patients need to meet the following criteria to participate;

Inclusion Criteria

- Recently diagnosed with COVID-19 (<12 weeks), confirmed with a positive (PCR or antigen by GGD).
- Persistent loss of smell (>4 weeks), objectified by TDI < 30.5 on SST.
- Age 18 years, or older, and capable of giving informed consent.
- Good understanding of the Dutch language.

Exclusion Criteria

- Pre-existing olfactory disorders.
- Chronic rhinitis or rhinosinusitis (with or without nasal polyps).
- Corticosteroid use (nasal, oral or intravenously) since positive COVID test.
- Pregnancy.
- Contra-indications of steroid use, which contains the following:
  - Diabetes mellitus for which drugs (either subcutaneously or orally) are used
  - Stomach ulcers/stomach bleeding
  - Psychoses
  - Ongoing oncological disease

Sample Size

Sample size was calculated based on means and standard deviations of an earlier pilot study [26]. With a power of 0.90, an alpha of 0.05, and a mean difference of 5.5 (sd 8.0) on SST-scores, total sample size is 92. To correct for possible non-parametric testing, the sample size is increased with 15%. As the study is limited in time and effort for the patient, a maximum of 10% dropout is expected. This gives a total sample size of 116 patients, with 58 in every group.

Randomization, blinding and treatment allocation

Patients will be randomly allocated to one of the two groups. The pharmacy in charge of preparation of treatment and placebo medication made a block randomisation sequence list, on which the patient subject number is linked to the study medication number. This pharmacy is a Dutch state-of-the-art Good Manufacturing Practice (GMP) compounding pharmacy independent from our department. To minimise seasonal effects between groups, randomisation occurs in blocks of 4 patients. Both groups carry the same weight.

Patients, physicians and outcome assessors are blinded for treatment allocation.
Only after finishing all analyses, the blinding of researchers and patients to the treatment allocation will be broken. If deblinding is necessary because of medical reasons this can be done by the clinical drug research pharmacy at any time.

Intervention

Group A will be treated with capsules of 40mg of prednisolone, once a day for ten days. Group B will receive capsules placebo medication, once a day, for ten days. Patients in both treatment groups will perform 12 weeks of olfactory training. In this training patients sniff out four odours (rose, lemon, eucalyptus, clove) twice a day [20]. Training compliance will be monitored by crossing off a daily schedule. Patients receive study medication and olfactory training kits at first visit.

Outcomes to be measured

At inclusion, demographic data, such as gender, age, and native language, will be collected. Medical status contains medication use, medical history, date of COVID-19 infection, date of smell loss, and vaccination status. Outcome measurements will be collected at the first and second visit to the outpatient department. At the first visit a nasendoscopy will be conducted, to eliminate other causes for loss of smell. For the primary outcome, SST is performed during this visit and can possibly still lead to exclusion when a TDI-score >30.5 is measured. Secondary outcomes will be assessed by the TST and questionnaires. Besides, the patients receives three questionnaires to fill in: the validated Sino-Nasal Outcome Test -22 questionnaire (SNOT-22) [1], the self-reported smell, taste, parosmia, trigeminal

sensations questionnaire by means of a Visual Analogue Scale (VAS) [28], and the translated Questionnaire of Olfactory Disorders (QoD) [29,30]. All patients will perform 12 weeks of smell training. 12 weeks after start therapy, the SST, the TST, and the same questionnaires will be administered in order to compare outomes. Both primary and secondary outcomes will be registered in an electronic Case Report Form (eCRF), the endorsed system Castor EDC.

Explanation of examinations

Olfactory function will be assessed with the SST, a widely used and well validated test that is commercially available [31]. The SST is produced by Burghart, a medically certified company (ISO 13485), indicating the odorants and their solvent pose no health risks. This test battery examines nasal chemosensory performance using pen-like odour devices filled with odorants and/or solvent. The test consists of three parts: a detection threshold (THR), a discrimination test (DIS), and an identification test (ID). The TDI-score is the sum of these three components, and ranges from 1-48. The higher the score, the better the smell function.

The THR will be measured with a standard series of pens with different concentrations of n-butanol. With a staircase procedure, three pens will be presented to participants in a randomized order. Of these pens, one contains the odour and two contain solvent. Participants have to indicate which pen contains the odorant. To measure DIS ability, 16 triplets of three odorants will be presented. The triplet contains two pens with the same odour and one with a different odour. Participants have to discriminate which pen smells differently. During the ID test, 16 pens with common odours will be presented. Participants have to choose the correct description form a list of four descriptors for each pen.

The TST is a validated test which uses filter-paper taste strips impregnated with different concentrations of the basic tastes sweet, salt, bitter, and sour [32]. The filter papers are impregnated with four concentrations of sweet (0.05, 0.1, 0.2, or 0.4 g/ml sucrose), salt (0.016, 0.04, 0.1 or 0.25 g/ml sodium chloride), sour (0.05, 0.09, 0.165 or 0.3 g/ml citric acid) or bitter (0.0004, 0.0009, 0.0024 or 0.006 g/ml quinine hydrochloride) taste. After placing a paper on the tongue, patients are asked to identify the taste with five possible answers (sweet, sour, salty, bitter or tasteless). Taste strips were presented in a semi-randomized forced choice procedure. Total taste score range from 0-16 since scores for each taste range from 0-4. High scores indicate a better taste function.

Explanation of questionnaires

- Sinonasal Outcome Test (SNOT-22): this questionnaire consist of 22 questions about nasal symptoms and health related quality of life. Patients need to score these symptoms on a 5 point Likert scale, higher scores implicates worse symptoms [28]. In patients with chronic rhinosinusitis, an improvement of 8.9 points after surgery is considered as a clinically

relevant difference [33].
- Self-reported smell, taste, parosmia, and trigeminal sensations by means of Visual Analogue Scores (VAS): this questionnaire subjectively measures olfactory, gustatory, and trigeminal function. Subjects will fill out a brief questionnaire on a 100 unit visual analogue scale, with questions pertaining to their current ability to smell, taste, and perceive trigeminal sensations [1]. Recovery is considered as an improvement of at least 80% of their pre-illness function.
- Questionnaire of Olfactory Disorders (QoD): to asses olfactory-specific quality of life, the translated QoD of the English validated version will be used. For the first 24 questions, answers are ranked by 4 options: agree, partly agree, disagree partly, disagree. Two questions require a yes or no answer and nine questions are answered using a 10 point Likert scale [29,30].

Statistical analyses

All statistical analyses will be performed using IBM SPSS Statistics 27.0 software and R statistical computing. A test for normality will be used to assess whether variables are normally distributed. We expect limiting missing data. Potentially missing data will be handled with multiple imputation, if the assumption for multiple imputation are met. Analyses will be performed on an intention-to-treat basis.
Primary study parameters: The primary study parameter is the difference on the TDI-score post-treatment on the SST, between the two groups (prednisolone or placebo). A difference of 5.5 on TDI-score is determined as a clinically relevant difference for the primary outcome [34]. Mean (or median) and standard deviation (or the range) will be reported. Depending on the distribution of the outcome we will use the unpaired t-tests or the Mann Whitney-U tests to determine the statistical differences between intervention and control group.
Secondary study parameters: Objective gustatory function by means of TST, assessing recognition thresholds and identification for the four basic tastes score range 0 to 16 [32]. Clinical improvement is set at >2 points [35]. The Taste Strip Test score and the scores on the different questionnaires are measured at start and 12 weeks after start. Mean (or median) and standard deviation (or range) will be reported. Depending on the distribution of the outcome we will use the unpaired t-tests or the Mann Whitney-U tests to determine the statistical differences between intervention and control group.

Ethics approval and Dissemination

The institutional Review Board of the University Medical Center Utrecht approved the research protocol (protocol number: 21-635/G-D, October 2021). This study will be conducted according to the principles of the Declaration of Helsinki (2013, Fortaleza) and in accordance with the Medical Research Involving Human Subjects Act (WMO), the EU GDPR (General Data Protection Regulation), and other guidelines, regulations and Acts, e.g. “Code

goed gebruik". For this protocol the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) checklist is used. The Consolidated Standards of Reporting Trials (CONSORT) will be used for the full RCT report.

All substantial amendments will be notified to the METC and to the competent authority. Data handling and protection is conducted according to the ISO standards (27001 & 9001), ICH-GCP, and applicable regulations. Confidentiality will be maintained at all times, participant information will not be disclosed to third parties. Only investigators directly involved in this study will get access to all of the collected research data. Patients will receive an unique identifier, after which the members of the research team will extract all necessary clinical parameters into the eCRF Castor EDC and IBM SPSS Statistics 27.0 which is secured by a password, and located in a locked room. A local monitor (UMCU) will monitor trial quality. AE will be recorded in Castor EDC and SAE's will be reported to the sponsor. The sponsor will report the SAE's through the portal *ToetsingOnline* to the accredited METC that approved the protocol.

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Prednisolone does not improve olfactory function after COVID-19: a randomized, double-blind, placebo-controlled trial.

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Abstract

Background

Prednisolone has been suggested as a treatment for olfactory disorders after COVID-19, but evidence is scarce. Hence, we aimed to determine the efficacy of a short oral prednisolone treatment on patients with persistent olfactory disorders after COVID-19.

Methods

We performed a randomized, double-blind, placebo-controlled, single-centered trial in the Netherlands. Patients were included if they were >18 years old and if they had persistent (>4 weeks) olfactory disorders within 12 weeks after a confirmed COVID-19 test. The treatment group received oral prednisolone 40mg once daily for ten days and the placebo group received matching placebo. In addition, all patients performed olfactory training. The primary outcome was the objective olfactory function on Sniffin' Sticks Test (SST) 12 weeks after the start of treatment, measured in Threshold-Discrimination-Identification (TDI) score. Secondary outcomes were objective gustatory function assessed by the Taste Strip Test (TST) and subjective self-reported outcomes on questionnaires about olfactory, gustatory and trigeminal function, quality of life, and nasal symptoms. The CONSORT 2010 guideline was performed**.**

Results

Between November 2021 and February 2022 we included 115 eligible patients, randomly assigned to the treatment (n=58) or placebo group (n=57). No difference in olfactory function between groups was obtained after 12 weeks. Median TDI score on SST was 26.8 (IQR 23.6-29.3) in the placebo group and 28.8 (IQR 24.0-30.9) in the prednisolone group, with a median difference of -1.5 (-3.0 to 0.25). There was similar improvement on olfactory function in both groups after 12 weeks. Furthermore, on secondary outcomes, we obtained no differences between groups.

Conclusions

This trial shows that prednisolone does not improve olfactory function after COVID-19. Therefore, we recommend not prescribing prednisolone for patients with persistent olfactory disorders after COVID-19.

Trial Registration

This trial is registered on the ISRCTN registry with trial ID ISRCTN70794078.

Background

Olfactory disorders are a common early feature in COVID-19 [1], occurring in about two of every three patients [2,3]. Though most patients recover within 4 weeks [4], it is reported that up to ~46% of patients still have olfactory disorders after 6 months [5,6], and 20-60% after a year [7,8]. The prevalence of long-term olfactory disorders varies widely because of the different methods of assessing olfactory function and a lack of follow-up. Patients with persistent olfactory disorders can have increased depressive symptoms and nutritional issues, reducing patients' quality of life [9].

At this time, the only therapeutic option for olfactory disorders in COVID-19 is olfactory training [10]. During olfactory training, a patient sniffs a set of known odors daily for a period of 6 months. Olfactory training may speed up and increase the extent of smell recovery however effects seem limited [11,12].

As the persistent loss of smell is thought to be caused by an inflammatory response [13], corticosteroids might be a treatment option. Some studies assessed corticosteroids in nasal spray, without beneficial effect [14–16]. Patients who were treated with a short oral prednisolone treatment experienced an improved sense of smell in two small studies [17–19]. However, these studies have a low level of evidence because of the limited number of cases (n = 9), short follow up (4-10 weeks) and the non-blinded study designs. Due to the uncertainty of the evidence, there is still no consensus on treatment [19].

If treatment with prednisolone in combination with olfactory training more effectively improves olfactory function, a long-term disability may be prevented for more patients. Side effects of prednisolone, such as stomach irritations and nervousness/restlessness need to be weighed against the potential benefit [20,21].

Since many patients suffer from olfactory disorders after COVID-19, we need to ensure the effectiveness of this treatment. Therefore, we investigated the efficacy of a treatment in combination with olfactory training in a randomized, double-blind, placebo-controlled, single-centered trial.

Methods

Study design

The corticosteroids for COVID-19 induced loss of Smell (COCOS) trial was a single-centre, randomized, double-blinded, placebo-controlled study in the Netherlands to determine the efficacy of a short prednisolone treatment on olfactory disorders after COVID-19. The trial consisted of a baseline visit at the outpatient Ear, Nose and Throat (ENT) clinic and a second visit (follow-up) after 12 weeks, (Figure 1). The institutional Review Board of the participating hospital approved the research protocol (protocol number: 21-635/G-D, October 2021). This study was conducted according to the principles of the Declaration of Helsinki (2013, Fortaleza). The CONSORT 2010 guideline was performed. The recruitment phase started November 2021 and ended February 2022. The trial ended May 2022.

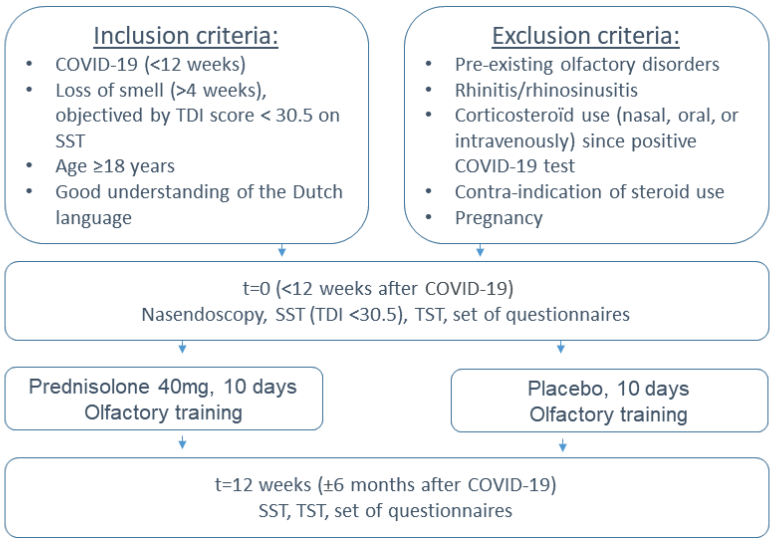

Figure 1. Study design. TDI score; Threshold-Discrimination-Identification score; SST; Sniffin' Sticks Test; TST; Taste Strip Test.

Participants

Participants were identified by the Dutch Patients Association for smell and taste disorders, and via the Dutch media that had been approached by the participating hospital. Interested patients could contact the research team via our website during the screening period. Investigators contacted interested patients by telephone to check inclusion and exclusion criteria, and medical status. Medical status consisted of medication use, medical history, date of confirmed positive COVID-19 test, and date of onset of the olfactory disorder. Patients were included if they were >18 years old, if they had persistent (>4 weeks) olfactory disorders within 12 weeks after COVID-19 diagnosis based on a positive test (PCR or antigen),

and if they understood the Dutch language. Patients were excluded if they used oral anticoagulants without stomach protection-, or if they suffered from pre-existing olfactory disorders including chronic rhinitis or rhinosinusitis-, or diseases which contra-indicate the use of steroids (diabetes mellitus for which drugs are used, stomach ulcers/bleeding, psychoses or ongoing oncological disease). Women who were pregnant, or who intended to become pregnant, were excluded. Eligible patients were invited for a baseline visit at the outpatient ENT clinic at the participating hospital. At the baseline visit, patients could still be excluded if they had no objective hyposmia (reduced loss of smell) or anosmia (total loss of smell) confirmed with a Threshold-Discrimination-Identification (TDI) score >30.5 on Sniffin' Sticks Test (SST), or if they had other causes for olfactory disorders objectified by nasendoscopy.

Procedures

We collected further baseline characteristics such as vaccination status and COVID-19 symptoms at first visit. Furthermore, we performed a nasendoscopy in order to eliminate other causes for olfactory disorders. Patients underwent objective smell and taste tests, and filled in three additional questionnaires. At the baseline visit, patients received their randomly allocated blinded study medication (40mg prednisolone once daily for 10 days, or matching placebo) and were instructed to start their 10 days of study medication the next morning. Researchers contacted patients by telephone ten days after the baseline visit to assess possible side-effects and treatment compliance. Patients in both groups performed 12 weeks olfactory training twice a day, coming to a total of 168 sessions. Patients crossed off a daily schedule allowing researchers to monitor olfactory training compliance. The follow-up visit was scheduled 12 weeks after the start of treatment. Outcome measurements were collected at the first visit (baseline) and second visit (follow-up) to compare outcomes (Figure 1). All outcomes were registered in an electronic case report form (eCRF), the endorsed system Castor EDC.

Randomization and blinding

Patients were randomly allocated to receive prednisolone or placebo. Half of the group was treated with capsules of 40mg of prednisolone, once daily for 10 days. The other half received capsules of placebo, once daily for 10 days. The pharmacy that prepared prednisolone and placebo capsules made a block randomization sequence list, on which the patient subject number was linked to the study medication number. This pharmacy is a Dutch state-of-the-art good manufacturing practice compounding pharmacy independent from our department. To minimize seasonal effects between groups, randomization occurred in block sizes of four patients. The blinding of researchers, physicians, outcome assessors and patients to the treatment allocation broke after all the analyses were finished.

Outcome measures

The primary outcome was the objective difference between the two groups on the TDI score post-treatment at 12 weeks, measured with the Sniffin' Sticks Test (SST, Burghart). The SST consists of three parts: a threshold test (score ranging 1-16), discrimination test (score ranging 0-16), and identification test (score ranging 0-16). The TDI score is the sum of these three tests and ranges from 1 to 48. The higher the score, the better the olfactory function. A score of ≤16.5 is considered as anosmia, a score of >30.5 as normosmia and scores between these values are considered as hyposmia. A difference of 5.5 on TDI score was determined as a clinically relevant difference for the primary outcome [22]. Secondary objective outcome was gustatory function measured by Taste Strip Test (TST, Burghart), assessing recognition thresholds and identification of the four basis tastes [23]. Total taste score range from 0 to 16 since scores for each taste range from 0 to 4. High scores indicate

a better taste function. Clinical improvement was set at >2 points [24].
Secondary subjective outcomes were olfactory, gustatory and trigeminal function, impact of smell/taste changes on quality of life, and nasal symptoms measured by subjective questionnaire outcomes. These contained the validated Sino-Nasal Outcome Test -22 questionnaire (SNOT-22) [25,26], self-reported smell, taste, trigeminal sensations questionnaire by medians of visual-analogue-scale (VAS), ranging 0- 10 [27], and the translated Olfactory Disorders Questionnaire (ODQ) [28,29]. All outcomes where assessed during both first and second visit. Details of all outcomes, examinations, and questionnaires are reported in the published study protocol [30].

Statistical analyses

All statistical analyses were performed using the IBM SPSS Statistics 26.0.0.1 software and R statistical computing. We performed analysis on an intention-to-treat basis. Sample size was calculated based on means and standard deviations of an earlier pilot study [31]. With a power of 0.90, an alpha of 0.05 and a mean difference of 5.5 (SD 8.0) on SST-scores, the total sample size was 92. To correct for possible non-parametric testing, the sample size was increased with 15%. As the study is limited in time and effort for the patient, a maximum of 10% dropout was expected. This gives a total sample size of initial 116 patients, with 58 in every group. A test for normality was used to assess whether variables were normally distributed. Since all our outcomes were not-normally distributed, a Mann- Whitney U test was performed to determine statistical significant differences between the prednisolone and placebo group. The differences in continuous variables between the groups was calculated using Hodges-Lehmann estimation. Confidence intervals for differences between groups were reported.

Patient and public involvement

The national patients association was involved in the conduct of the study, applying for the funding, and in recruiting patients. No patients were involved in the research questions or outcome measurements. We acknowledged and thanks the participants of our trial for their contribution. Patients who participate in this trial, who prefer, will be informed of the results.

Results

Patients

In total, 115 eligible patients came for their first visit to the outpatient ENT department in the participating hospital. We initially planned 116 patients by telephone assessment, but there was one no show after the medication was already prescribed and collected from the pharmacy and could therefore not be reused. This led to 115 patients who were enrolled, who gave informed consent, and who were randomly assigned to the prednisolone group (n =58) or placebo group (n=57), (Figure 2). There were no patients with a TDI score >30.5 or abnormalities at nasendoscopy on first visit. Trial participants were recruited during the fourth COVID-19 wave, presumably largely the Delta variant (July, 2021 to January 2022) and were distributed from all over the Netherlands. Three patients had long-term COVID-19 related symptoms at the first visit such as fatigue, reduced cognitive function and reduced physical condition which had all improved at second visit. The rest had experienced mild COVID-19 related symptoms such as cough, fever or a cold during the infection or have had no complaints at all. No patients had been hospitalized.

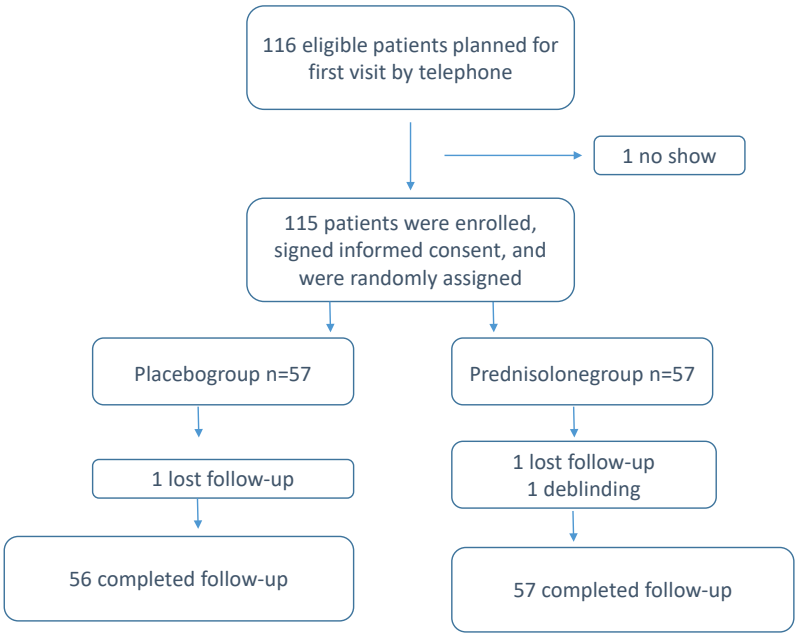

Figure 2. Participant flow-chart.

Baseline Characteristics

Baseline characteristics are reported in Table 1. At baseline there were no differences between age, gender, vaccination status and time since confirmed positive COVID-19 test. Median age of all study patients was 49 years (IQR 41- 57), with a minimum of 20 and a maximum of 78 years. Of these patients, 73 (63.5%) were female and 42 (36.5%) were male. In the placebo group were 50 patients (87.7%) vaccinated and in the prednisolone group 41 patients (70.7%). Median duration since confirmed COVID-19 test for all study patients was 56 days (IQR 44- 69), with 53 days (IQR 43.5- 67.0) in the placebo group and 59.5 days (IQR 46.5- 73.0) in the prednisolone group. Median objective TDI score in the placebo group was 20.5 (IQR 17.5- 24.3) and 22.9 (IQR 19.9- 25.1) in the prednisolone group. Median self-reported smell function on VAS-score was 1.1 (IQR 0.3- 3.1) in the placebo group and 1.4 (IQR 0.5- 2.8) in the prednisolone group. Both objective and subjective gustatory function did not differ at baseline between the two groups. Scores on TST were equal in both groups with a median of 10 (IQR 7-12) and the median self-reported taste function on VAS-score was 3.4 (1.2- 5.8) in the placebo group and 3.7 (IQR 1.0- 5.8) in the prednisolone group. Moreover, we obtained no differences between group on quality of life, nasal symptoms or self-reported trigeminal function (Table 1).

	Placebo group (n=57)	Prednisolone group (n=58)
Age, years	46 (39.5- 55.0)	51 (42.5- 59.3)
Sex		
Female	39 (68.4%)	34 (58.6%)
Male	18 (31.6%)	24 (41.4%)
Vaccinated	50.0 (87.7%)	41 (70.7%)
Time since positive COVID-19 test, days	53.0 (43.5- 67.0)	59.5 (46.5- 73.0)
Sino-nasal Outcome Test (SNOT-22)	23.0 (14.5- 37.0)	20.5 (13.5- 44.0)
Sniffin' Sticks Test (SST)		
TDI score	20.5 (17.5- 24.3)	22.9 (19.9- 25.1)
Threshold	1.3 (1.0- 3.4)	1.5 (1.0- 3.8)
Discrimination	9 (7- 11)	10 (8- 11)
Identification	9 (7- 11)	10 (9- 12)
Taste Strip Test (TST)		
Total score	10 (7- 12)	10 (7- 12)
Sweet	3 (2- 4)	4 (2. 8-4)
Sour	2 (1- 3)	2 (1- 3)
Salty	2 (1- 3)	3 (2- 3)
Bitter	3 (1- 3)	2 (1- 3)
Olfactory Disorders Questionnaire (ODQ)		
Total score	0.5 (0.4- 0.6)	0.5 (0.4- 0.5)

	Placebo group (n=57)	Prednisolone group (n=58)
Self-reported visual analogue scale (VAS)		
Sense of smell	1.1 (0.3- 3.1)	1.4 (0.5- 2.8)
Sense of taste	3.4 (1.2- 5.8)	3.7 (1.0- 5.8)
Trigeminal sensations	3.8 (2.1- 5.8)	5.2 (2.6-6.8)
Olfactory training compliance, frequency	136 (101- 150)	123 (83- 152)

Table 1. Baseline characteristics, intention to treat population. Data are presented as median (IQR) or n (%), except where otherwise stated. Outcome ranges were as follows: SNOT-22 0-50; TDI 1-48; T 1-16; D; 0-16; I 0-16; TST 0-16; Sweet, Sour, Salty, Bitter 0-4; ODQ 0.13-1.0; VAS 0-10; Frequency Olfactory training 0-168.

Follow-up

After 12 weeks all patients were eligible for follow-up since analysis was performed on the intention-to-treat base. Two patients were lost to follow-up (Figure 2). One patient in the placebo group had to receive a prednisolone treatment for her asthma (7 days of 20mg prednisolone once daily), between first and second visit, but after 10 days of study treatment.

Outcomes

Primary and secondary outcomes are presented in Table 2. At 12 weeks of follow-up, patients treated with prednisolone showed no better olfactory function than patients treated with placebo. Median TDI score was 26.8 (IQR 23.6- 29.3) in the placebo group and 28.8 (IQR 24.0- 30.9) in the prednisolone group, with a median difference of - 1.5 (95% CI -3.0 to 0.25). There was similar improvement on olfactory function in both groups after 12 weeks. Separate TDI scores did not show any significant or clinically relevant difference (Table 2). Self-reported smell function on VAS-score was 3.2 (IQR 1.8- 6.5) in the placebo group and 3.6 (IQR 1.0- 5.8) in the prednisolone group with a median difference of 0.3 (95% CI -0.9 to -1.3, p=0.53). Additionally, no effect was obtained on objective gustatory function in the prednisolone group compared to the placebo group. Both groups showed a median of 11 on TST (IQR 9- 13, p=0.50). Self-reported taste function on VAS-score was 5.6 (IQR 2.3- 7.6) in the placebo group and 5.0 (IQR 2.0- 7.8) in the prednisolone group with a median difference of 0.1 (95% CI -1.0 to 1.3, p=0.80). Moreover, no differences between groups were obtained in quality of life, nasal symptoms or self-reported trigeminal function on questionnaires (Table 2).

The daily olfactory training schedule was not obtained in 2 of 113 patients. There were no major differences between compliance of olfactory training between the groups. Compliance of olfactory training is expressed in frequencies (Table 1).

	Placebo group (n=56)	Prednisolone group (n=57)	Difference (95% CI)	*P* value
Sniffin' Sticks Test (SST)				
TDI score	26.8 (23.6- 29.3)	28.8 (24.0- 30.9)	-1.5 (-3.0 to 0.25)	P = 0.10
Threshold	4.3 (3.3- 5.4)	4.5 (3.1- 6.4)	-0.25 (-1.0 to 0.5)	P = 0.47
Discrimination	11 (10- 12)	12 (10.5- 13)	-1.00 (-1.00 to 0.00)	P = 0.12
Identification	11.5 (10- 12)	11 (10- 13)	0.00 (-1.00 to 1.00)	P = 0.45
Taste Strip Test (TST)				
Total score	11 (9.3- 13)	11 (9- 13)	0.00 (-1.00 to 1.00)	P = 0.50
Sweet	4 (3- 4)	4 (3- 4)	0.00 (0.00 to 0.00)	P = 0.66
Sour	2 (2- 3)	2 (2- 3)	0.00 (0.00 to 0.00)	P = 0.84
Salty	3 (2- 4)	3 (2- 4)	0.00 (0.00 to 1.00)	P = 0.31
Bitter	3 (2- 4)	3 (2- 3.5)	0.00 (0.00 to 1.00)	P = 0.47
SNOT-22				
Total score	16 (10- 26)	19 (10- 32)	-1.00 (-7.0 to 4.0)	P = 0.69
ODQ				
Total score	0.4 (0.3- 0.6)	0.4 (0.3- 0.5)	0.00 (-0.06 to 0.06)	P = 0.89
VAS				
Sense of smell	3.2 (1.8- 6.5)	3.6 (1.0- 5.8)	0.3 (-0.9 to 1.3)	P = 0.53
Sense of taste	5.6 (2.3- 7.6)	5.0 (2.0- 7.8)	0.1 (-1.00 to 1.3)	P = 0.80
Trigeminal sensations	5.1 (2.9- 7.4)	5.3 (2.4- 7.9)	-0.2 (-1.3 to 1.00)	P = 0.76

Table 2. Primary and secondary outcomes at 12 weeks. Data are presented as median (IQR) or n (%), except where otherwise stated. Differences are expressed as rate differences or Hodges-Lehmann estimator and 95% CI. Outcome ranges: SNOT-22 0-50; TDI 1-48; T 1-16; D; 0-16; I 0-16; TST 0-16; Sweet, Sour, Salty, Bitter 0-4; ODQ 0.13-1.0; VAS 0-10; Frequency Olfactory training 0-168.

Harms

We reported three adverse events in the prednisolone group in Table 3. Adverse events contained severe side-effects for which intervention, discontinuation, or deblinding of treatment was needed. No serious adverse events occurred. For one patient in the prednisolone group we requested to break the blinding of the study medication due to psychological disorders and sleeplessness after full treatment compliance. This was the only patient with a deblinding before the end of the study. Three patients stopped the treatment because of side-effects after a minimum of six days, of which two patients were allocated in the placebo group and one in the prednisolone group. The rest of the patients in both groups complied with their treatment.

Adverse Events	N=3
Sleeplessness with psychological disorders after 10 days of treatment for which blinding of treatment was broken	1
Restlessness for which patient stopped treatment after 8 days	1
Stomach irritation for which omeprazole was needed, full treatment compliance	1

Table 3. Adverse Events.

We reported 14 patients with mild side-effects, of which 9 (15.5%) in the prednisolone group (n=58) and five (8.8%) in the placebo group (n=57). The most reported side-effects were nervousness/restlessness and stomach irritation. All side-effects were mild, common, and lasted a short time or stopped immediately after finishing the ten days of treatment.

Discussion

This randomized double-blind, placebo-controlled trial for patients with persistent olfactory disorders after COVID-19 showed that patients who received a short prednisolone treatment had no better olfactory function than patients who received placebo treatment.
Two previous studies did show a possible better olfactory function in patients who received a short prednisolone treatment [17,18]. This study failed to support that claim. The reason for the discrepancy in outcomes, is most likely due to the biases included in these previous studies. Only nine patients were treated in each study on their own request. The studies were not blinded or randomized. Even though both studies used an objective outcome measure of smell, the investigator taking the test might have influenced the outcome. In our study design we eliminated these biases and ensured sufficient power. There are however limitations in our trial we have to take in consideration. In our study we treated patients with 10 days of 40mg prednisolone, starting at least 4 weeks (median ~ 59.5 days) after the initial infection. Prednisolone dosage and timing could have influenced outcome. With the limited available evidence, we choose to use a comparable dosage schedule as used in the previous studies. Higher dosage might have increased effectiveness, but also would have increased side effects, both in number and severity. The short prednisolone treatment schedule is well known in otorhinolaryngology practice. The same schedules are used in sensorineural hearing loss and Bell's palsy. However in these diseases prednisolone treatment starts preferably within 72h after the start of symptoms [20,21]. Nevertheless we started treatment after 4 weeks in this trial. Firstly, because most patients regain spontaneous normal smell and taste function within this 4 week period, so treating them in that phase could risk overtreatment. Secondly, the immune system against COVID-19 can be inhibited by prednisolone which can lead to a prolonged infection.
Due to the timing of this study, it is likely that mainly patients with the COVID-19 Delta

variant are included, although we did not test for this specifically. Up to now it is unknown how different COVID variants influence outcome in olfactory function. The Omicron variant though, has proven to have less negative effect on smell and taste, than the Delta variant [32]. Possibly and hopefully, fewer Omicron patients will face long-term disability in olfactory disorders, compared to Delta patients. We presume, that it is unlikely that the COVID variants influenced the outcome of this study.

The only current treatment option for persistent olfactory disorders after COVID-19 is olfactory training. In this study both groups improved substantially on their olfactory function on the second visit. This suggests that even after a longer period of time, smell will continue to improve. Therefore we intend to retest our study population 1 year after the initial infection, to gain better insight into the course of the olfactory function. Furthermore, if we gain a better understanding of the pathophysiological mechanisms underlying olfactory disorders, we may be able to develop new treatments.

Conclusions

Our randomized, double-blind, placebo-controlled trial showed no beneficial effect of a prednisolone treatment (40mg daily, for 10 days) over placebo treatment in patients with persisting olfactory disorders (>4 weeks) after COVID-19 (<12 weeks). Therefore, we recommend not to prescribe prednisolone for patients with olfactory disorders after COVID-19. However we have to take in consideration that our trial assessed outcomes on a specific population, treatment dosage and time. As variants changes, as countries may have different COVID-19 treatment protocols, results may vary. Other studies focusing on different treatment schedules, severity of illness, and COVID-19 variants could help to confirm our findings.

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The effect of smell training on COVID-19 induced smell loss.

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Abstract

Objective: while smell training appears to be effective for post viral smell disorders, its effectiveness in COVID-19 induced smell disorders is currently not well known. Therefore, we aim to investigate the potential effect of smell training on patients with COVID-19 induced smell loss.

Methods: we conducted a case-control study with two comparable cohorts. One of which (n=111) was instructed to perform smell training twice daily for 12 weeks, therapeutical adherence was monitored on a daily schedule, while the other cohort (n=50) did not perform smell training. The Sniffin' Sticks Test (SST) was used to objectify participants' sense of smell at baseline and after 12 weeks, reported as a Threshold, Discrimination, and Identification (TDI) score. We also determined the association between therapeutical adherence and the TDI scores.

Results: we found a significant difference in psychophysical smell function between patients with COVID-19 induced smell disorders who performed 12 weeks of smell training (TDI score 27.5, IQR 23.75-29.75) and those who did not (TDI score 25.75, IQR 17.88-29.13). Median TDI difference between groups was 2.00 (95% CI 0.00-4.00, p=0.038). However, there was no association between the therapeutical adherence and olfactory function.

Conclusion: we discovered a significant moderate difference in psychophysical smell function between patients with COVID-19-induced smell disorders who performed smell training and those who did not, implying a possible advantage of training. However, no relationship was found between therapeutical adherence of smell training and olfactory function.

Introduction

The COVID-19 pandemic has resulted in a significant burden of patients with persistent smell loss [1,2]. Given the limited treatment options for COVID-19 induced olfactory disorders [3–5] and the decreased quality of life experienced by these patients [6], there is a clear need for an effective intervention [7].

The pathophysiologic mechanism underlying COVID-19 induced smell disorders is believed to be damage to sustentacular cells of the olfactory epithelium. These cells support the Olfactory Receptor Neurons (ORNs) in processing odors and the olfactory epithelium in the transduction cascade, both required for a functional olfactory system [8–11]. Therefore, harm to the sustentacular cells indirectly affects the ORNs and the olfactory epithelium, leading to olfactory disorders.

Olfactory training has been proposed as a potential treatment option for COVID-19 induced olfactory disorders [12–19] as it has been shown to promote the regeneration of the number and activity of ORNs through repetitive odor exposure [20,21]. Previous studies have demonstrated the efficacy of olfactory training in other types of olfactory disorders (e.g., post-traumatic and post-infectious olfactory disorders) [20,22–25].
However, current studies investigating the effect of olfactory training in COVID-19 patients have small sample sizes [14–16,26], no generalizable psychophysical measurements [14], lack a control group [15,17,27], or monitoring of therapy compliance [12,14,17] which limits the ability to draw conclusions [13,25].

Smell training compliance can be challenging, this could be due to its repetitive nature, the nuanced noticed effect and a lack of motivation. Autonomous (willingness-based) and controlled (external pressure-based) motivation are crucial for optimal compliance. Healthcare practitioners can enhance autonomous motivation through improved therapy communication [28], while monitoring, such as using a treatment diary, aids controlled motivation and overall compliance [29].

We observed a different recovery trajectory between two comparable study cohorts within the same project [30], in which one group was stimulated to perform smell training and the other group did not perform smell training. Therefore we performed a case-control study with psychophysical tests of olfactory function to investigate the efficacy of smell training in comparison to a control group without smell training. In addition, we aim to explore the potential association between the frequency of smell training and its impact on olfactory function. If adherence to the treatment demonstrates a beneficial effect on TDI scores, this could contribute to the instructions we give to patients, e.g. encourage them to strictly

perform the smell training consequently and explain the possible consequences of a limited effect when performing the training inconsequent.

Methods

We performed a case-control study in the Netherlands, analyzing two comparable prospective study cohorts of COVID-19 patients. Both studies are part of the national research project 'Sniffing Out COVID', funded by the Dutch Organization for Health Research and Development, project nr 10430102110001. Data from patients who participated in the COCOS study (COrticoisteroids for Covid-19 Induced loss of Smell) [31] and data from patients participating in the COVORTS study (COVid-19 cohORT for Smell loss) were compared [30]. The University Medical Center Utrecht's Institutional Review Board approved the original research protocol for the COCOS trial (21-635/G-D, October 2021). The Medical Ethical Assessment Committee (METC) in the East of the Netherlands approved the COVORTS study (2021-11687, NL77954.091.21).

Study cohorts

The COCOS study (smell training cohort) was a randomized, double-blind, placebo-controlled trial determining the possible benefit of an oral prednisolone treatment (10 days 40mg) on the olfactory function in patients with COVID-19 induced smell disorders. Results showed no difference in olfactory function between patients who received prednisolone and those who received placebo [31]. Consequently, we combined the patients from both groups into a single cohort since there was no distinction between the placebo and prednisolone group. Patients in both placebo and prednisolone group performed olfactory training for 12 weeks, consisting of repeated exposure to four different intense odors; (rose, eucalyptus, lemon, and cloves) [20]. To monitor compliance to the training, patients filled in a daily schedule, in which they wrote if they did the training. Therapeutical adherence was registered as frequency. They were recommended to do the training each morning and evening. The maximum achievable score was 168, representing the ideal scenario of performing the training twice a day for a duration of 12 weeks.

The COVORTS study (no smell training cohort) was a prospective cohort study in order to assess olfactory function and recovery over time, without any interventions [30].

Patients in both studies underwent the same psychophysical Sniffin' Sticks Test (SST) and reported their sense of smell in a Visual Analogue Scale (VAS) at baseline (first visit) and after 12 weeks (second visit).

Patients

Patients were recruited via the Dutch media, the National Institute for Health and the Environment (RIVM), and the National Patients Association 'Reukensmaakstoornis.nl'. All patients signed informed consent in order to participate. Inclusion and exclusion criteria for both studies were similar [30,31] apart from a maximum age of 60 years in the COVORTS cohort, whereas there was no maximum age for participation in the COCOS study. Patients in the COCOS study underwent a nasendoscopy at baseline in order to eliminate other potential causes for their loss of smell. Both studies included patients with at least 4 weeks of COVID-19 induced smell loss objectified by the SST, with a Threshold-Discrimination-Identification (TDI) score of <30.5 at first visit (<12 weeks following a confirmed COVID-19 diagnosis by PCR). So, in both cohorts only patients with a minimum of 4 weeks and a maximum of 12 weeks of COVID-19 induced smell loss were included. The researchers of the COVORTS study made an amendment to include patients with only a positive self-home test as PCR testing had become less common in the Netherlands during their recruitment period. For this study we used one patient with a positive self-home test without confirmed PCR. The Medical Ethical Assessment Committee (METC) in the East of the Netherlands approved the amendment in July 2022. In the COCOS study 115 patients completed their first visit, two patients lost follow-up at second visit. Of these 113 patients, two patients were excluded by not fulfilling the olfactory training diary, resulting in 111 patients for the analysis.

In the COVORTS study, 60 patients finished first visit. Without being encouraged, ten patients performed smell training on their own initiative during the study period. These patients were excluded, resulting in 50 patients for the analysis (Figure 1).

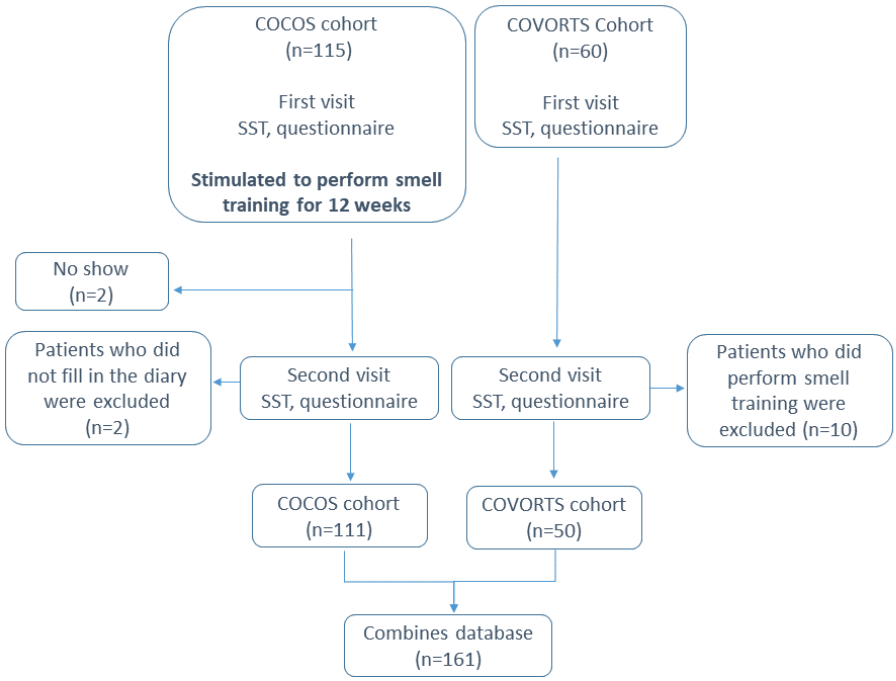

Figure 1. Patient flow-chart and study design.

Procedures

The patients in the COCOS study visited the Outpatient clinic for Ear, Nose, and Throat twice for the assessment of the SST, and to fill out a VAS questionnaire (Figure 1).

The start of recruitment and the assessment of the baseline data started for the COCOS study in November 2021 and ended in February 2022, all second visits were between February 2022 and May 2022. For the COVORTS study, the start of recruitment and the assessment of baseline data used for this study, started as well in October 2021, and ended in November 2022. Second visits were between February 2022 and February 2023. At first visit, all patients received an olfactory trainings set, were stimulated to perform smell training twice daily for 12 weeks and were closely monitored by crossing off a treatment diary. At second visit (approximately 12 weeks after first visit), patients administered the same smell tests and VAS questionnaire. Patients who did not fill out the treatment diary were excluded (n=2).

Patients in the COVORTS study were visited at home twice in order to perform the SST, and to fulfill the same VAS questionnaire (Figure 1). Patients were asked at first and second visit

whether they performed olfactory training, and were excluded from the analysis if they stated they had (n=10).

Outcome measures

The primary outcome of this study is the comparison of TDI scores reflecting psychophysical olfactory function, obtained from the SST, between the smell training (COCOS) and no smell training (COVORTS) cohorts after 12 weeks. The TDI score ranges from 0 to 48, with a higher score indicating a better olfactory function. The TDI score is derived from three tests: Threshold (score range 1-16), Discrimination (score range 0-16), and Identification (score range 0-16) [32], with a clinically relevant difference defined as 5.5 points [33]. We also collected data from the self-reported sense of smell on a Visual Analogue Scale (VAS) ranging from 0-10 [3].

Statistical analyses

All statistical analyses were conducted using IBM SPSS Statistics 26.0.0.1 software. Our data indicated non-normal distribution for all outcomes, so we performed a Mann-Whitney U test for differences on the outcome measures between the COCOS and COVORTS cohort. Median differences between outcomes were calculated using Hodge-Lehmann estimators, and confidence intervals and p-values were reported. An univariable linear regression analysis was conducted on the smell training cohort to explore the potential association of the frequency of smell training on TDI score outcomes at second visit.

Results

Baseline characteristics (first visit)

The smell training cohort had a median age of 49 years (IQR 41-57), with 40 (36.0%) male patients and 71 (64.0%) female patients. Median duration between COVID-19 infection and first measurements was 56 days (IQR 44-69). Out of 111 patients, 88 patients (79.3%) were vaccinated for COVID-19. Median TDI score on the SST was 21.50 (IQR 18.25-24.75). Patients' median self-reported sense of smell scores on the Visual Analogue Scale (0-10) was 1.2 (IQR 0.4-3.0) (Table 1).

The no smell training cohort had a median age of 51 years (IQR 45-55), with 10 (20.0%) male patients and 40 (80.0%) female patients. Median duration between COVID-19 infection and first measurements was 88 days (IQR 71-98.5)

Median TDI score on the SST was 24.0 (IQR 19.20-27.31). Patients rated their sense of smell on the Visual Analogue Scale (0-10) as 2.1 (IQR 0.6-2.9) (Table 1).

	Smell training cohort n = 111 (COCOS)	No smell training cohort n = 50 (COVORTS)
Gender		
Male	40 (36.0)	10 (20.0)
Female	71 (64.0)	40 (80.0)
Age, years	49 (41-57)	51 (45-55)
Vaccination status	88 (79.3)	39 (78.0)
Duration between first visit and COVID-19, days	56 (44-69)	88 (71-98.5)
Median frequency of performing smell training	129 (86-151)	-
TDI Score	21.50 (18.25-24.75)	24.0 (19.20-27.31)
Threshold	1.5 (1.0-3.5)	4.5 (1.5-6.5)
Discrimination	9.0 (8.0-11.0)	9.0 (7.8-11.0)
Identification	10.0 (8.0-11.0)	10.0 (7.0-12.0)
Self-reported sense of smell, VAS (0-10)	1.2 (0.4-3.0)	2.1 (0.6-2.9)

Table 1. Baseline characteristics and outcomes. Data is reported n (%) or in medians (IQR), unless where otherwise stated. TDI 1-48; T 1-16; D 0-16; I 0-16; VAS 0-10.

Outcomes after 12 weeks (second visit)

Median duration between first and second visit was 12 weeks (IQR 11-13). Median frequency of performing smell training was 129 times (IQR 86-151), with a maximum of 168 times. The distribution of the frequency of performing olfactory training is shown in Figure 2.

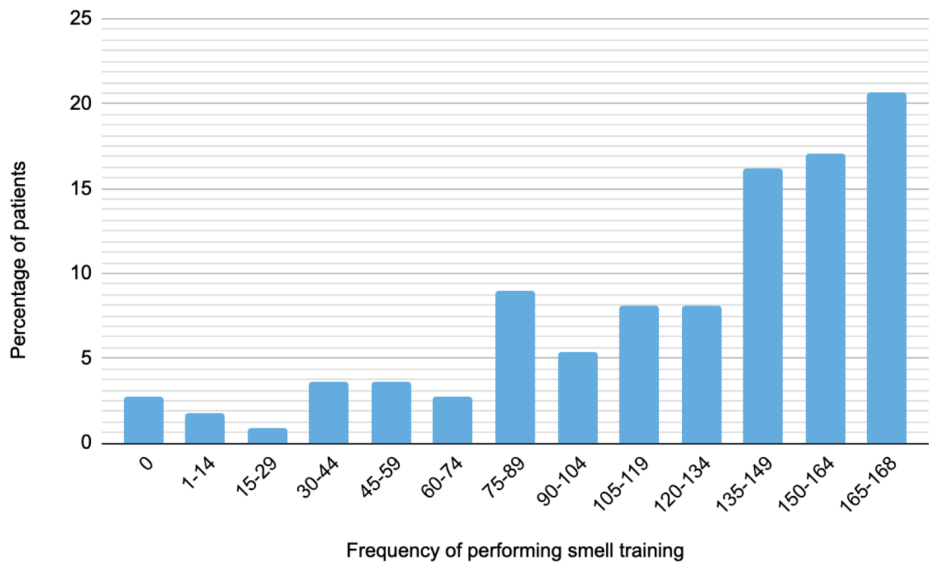

Figure 2. Distribution of performing olfactory training in frequency over 12 weeks.

The smell training cohort scored a median TDI score of 27.5 (IQR 23.75-29.75). They rated their sense of smell on the Visual Analogue Scale (0-10) as 3.2 (IQR 1.4-5.9) (Table 2).

The no smell training cohort had a median TDI score of 25.75 (IQR 17.88-29.13). They rated their sense of smell on the Visual Analogue Scale (0-10) as 4.3 (IQR 1.6-6.0) (Table 2).

Median TDI difference between groups was 2.00 (95% CI 0.00 to 4.00, p = 0.038). Median difference in self-reported sense of smell was 0.01 (95% CI -0.70 to 1.10, p = 0.711) (Table 2).

	Smell training cohort (COCOS) N = 111	**No smell training cohort (COVORTS) N = 50**	**Difference (95% CI)**	**P-value**
TDI Score	27.50 (23.75-29.75)	25.75 (17.88-29.13)	2.00 (0.00 to 4.00)	0.038
Threshold	4.50 (3.3-5.5)	4.6 (1.8-7.1)	0.25 (-0.50 to 1.25)	0.387
Discrimination	11 (10-13)	10 (8-12)	2.00 (1.00 to 2.00)	0.000
Identification	11 (10-13)	10 (8-11.3)	1.00 (1.00 to 2.00)	0.000
Self-reported sense of smell, VAS.	3.2 (1.4-5.9)	4.3 (1.6-6.0)	0.01 (-0.70 to 1.10)	0.711

Table 2. Primary and secondary outcomes at the second visit. Data is reported in medians (IQR).
TDI 1-48; T 1-16; D 0-16; I 0-16; VAS 0-10.

Compared to baseline the smell training cohort showed an improvement in TDI score of 6 points. The improvement of TDI score in the no smell training cohort was 1.75 points (Figure 3).

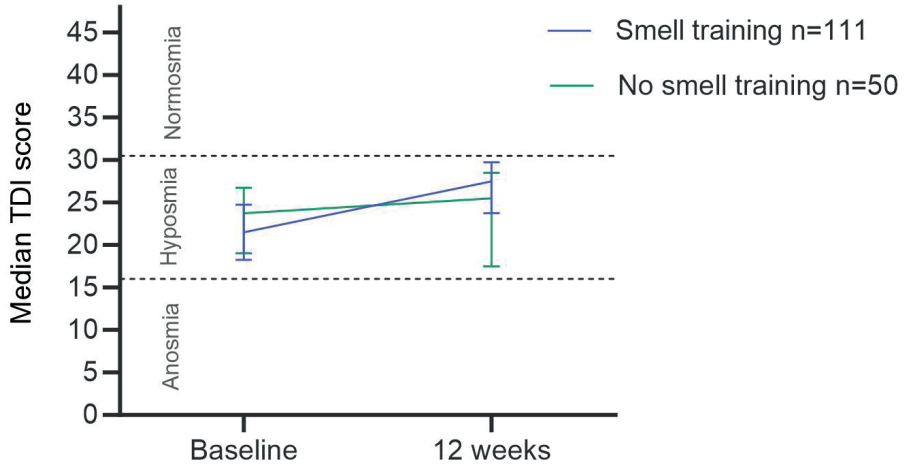

Figure 3. TDI scores over time in smell training cohort (COCOS) and in no smell training cohort (COVORTS). Data is visualized in medians and IQR.

Frequency of smell training

Table 3 shows an univariable linear regression analysis performed on the smell training (COCOS) cohort. No statistical significant association between the frequency of performed smell training and the TDI score or self-reported sense of smell (VAS) was found.

	Regression Coefficient (95% CI)	Standard Error	P-value
TDI score	-0.007 (-0.028 to 0.014)	0.011	0.507
Self-reported sense of smell, VAS	0.004 (-0.008 to 0.016)	0.006	0.491

Table 3. Linear regression of the frequency of smell training associating with TDI score and self-reported sense of smell (COCOS cohort).

Discussion

This case-control study investigated the efficacy of stimulated and monitored smell training in patients with COVID-19 induced smell loss, compared to those who did not perform smell training. The median difference of the smell training cohort on TDI score in 12 weeks is 6 points, which is a clinically relevant improvement. The median difference on TDI score of the no smell training cohort was 1.75 points, which is not a clinically relevant improvement. We want to emphasize that the main outcome of our study is the difference between the

groups, not the difference between the pre and post measurement. The difference between the two types of methodology is not too difficult to explain. What we aimed to do here is to assess the differences between using two types of therapies. The best way to do that is by comparing two groups, ideally in a randomized controlled trial. We took the second best, a comparative study of two groups. Assessing the pre-and post-measurement alone will leave us with more known and unknown confounders than using the comparative design. Therefore, the main outcome of our study is the difference between the TDI scores between the groups. There is a significant difference in TDI score between the groups after 12 weeks, however, both in the hyposmic range and with a moderate difference. Furthermore, we determined the association between frequency of smell training and TDI scores at the second visit in order to inform patients better about the possible effect of optimal therapeutical adherence. The reason for using the association at second visit is because using Delta as an outcome would lead to bias due to regression to the mean [34]. However, the frequency of smell training was not associated with smell function. We must acknowledge that this study was not suitable for fully assessing this question, because almost all of our patients were compliant with the smell training. Thus, the regression cannot truly be discriminative, as the distribution of frequency of performing smell training is not evenly distributed. Therefore we failed to provide patients with comprehensive information about the significance of treatment compliance or the enhancement of the treatment.

We aimed to addresses limitations of current literature by utilizing a large sample size, conducting thorough psychophysical and subjective measurements, and monitoring therapeutical adherence. Additionally, we included a control group to address the potential confounding effect of spontaneous recovery over time, enabling a more accurate evaluation of the difference between olfactory training and non-intervention. Moreover, we only included patients who had a confirmed COVID-19 diagnosis.

There are however limitations in this study to acknowledge. Firstly, the gold standard for assessing the effect of an intervention is a randomized controlled trial (RCT), since RCTs minimize the effect of confounding factors. In the present paper we describe a secondary outcome of both the COCOS and the COVORTS study, therefore we did not assess this topic in an RCT. However, the largest issue with non-RCTs when assessing the effect of an intervention is confounding by indication, in which the confounding is caused by the presence of an indication for the exposure. That confounding by indication might have been the case in our study, because individuals who responded to participate in either the COCOS or in the COVORTS cohort, might have had different characteristics. The difference in treatment between the two cohorts could initially have attracted patients who suffered from a higher degree of smell loss to participate in the smell training (COCOS) study. The smell training cohort had a lower starting point on TDI score, and therefore more to gain.

They also experienced less days of smell loss at baseline (56 days) in comparison to the no smell training cohort (88 days). Nonetheless, both cohorts shared the same inclusion and exclusion criteria, the same recruitment and testing period, and the same virus variants and vaccines available. Secondly, patients may have exhibited socially desired behavior when filling out the training diary, making the frequency of performed smell training sessions uncertain.
Another noteworthy distinction between the compared cohorts is the likelihood that patients in the smell training cohort exhibited more attention towards their sense of smell due to receiving treatment and performing smell training. However, the SST is a validated psychophysical test, which is used to assess the most objective smell function possible. Therefore it is unlikely that patients in the COCOS trial might have displayed a positive placebo effect that could have influenced the results in comparison to patients from the COVORTS trial.

Extending the period of smell training could potentially result in further improvement of smell function, as earlier recommended by previous studies ranging from 6 to 12 months [17,22]. We recommend conducting a randomized controlled trial with a prolonged follow-up period in order to gain more insight on the long-term effects of smell training. Though there are ethical considerations by withholding a control group from olfactory training and there is a challenge ensuring therapeutical adherence for an extended period. By incorporating both stimulation and monitoring (e.g. keeping a treatment diary or using a smartphone application) individuals are more likely to remain motivated and committed to the therapy [28,29]. Although our study reveals a significant moderate difference between the two groups after 12 weeks, there is no association between smell training frequency and olfactory function. There are two explanations for this outcome: first the distribution of the smell training was disbalanced in the cohort, the majority of patients performed the training to a limited extent. A RCT, designed to objectify the significance of frequency, could have led to a more balanced distribution of participants across different frequencies. However, it is worth considering that in real-world clinical settings, patients perform smell training with a wide range of frequencies. Besides, our primary objective was not to focus on the frequency, but to examine the differences between a group that received a training set and explicit instructions to perform smell training, and a group that was not provided any instruction or stimulation for smell training and was even excluded if they engaged in smell training on their own initiative. The other explanation might be that it is not the frequency of the therapy but the awareness of focusing on smell, that has the most effect.

Nevertheless, based on our findings and previous research indicating possible benefits [12–17,25], we highly encourage patients with smell loss following COVID-19 to perform olfactory training.

Conclusion

We found a statistical significant difference in smell function between patients with COVID-19-induced smell disorders who performed smell training and those who did not, implying a possible advantage of smell training. However, no association was found between the frequency of smell training and olfactory function. A randomized controlled trial with an extended follow-up period would be desirable to obtain more conclusive and validated results.

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Part III

Epidemiology & Clinical Course

6

One year psychophysical evaluation of COVID-19 induced olfactory disorders: a prospective cohort study.

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Abstract

Background: olfactory disorders are common in COVID-19. While many patients recover within weeks, a notable number of patients suffer from prolonged olfactory disorders. Much research has focused on the acute phase of olfactory disorders in COVID-19, however there is still inconsistency regarding the prognosis. We aim to assess both objective and subjective olfactory function in patients with persisting olfactory disorders following COVID-19, one year after diagnosis.

Methods: we objectively measured olfactory function in 77 patients who initially had COVID-19 induced smell disorders, one year after confirmed diagnosis. These patients previously underwent two objective measurements at approximately three and six months after COVID-19, in the context of the COCOS trial (COrticosteroids for COvid-19 induced loss of Smell). The main outcome measurement was TDI score (Threshold-Discrimination-Identification) on Sniffin' Sticks Test (SST). Secondary outcomes included objective gustatory function on Taste Strip Test (TST), self-reported olfactory, gustatory and trigeminal function on a Visual Analogue Scale (VAS) and outcomes on questionnaires about quality of life, and nasal symptoms.

Results: the findings of this study show that one year following COVID-19, the median TDI score increased to 30.75 (IQR 27.38-33.5), regarded as normosmia. The median TDI score started at 21.25 (IQR 18.25- 24.75) at baseline and increased to 27.5 (IQR 23.63-30.0) at six months following COVID-19. The increase of 9.5 points on the TDI score between baseline and one year after COVID-19 marks a clinically relevant improvement. Regarding the self-reported VAS score (1-10) on sense of smell, it increased from 1.2 (IQR 0.4-3.0) at baseline to 3.2 (IQR 1.4-6.0) at six months and further improved up to 6.1 (IQR 2.7 -7.5) after one year. Objective gustatory function increased with 2 points on TST a year after diagnosis. Self-reported olfactory, gustatory, and trigeminal functions also improved over time, as did quality of life.

Conclusions: objective and self-reported olfactory function continued to improve one year after COVID-19. The median TDI score of 30.75 (IQR 27.38-33.5) is regarded as normosmia, which is a favorable outcome. However, the rate of improvement on TDI score reduces over time.

Background

The importance of smell is often only recognized when it is lost. The COVID-19 pandemic has emphasized the impact of olfactory disorders, with recently reported over 50% of COVID-19 patients experience olfactory disorders [1,2]. Although many patients have temporary olfactory disorders which resolve within weeks [2–4], according to a recent meta- analysis, about 5% of patients who initially experienced olfactory disorders will continue to have symptoms six months later [5].

Affected patients with persisting smell loss can suffer from decreased quality of life and malnutrition [6]. Given this significant impact olfactory disorders can have on a person's life and the need for medical professionals to provide accurate information about recovery expectations, it is critical to increase our understanding of the clinical course of olfactory disorders after COVID-19. Although olfactory disorders in the early phase of COVID-19 have been thoroughly examined, knowledge about duration and prognosis is limited [7]. Most studies rely on self-reported sense of smell [8–18], but objective psychophysical tests can provide more precise and comparable information [19–22]. Psychological testing has been done in studies, however comparative baseline data or a prolonged follow-up are lacking [13,17,23–25]. We previously reported objective improvement in a cohort of patients with COVID-19 induced persisting smell disorders (>4 weeks) who had psychophysical measurements taken at approximately three and six months after diagnosis [26]. In the present study we aim to determine whether this improvement in smell function is still maintained one year following COVID-19, by conducting a prospective cohort study with the same patients.

Methods

Study design

This study is a prospective cohort study, and a follow up of the COCOS trial [26,27]. The original COCOS trial was a randomized, double-blind, placebo-controlled trial with 113 patients suffering from persisting (>4 weeks) smell disorders within three months after confirmed COVID-19. Patients visited the outpatient clinic for Ear- Nose- and Throat twice in order to measure their smell function at approximately three and six months after COVID-19 diagnosis. The first visits (baseline) were between November 2021 and February 2022. Most likely, the majority of patients was infected with the Delta variant, considering this was the dominant COVID-19 variant during that period [28]. The second visits occurred from February to May 2022. Half of the patients were treated with 40mg oral prednisolone for ten days. The other half received matching placebo. Researchers, physicians and patients were blinded until the analysis was finished. All patients were instructed and advised to perform olfactory training twice a day for at least 12 weeks. Therapy compliance was monitored by having patients fill out a daily schedule. The COCOS trial cohort (n=113) performed olfactory training with median of 129 times in 12 weeks (IQR 85-151). Both olfactory training and study treatment started the day after first visit. Results showed no effect of prednisolone on smell function in comparison with placebo [26]. However, we found in both groups the same median improvement in all outcomes at approximately 6 months after their COVID-19 diagnosis.

For the present study we aim to determine whether this observed improvement continues one year after COVID-19. To achieve this, we prolonged the follow-up period, conducting a third measurement approximately one year after the initial COVID-19 diagnosis (Figure 1). Our unique advantage lay in having a cohort of patients with confirmed COVID-19 diagnosis and smell loss, with both objective and subjective measurements. We had the opportunity to conduct an additional set of measurements, enabling this subsequent prospective cohort study. This study is solely based on observing the possible progression of improvement, without any further interventions. The institutional Review Board of the participating hospital approved an amendment of the COCOS protocol, which allowed for this third visit (protocol number: 21-635/Gm-A) in July 2022.

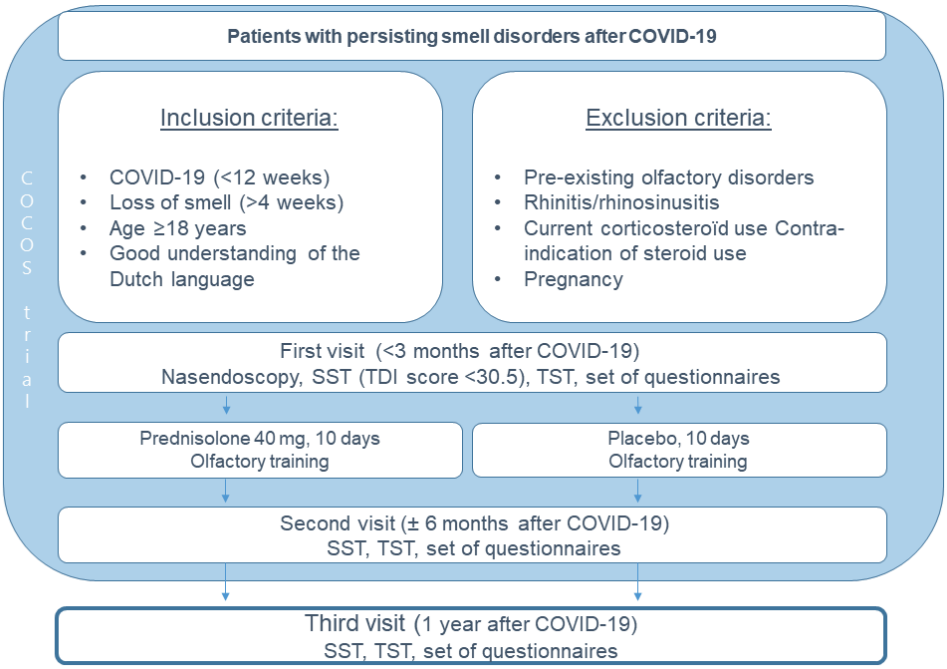

Figure 1. Study design. TDI score; Threshold-Discrimination-Identification score; SST; Sniffin' Sticks Test; TST; Taste Strip Test.

Participants

For this study we approached participants from the COCOS trial. They were contacted by phone or email and received updated patient information forms. Results about the initial COCOS trial were already provided to the patients. The recruitment period for this study started in September 2022 and ended in January 2023. After informed consent participants underwent the third round of measurements.

Procedures

The third visits for the study participants took place at the outpatient Ear, Nose and Throat (ENT) clinic between September 2022 and January 2023. At this visit, the same smell and taste tests and questionnaires were administered as in the original COCOS trial to compare outcomes (Figure 1). In some cases the third measurement was conducted at the participants' home. All measurements were recorded in an electronic case report form (eCRF) using the Castor Electronic Data Capture (EDC) system.

Outcome measurements

Main outcome was the Threshold-Discrimination-Identification (TDI) score on the objective Sniffin' Sticks Test (SST). The TDI score is the sum of three different tests: a Threshold (score 1-16), Discrimination (score 0-16) and Identification test (score 0-16). The TDI score ranges from 1-48, a higher score is considered as a better olfactory function. Scoring ≤ 16 points is considered as anosmia, ≤ 30.5 as hyposmia and ≤ 41.25 as normosmia. Scoring 41.5 points or above is considered as a super smeller [29]. A difference of 5.5 on TDI score was considered a clinically relevant difference [30].

Secondary objective outcome was gustatory function, measured by the Taste Strip Test (TST), which assesses recognition thresholds and identification of the four basic tastes; sweet, salty, sour and bitter. The total score ranges from 0 to 16, with high scores indicating a better taste function. Clinical improvement was defined as a score increase of >2 points [31]. The secondary subjective outcomes were assessed through several validated questionnaires and self-reported scales. The questionnaires included the Sino-Nasal Outcome Test-22 (SNOT-22), a visual analog scale (VAS) for self-reported smell, taste, and trigeminal sensations, and the Olfactory Disorders Questionnaire (ODQ). These questionnaires were used to measure olfactory, gustatory and trigeminal function, the impact of smell/taste changes on quality of life, and nasal symptoms. The outcomes were assessed at first, second and third visit. Further details on the outcome measurements, examinations, and questionnaires can be found in the protocol, section 7.5 and 8.1.2 [27]

Statistical analysis

The analysis was conducted using IBM SPSS Statistics 26.0.0.1. For this follow up study no sample size was calculated. We used descriptives to report the data. Since the data was not-normally distributed, we used medians with interquartile ranges (IQR).

Results

Patients

All 113 patients who completed the COCOS trial (first and second visit) were approached to participate for this follow-up study for which a third visit was required. There were 36 (31.9%) drop-outs, leading to 77 (68.1%) participating patients for this follow-up study and completing a third measurement (Figure 2).

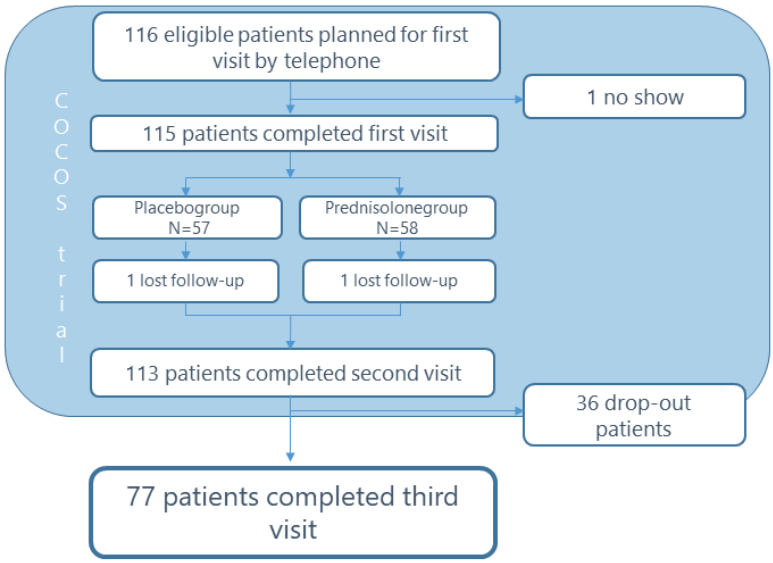

Figure 2. Participant flow-chart.

Drop-out patients

Out of the original COCOS cohort, we did not conduct a third measurement at one year after diagnosis in 36 patients. Of these patients, 23 did not respond to the invitation to participate in this follow-up study. Additionally, three participants declined due to poor olfactory function, while one participant declined due to excellent olfactory function. Nine participants had personal reasons, such as relocation, lack of time, or physical limitations which prevented them from participating. Table 1 describes the characteristics and outcome measurements at second visit compared between participating patients and patient drop-outs. Descriptive data at baseline, between the prednisolone and placebo arm are described elsewhere [27]. Age and sex of both participating and drop-outs were comparable. Median age of participants in this study was 52 years old (IQR 42-59) and in the drop-out patients 45.5 years old (IQR 38.8-56.5). Median TDI score on second visit was 28.0 (IQR 23.5-30.25) in participants and 27.0 (IQR 25.38-29.69) in drop-outs. In both participating and drop-out patients the median TST at second visit was 11 (IQR 9-13) Scores on quality of life, self-reported smell and taste tests and nasal symptoms seemed more favorable in patients who dropped out.

	Participating patients N=77	Drop out patients N=36
Age, years	52 (42-59)	45.5 (38.8-56.5)
Sex		
Female	51 (66.2)	21 (58.3)
Male	26 (33.8)	15 (41.7)
TDI score	28.0 (23.5-30.25)	27.0 (25.38-29.69)
TST score	11 (9.5-13)	11 (9-13)
VAS score		
Sense of smell	2.8 (1.4-5.8)	4.35 (1.8-6.6)
Sense of taste	4.9 (1.6-7.2)	5.6 (3.0-8.0)
ODQ	0.38 (0.27-0.53)	0.37 (0.23-0.47)
SNOT-22	19 (10-30)	17 (8.5- 3)

Table 1. Characteristics and outcome measurements at second visit compared between participating patients and patient drop-outs. TDI score; Threshold-Discrimination-Identification score; TST; Taste Strip Test; VAS; Visual Analogue Scale; ODQ; Olfactory Disorders Questionnaire; SNOT-22; Sino-Nasal Outcome Test. Data are presented as median (IQR) or n (%). Outcome ranges were as follows: TDI 1-48; TST 0-16; VAS 0-10; ODQ 0.13-1.0; SNOT-22 0-110.

Characteristics participating patients

Table 2 describes characteristics and outcomes during this follow-up study (third visit), and during the original COCOS trial (first and second visit) in order to compare outcomes. At third visit, median age was 51 years (IQR 41-58). Among 77 patients, 51 (66.2%) were female and 26 (33.8%) were male. At least one COVID-19 vaccination was administered to 62 patients (80.5%). Median time between COVID-19 diagnosis and third visit was 368 days (IQR 355-379). Between second and third visit, 21 patients have had a reinfection, with mild or no complains. We did not collect specific data on the frequency and duration of olfactory training after the second visit, but 22 patients stated that they continued olfactory training on occasion.

Outcomes

Table 2 provides outcome measurements. The median TDI score started at 21.25 (IQR 18.25- 24.75) at baseline and increased to 27.5 (23.63-30.0), at six months after COVID-19 (Figure 3) [26].

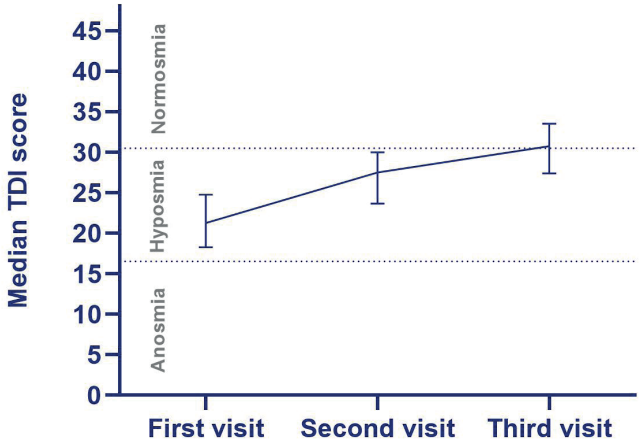

Figure 3. TDI scores over time.

The findings of this study show that one year following COVID-19, the median TDI score was 30.75 (IQR 27.38-33.5), regarded as normosmia (Figure 3). The degree of improvement decreases over time, the overall improvement of 9.5 points on the TDI score exceeds the minimum clinically important difference of 5.5 points [30]. Reported VAS score (1-10) on sense of smell, increased from 1.2 (IQR 0.4-3.0) at baseline to 3.2 (IQR 1.4-6.0) at six months to 6.1 (IQR 2.7-7.5) after one year.

The median score on TST one year after COVID-19 was 12 (IQR 9-14), starting with 10 (IQR 7-12) at baseline and 11 (9.0-13.0) at six months after diagnosis.

Self-reported VAS score on sense of taste also showed improvement, starting at 3.4 (IQR 1.1-5.7) and increased to 5.3 (IQR 2.3-7.7) at six months, to 7.0 (IQR 3.4-7.9) after one year. The ODQ score reduced to 0.30 (IQR 0.21-0.43) one year after COVID-19 in comparison with 0.48 (IQR 0.41-0.57) at baseline and 0.38 (IQR 0.26-0.53) at six months. Nasal symptoms scored 17.0 (IQR 8.5-30.0) at SNOT-22 questionnaire and improved with 4 points in comparison with the baseline score of 21.0 (IQR 14.0-39.0). Although not the focus of our study, median TDI score for patients who received a placebo (N=35, with 22 missing) was 31.5 (IQR 27.5-33.5). For patients who received the prednisolone (N=41, with 15 missing) the median TDI score was 30.0 (IQR of 26.5 to 33.5).

	First visit (<3 months after diagnosis) N=115	Second visit (±6 months after diagnosis) N=113	Third visit (1 year after diagnosis) N=77
Age, years	48 (41-57)	50 (40.5-57)	51 (41-58)
Sex			
Female	73 (63.5)	72 (63.7)	51 (66.2)
Male	42 (36.5)	41 (36.3)	26 (33.8)
Time from confirmed COVID test, days	43.0 (42.5- 69.5)	140 (128-154)	368 (355-379)
Time from start of smell loss, days	55.0 (42.0-66.8)	137 (126.3-152)	365.5 (352-376.8)
Sino-nasal Outcome Test (SNOT-22)	21.0 (14.0-39.0)	18.0 (10-28)	17.0 (8.5-30.0)
Sniffin' Stick Test (SST)			
TDI score	21.25 (18.25- 24.75)	27.5 (23.63-30.0)	30.75 (27.38-33.5)
Threshold	1.5 (1.0-3.5)	4.5 (3.3– 5.6)	6.25 (4.1- 7.5)
Discrimination	9.0 (8.0-11.0)	11.0 (10.0-13.0)	12.0 (10.0-13.0)
Identification	10.0 (8.0-11.0)	11.0 (10.0-13.0)	13.0 (11.0-14.0)
Taste Strip Test (TST)			
Total score	10.0 (7.0-12.0)	11.0 (9.0-13.0)	12.0 (9.0-14.0)
Sweet	4.0 (2.0-4.0)	4.0 (3.0-4.0)	3.0 (3.0-4.0)
Sour	2.0 (1.0-3.0)	2.0 (2.0-3.0)	2.0 (2.0-3.0)
Salty	2.0 (1.0-3.0)	3.0 (2.0-4.0)	3.0 (2.0-4.0)
Bitter	2.0 (1.0-3.0)	3.0 (2.0-4.0)	3.0 (2.0-4.0)
Olfactory Disorders Questionnaire (ODQ)			
Total score	0.48 (0.41-0.57)	0.38 (0.26-0.53)	0.30 (0.21-0.43)
Self-reported Visual Analogue Scale (VAS)			
Sense of Smell	1.2 (0.4-3.0)	3.2 (1.4-6.0)	6.1 (2.7-7.5)
Sense of Taste	3.4 (1.1-5.7)	5.3 (2.3-7.7)	7.0 (3.4-7.9)
Trigeminal Sensations	4.5 (2.2-6.6)	5.3 (2.8- 7.6)	6.10 (2.5-8.0)

Table 2. Characteristics and outcome measurements over time. Data are presented as median (IQR) or n (%). SNOT-22; Sino-Nasal Outcome Test; TDI score; Threshold-Discrimination-Identification score; SST; Sniffin' Sticks Test; TST; Taste Strip Test; ODQ; Olfactory Disorders Questionnaire; VAS; Visual Analogue Scale. Outcome ranges were as follows: SNOT-22 0-110; TDI 1-48; T 1-16; D 0-16; I; 0-16; TST 0-16; Sweet, Sour, Salty, Bitter 0-4; ODQ 0.13-1.0; VAS 0-10.

Discussion

We aimed to investigate the prognosis of patients with COVID-19 induced olfactory disorders. The results of this study demonstrate a favorable outcome, with a median TDI score on SST of 30.75 (IQR 27.38-33.5) one year after diagnosis, regarded as normosmia. Total improvement in TDI score between three months and one year after COVID-19 was 9.5 points, which exceeded the minimum clinically important difference of 5.5 points [30]. This

indicates a continued recovery of olfactory function, even after a prolonged time. We already knew improvement on smell function occurs during the initial period following COVID-19 [26]. This study showed a persisting recovery process a year after diagnosis, albeit with deceleration over time. While the recovery to normosmia seems promising, it does not necessarily mean that every individual will regain their pre-COVID-19 sense of smell.

Our secondary outcomes demonstrated ongoing improvement as well. This included continued improvement on objective gustatory function on TST, as well as improvements in self-reported quality of life, sense of smell- and taste. The favorable outcome observed in our study is likely due to a natural course of recovery, although it is also possible that the olfactory training our cohort performed in the early phase after COVID-19 may have had a positive effect.

Other studies using psychophysical tests, lack long-term follow-up [13,23], and studies that do have long-term follow-up lack psychophysical measurements [9–12,14–16,32]. There have been studies with psychophysical tests performed a long time after COVID-19, however without comparative baseline data. One study utilized the psychophysical University of Pennsylvania Scent Identification Test (UPSIT) one year after COVID-19, which had a median score of 31 (IQR = 5.0) [17]. This is categorized as mild hyposmia in the UPSIT score [33]. Another case-control study conducted a SST at 401 days after COVID-19, showing a median TDI score of 31.5 [24]. Our TDI scores one year after COVD-19 fit within the range of these TDI scores. Two other studies performed the SST from one up to two years after COVID-19 and found that while some individuals continued to recover over time, others still exhibited olfactory disorders after two years [25,32]. Finally, one study compared olfactory disorders between patients in the first and second waves of COVID-19 using extended SST at various time points after infection, and found just like our results that most recovery occurred in the early stage after COVID-19 [34].

While these studies contribute to our understanding of the course of olfactory disorders following COVID-19, they either lack comparable baseline data, or extended psychological tests, or the patients included did not initially suffer from objective olfactory disorders after COVID-19. As a result, their study designs are not intended to observe the course of olfactory function in patients with olfactory disorders over time.

With this study we present the one year results of a cohort with COVID-19 induced olfactory disorders. We conducted psychophysical testing at baseline, intermediate and one year follow-up stages of COVID-19. This allows for a comparative analysis of TDI scores over time, providing objective data on improvement. We used a standardized protocol, combining objective and self-reported subjective data. Thereby, covering all outcomes important for

assessing the course of COVID-19 induced olfactory disorders. Besides, our study was solely focused on patients with smell disorders following COVID-19. This ensured that patient participation was not biased towards any other particular post-COVID-19 syndrome. Additionally, all patients had a confirmed COVID-19 diagnosis and follow-up period was prolonged.

There are however some restrictions of our study to take in account. The most important one being that the one year measurement was not foreseen in the initial set-up of the study, therefore the outcomes were not taken into account in the first manuscript [26]. This might also reflect in the participation numbers to the one year measurement. Due to some patients' dropouts, there may be some possible patient selection bias in this follow-up study. We were unable to determine for all of the drop-out patients, their reasons for declining to participate in this follow-up study. Though, as reported, drop-outs and participants were comparable in characteristics. The study's generalizability could also be restricted as it solely included participants from the Netherlands. Besides, the inquiry into the impact of COVID-19 reinfections on the recovery process is an intriguing question. We inquired with our participants regarding COVID-19 reinfections, but the results in the third measurement phase were unreliable due to inconsistent testing practices and reduced testing needs. Secondly, because of the timing of our study, patients were mainly infected by the Delta-variant. COVID-19 variants and vaccination status might influence the speed and extent of recovery. Thirdly, during the cohort recruitment phase, the prevailing knowledge suggested that most patients spontaneously recover their normal smell and taste function within four weeks [35]. Therefore, intervening during this period carried the risk of overtreatment. Additionally, the use of prednisolone to manage COVID-19 could potentially inhibit the immune system and prolong the infection. In light of our current knowledge, we might have considered enrolling participants with a more extended duration of persistent smell loss as eligibility criteria [9,36].

Lastly, it should be noted that approximately half of the participants in our follow-up study was earlier treated with ten days of 40mg oral prednisolone in the context of the COCOS trial. Since no effect of prednisolone was shown on olfactory function, it is unlikely to have influenced our outcomes.

In terms of clinical implications, patients could be reassured by the findings of our study, which indicate a continued recovery of olfactory function after a year, albeit at a slower rate over time. Not only objective results are favorable, quality of life and self-reported smell function improved as well. Despite this promising news for patients, healthcare workers face a challenge due to the large numbers of patients suffering from olfactory disorders with limited treatment options. Considering the findings of McWilliams [11], which showed

self-reported sense of smell two years after COVID-19 with 7.5% reporting no recovery, future research may further prolong follow-up period with psychophysical tests. Above that, the potential impact of olfactory training on the observed improvements in this cohort warrants further investigation.

Conclusion

Our study demonstrated a continued recovery process on COVID-19 induced olfactory disorders, one year after diagnosis. Median objective smell function surpassed the threshold of normosmia and rate of improvement between baseline and the one year measurement was considered as clinically relevant. However the rate of improvement reduces over time. Aside from smell function, objective gustatory function on Taste Strip Tests continued to improve after a year, as did self-reported quality of life and sense of smell- and taste function.

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7

A comparative analysis of the incidence, severity and duration of smell and taste loss in COVID-19 cases versus non-COVID-19 cases: a longitudinal cohort study.

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Abstract

The COVID-19 pandemic has raise awareness of olfactory and gustatory disorders. However, these symptoms can also be caused by various other factors. In this study we aimed to compare the incidence, severity and duration between COVID-19 related and non-COVID-19 related smell and taste disorders. We conducted a longitudinal cohort study using data from the Dutch biobank Lifelines, which includes over 167,000 participants. The data were collected using 27 questionnaires distributed between March 2020 and May 2022. Descriptive data and the incidence of smell and taste loss in both groups were calculated. To visualize the proportion of severity rates of symptoms, a heatmap was created. A survival analysis was conducted and presented in a reversed Kaplan–Meier curve to show the probability of having persistent smell loss in both groups. The study included 235,722 participants. The incidence of smell loss was higher in the COVID-19 positive group, when compared to the COVID-19 negative group. We found varying degrees of symptom severity in COVID-19 positive cases, ranging from mild to severe, while non-COVID-19 related cases mostly reported mild symptoms. The survival outcome for smell and taste loss was 0.12 (SE 0.03, 95% CI 0.07–0.21) in COVID-19 related cases, and was 0.17 (SE 0.03, 95% CI 0.12–0.24) in cases related to other causes. This study reveals a higher incidence and severity of smell and taste loss in individuals with COVID-19 compared to non-COVID-19 related cases. However, non-COVID-19 related smell and taste loss tend to have a longer duration.

Introduction

The COVID-19 pandemic has exposed the world to unusual symptoms, namely olfactory and gustatory disorders. The prevalence of non-COVID-19 related smell disorders is reported to be between 1% and 3% [1–3]. These causes are mainly sinonasal disorders, post-infectious or post-traumatic disorders, aging and neurodegenerative diseases [1,3–9]. A recent study revealed that one-third of a general 65-year-old population suffered from impaired smell function and more than one-quarter from impaired taste function [4]. The loss of smell or taste can greatly influence health and overall well-being [5,6]. In COVID-19 cases, smell and taste disorders are among the most common symptoms experienced, which sheds light on the importance of these senses [7,8]. Changes or (partial) loss of the sense of smell or taste have been added to the clinical screening profile for COVID-19 and to the official list of symptoms that persist after COVID-19 by the Centers for Disease Control and Prevention and the World Health Organization [9]. In the majority of individuals with COVID-19, related smell or taste loss recover to normal levels after a few days or weeks [10]. However, it has been established that, 90–150 days after infection, 7.6% of patients still experienced at least a moderate severity of these symptoms (scored on a Likert scale of 1–5) [11]. While COVID-19 induced smell and taste disorders are regularly studied, there is a large heterogeneity in these studies, resulting a large variety in their outcomes. This is mainly due to differences in the studied population, reporting systems, healthcare systems, the method of diagnosing COVID-19 and measurement of smell and taste function. Most follow-up data are based on hospitalized COVID-19 patients, which gives a different perspective than information from the general population. Regrettably, data from non-hospitalized patients are not often reported [12]. Moreover, prior to the pandemic, there was limited attention paid to olfactory and gustatory disorders in clinical practice and research [13]. The presence of all of these factors poses challenges when conducting research to compare the course of smell loss between COVID-19 and non-COVID-19 cases. Therefore, we aim to determine the incidence, severity and duration of COVID-19 induced smell and taste loss, and compare this with non-COVID-19 related smell and taste loss in a general population.

Methods

Study Design and Participants

We conducted a longitudinal cohort study. The data used for this study were collected from a Dutch biobank called Lifelines. Lifelines is a multi-disciplinary prospective population-based cohort study examining in a unique three-generation design the health and health-related behaviors of 167,729 persons living in the north of the Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic,

behavioral, physical and psychological factors that contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics [14,15]. Participants were recruited by general practitioners or online self-registration. Data from participants was assessed by using digital questionnaires. Lifelines uses rigorous protocols for comprehensive data collection to ensure reliability and accuracy of the data [15,16]. The Medical Ethical Committee of University Medical Center Groningen (2007/152) approved the Lifelines cohort study [14–17]. All Lifelines participants signed informed consent [16]. The Lifelines study does not use inclusion criteria; however, severe mental illness, short life expectancy (<5 years), the inability to visit a general practitioner and insufficient understanding of the Dutch language are exclusion criteria [14]. Since April 2020 additional COVID-19 questionnaires have been sent out to Lifelines participants. These participants were at least 18 years old and able to complete digital questionnaires via a valid email address [14,15,17]. Follow-up questionnaires were sent out once a week (questionnaire 1–6) and later on a bi-weekly or monthly basis (questionnaire 7–26) [17]. More than 305,500 Lifelines participants were invited to fill in the COVID-19 questionnaires used for this study. Over time, these questionnaires were adjusted; new, relevant topics on COVID-19 were added and some questions were omitted [17].

Procedures

Participants were asked to indicate the extent to which they experienced various symptoms over the past seven days, from March 2020 to May 2020. From May 2020, in order to align with the regular questionnaire distribution schedule (bi-weekly or monthly), the timeframe was adjusted to the past 14 days. Loss of sense of smell or taste was assessed with a 5-point Likert scale (1 = not at all, 5 = extremely). Data were collected from a total of 27 different questionnaires administered between 30 March 2020 and 4 May 2022 (Appendix A). The questionnaires were conducted digitally, and all data, except for age and gender, were self-reported. The definition of a COVID-19 diagnosis has evolved over time. Initially, until 15 May 2020 (questionnaire 7), it was based on a doctor's diagnosis, due to limited testing options in the Netherlands until August 2020 [18]. From 13 October 2020 to 13 October 2022 (questionnaires 6–14), a diagnosis was established through a doctor's diagnosis or through a positive PCR test conducted in a healthcare facility. During the period of questionnaire 14 to questionnaire 20 (13 October 2022 to 26 April 2021), only a PCR test conducted in a healthcare facility was considered as a COVID-19 diagnosis, therefore the question about a doctor's diagnosis was omitted. From 26 April 2021 (questionnaire 20) until 4 May 2022 (questionnaire 26), a positive self-administered home test or a positive PCR test conducted at any organization, such as official testing for events, at work, or at school was considered as a COVID-19 diagnosis. Therefore, individuals who did not receive a doctor's diagnosis during the specific timeframes when this was required to determine their COVID-19 status, or those who did not undergo testing from questionnaire 14 onwards,

were unable to ascertain whether they were positive or negative. Consequently, this led to missing data for these patients, as their COVID-19 status remained unknown.

Statistical Analysis

All analysis were conducted using SPSS 26.01 and R 4.2.2 statistics software. Data from all 27 different questionnaires were combined and transformed into one dataset. Descriptive statistics were calculated, comparing participants who reported ever experiencing smell loss with participants who never reported experiencing smell loss. The incidence of COVID-19 positive and COVID-19 negative individuals was calculated for every separate questionnaire, each representing a time moment. The incidence is counted as the number or percentage of new cases per moment, and can differ at each time point. To visualize the proportion of the severity rates, a heatmap was created using R 4.2.2 statistics software [19,20]. The time for the heatmap is recoded as the moment when the participants first experience smell or taste loss and not based on the moment of completing the questionnaire. Moments 1 to 10 represent the 10 subsequent questionnaires following onset of these symptoms. The reason for including only the 10 subsequent questionnaires after onset of symptoms is due to a high number of participants who had reported no longer experiencing smell loss or due to missing data. To investigate the duration of smell loss in both COVID-19 positive and COVID-19 negative participants, a survival analysis was conducted and visualized by the reversed Kaplan–Meier method using R 4.2.2 statistics software. As well as in the heatmap, only participants who ever reported experiencing smell or taste loss were included in the survival analysis. Having smell or taste loss was defined by a score of 2 or higher on the Likert Scale (1 = not at all, 2 = a little bit, 3 = moderately, 4 = quite a lot, 5 = severely bothered by the symptom). To account for the missing data, participants were combined into two groups (those who ever reported being COVID-19 positive and those who always reported being COVID-19 negative). This grouping was based on all collected data of the 27 questionnaires used for this study, regardless the timing of the participants' diagnosis (Appendix A). This amalgamation was necessary to compare these two groups in both the heatmap and in the survival analysis. In these analyses, we matched these two groups (COVID-19 positive or negative) to their reported smell loss.

Results

The overall average response rate for all questionnaires was 36.3%. The average number of responding participants per questionnaire was ~35,000 responders, with the initial questionnaire consisting of ~53,000 responders, gradually reduced to ~20,000 responders in the final questionnaire [17]. The total number of patients who responded to the COVID-19 questionnaire at least once was 235,722. The median age was 56 years (IQR 49–65). Of the participants, 13,058 (5.5%) reported having smell loss at least once, and 96,300 (40.9%) participants reported never having smell loss. In 126,364 participants (53.6%), this information was missing. Of the patients who ever had smell or taste loss, 6480 participants (49.6%) were female and 3809 (29.2%) were male. There were 4668 (35.8%) participants who were ever diagnosed with COVID-19 and 7110 (54.4%) participants who were never diagnosed with COVID-19; for 1280 patients (9.8%), this information was missing (Table 1).

	Ever smell/taste loss n=13058	**Never smell/taste loss n=96300**
Age, years	56 (48-64)	57 (49-65)
Female	6480 (49.6%)	46769 (48.5%)
Male	3809 (29.2%)	29740 (30.9%)
Missing	2769 (21.2%)	19791 (20.6%)
Ever had COVID-19	4668 (35.8%)	8045 (8.4%)
Never had COVID-19	7110 (54.4%)	54122 (56.2%)
Missing	1280 (9.8%)	34133 (35.4%)
Hospitalized	158 (1.2%)	104 (0.1%)
Not hospitalized	4598 (35.2%)	8880 (9.2%)
Missing	8302 (63.6%)	87406 (90.7%)

Table 1. Descriptive data. Data are presented as median (IQR) or as n (%).

Figure 1 shows the number of COVID-19 positive participants (orange bars) and the number of COVID-19 negative participants (blue bars) per time point, starting at questionnaire 1 (30 March 2020) and going up to questionnaire 26 (4 May 2022). Figure 1 also demonstrates the percentage of participants with smell or taste loss in both the COVID-19 positive group (orange line) and COVID-19 negative group (blue line) for each questionnaire.

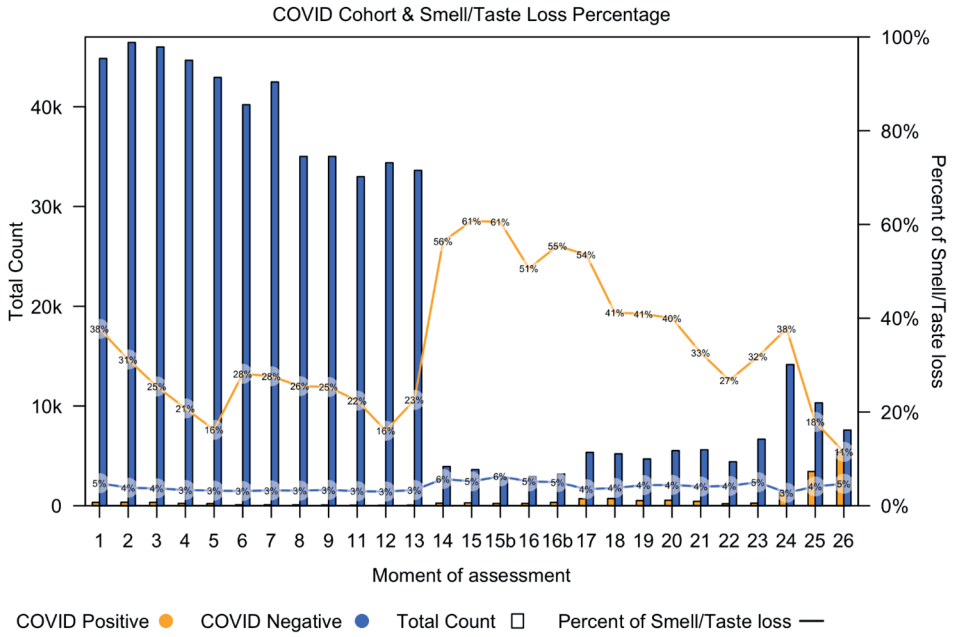

Figure 1. Incidence of participants categorized as COVID-19 positive or COVID-19 negative from first questionnaire (30 March 2020) up to last questionnaire (4 May 2022). The x-axis shows the subsequent questionnaires, each representing a different moment in time. Questionnaire 10 did not contain information about smell and taste or COVID-19 (Appendix A). The orange bars demonstrate the total count of COVID-19 positive participants and the blue bars the total count of COVID-19 negative participants. The orange line demonstrates the percentage of COVID-19 positive participants with smell or taste loss, and the blue line demonstrates the percentage of COVID-19 negative participants with smell or taste loss.

From timepoint 14, the number of participants decreased, which is attributed to changes in the criteria for considering a COVID-19 positive or negative status. A doctor's diagnosis was omitted from the questionnaire and only patients with a PCR test were included. As a result, patients without a PCR test were considered as missing data, leading to a reduced number of participants classified as either having or not having COVID-19. From questionnaire 20 onwards, self-administered home tests were added into the questionnaires. All participants, regardless of being tested for COVID-19, filled in the questions about smell or taste loss. This could be the reason for the increased incidence of smell loss in COVID-19 cases at timepoint 14. As shown in the figure, the total number of COVID-19 negative participants is higher than the number of COVID-19 positive participants at every time point. The incidence of smell loss was higher in the COVID-19 positive group, as a consistently higher percentage of COVID-19 diagnosed participants were experiencing smell loss at all time points.

Figures 2 and 3 demonstrate the proportion of the severity of smell or taste loss within the COVID-19 positive (Figure 2) and the COVID-19 negative (Figure 3) group. The y-axis presents

the rate of the severity. The x-axis time values are recoded; moment 1 represents the point when participants initially experienced a loss of smell or taste, irrespective of the questionnaire completion moment. The subsequent time points correspond to subsequent questionnaires in which the participants reported their ongoing loss of smell. In COVID-19 related smell or taste loss, the severity was widely distributed from 'a little bit' to 'severely' (Figure 2). In non-COVID-19 related smell or taste loss, the severity was primarily reported as 'a little bit' (Figure 3). These findings show that participants with COVID-19 related smell or taste loss experienced a higher severity than participants with other causes of these symptoms.

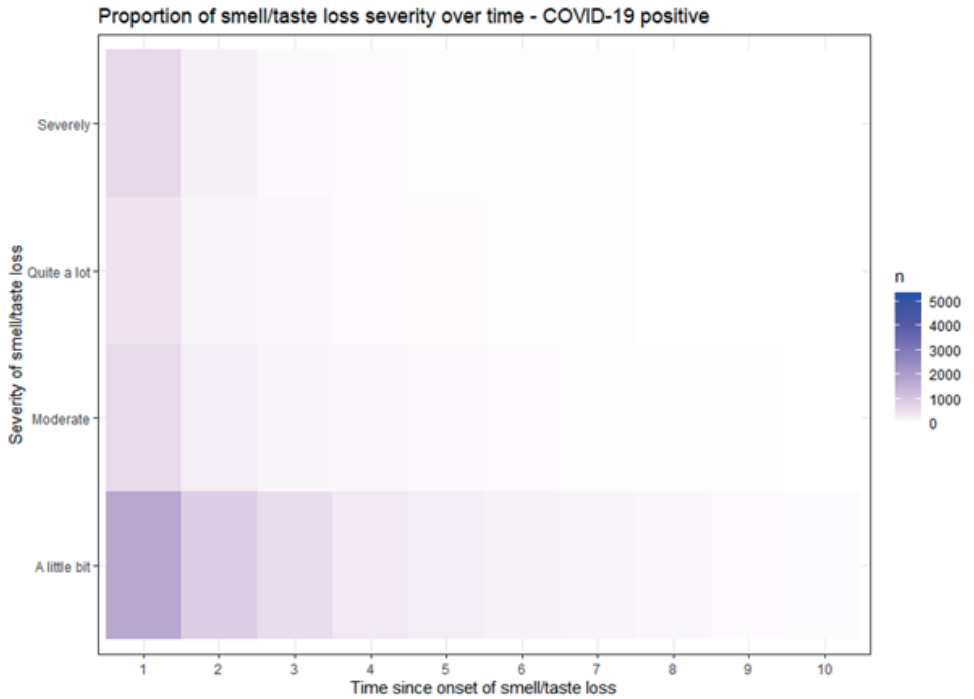

Figure 2. Proportion of severity of smell or taste loss in COVID-19 positive participants, presented in a heatmap. The y-axis shows the rate of severity of smell loss. The time on the x-axis is recoded and not based on the moment of questionnaire assessment, but on the onset of smell or taste loss and subsequent questionnaires with reported ongoing symptoms.

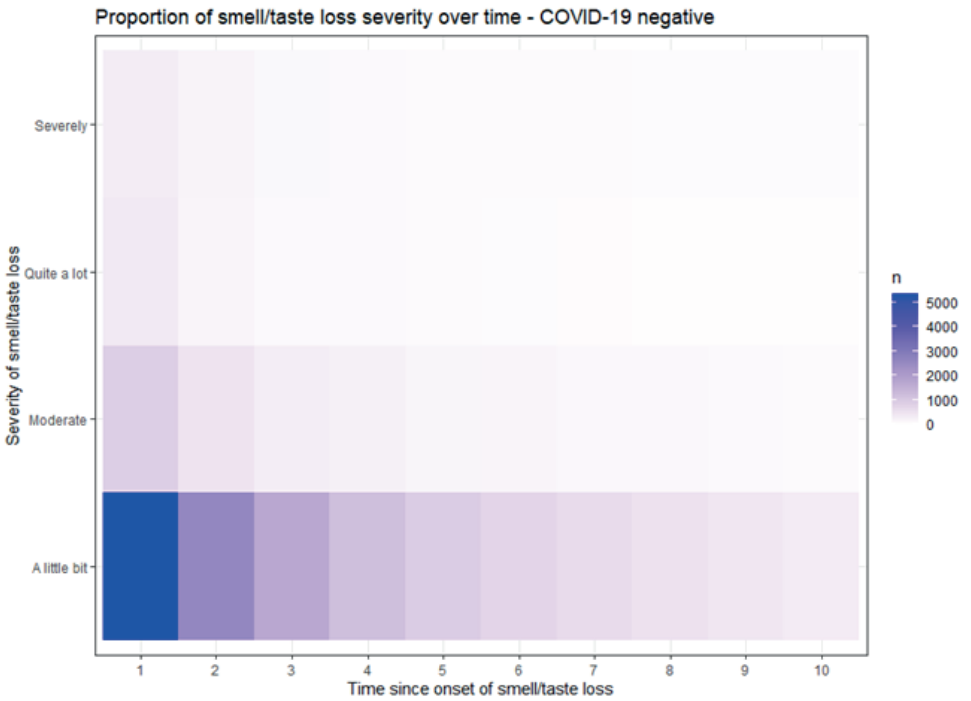

Figure 3. Proportion of severity of smell or taste loss in COVID-19 negative participants, presented in a heatmap. The y-axis shows the rate of severity of smell loss. The time on the x-axis is recoded and not based on the moment of questionnaire assessment, but on the onset of smell or taste loss and subsequent questionnaires with reported ongoing symptoms.

The duration of smell or taste loss in both groups was analyzed in a survival analyses and presented in a reversed Kaplan–Meier curve (Figure 4). At the latest measured time point with ongoing symptoms, the survival outcome for COVID-19 related smell or taste loss was 0.12 (SE 0.03, 95% CI 0.07–0.21). The survival outcome for non-COVID-19 related smell or taste loss was 0.17 (SE 0.03, 95% CI 0.12–0.24). These findings suggest that non-COVID-19 related cases exhibit a more prolonged duration, compared to those associated with COVID-19.

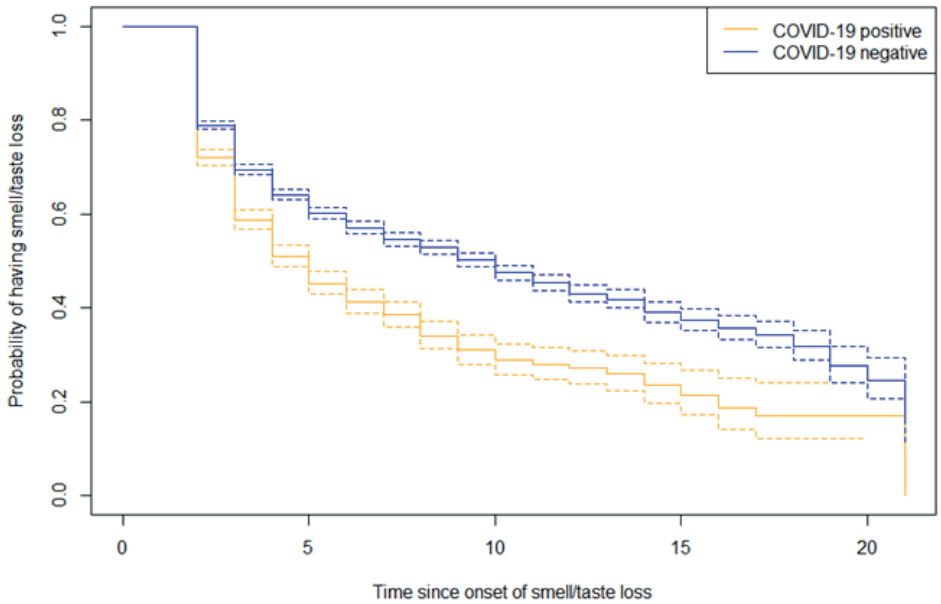

Figure 4. Survival analysis expressed in a reversed Kaplan–Meier curve. The y-axis shows the probability rate of having smell or taste loss. The time on the x-axis is recoded and not based on the moment of questionnaire assessment, but on the onset of smell or taste loss and subsequent questionnaires with reported ongoing symptoms.

Discussion

There are three main findings of this study. Firstly, we found a higher incidence of smell or taste loss among participants diagnosed with COVID-19, compared to those without COVID-19. It is noteworthy that the majority of participants, whether COVID-19 positive or negative, did not experience these symptoms. Secondly, we observed that participants with COVID-19 related smell or taste loss experienced more severe symptoms, when compared to non-COVID-19 related cases. This could be attributed to the fast and complete onset of olfactory loss in COVID-19, resulting in significant challenges in daily life [21,22]. Thirdly, the

duration of smell or taste loss demonstrated a more favorable outcome in COVID-19 participants, as non-COVID-19 related cases were estimated to have a higher likelihood of prolonged symptoms. Consequently, individuals with smell or taste loss originating from other causes than COVID-19 may be prone to extended symptoms or potentially no complete recovery. This is not surprising, since common causes of non-COVID-19 related smell loss are mostly chronic conditions such as sinonasal disorders, post-infectious or post-traumatic disorders, aging or neurodegenerative diseases [1,3,4,13,23–25]. The nature of this study, that relied on questionnaire data collected at various time points, comes with certain methodological limitations. The first is missing data, which resulted in temporal gaps between the presented time points. In the heatmap and survival analysis, we therefore modified the time variable to subsequent completed data since onset of symptoms. Furthermore, it is important to acknowledge the possibility that participants may be COVID-19 positive but experience non-COVID-19 related conditions such as rhinosinusitis or allergies. In such situations, participants were allocated to the COVID-19 positive group. Alongside the fact that there were variations in criteria used for determining COVID-19 positivity or negativity throughout the study period, these factors may have led to either an underestimation or overestimation of the true underlying cause of smell and taste loss. Despite the inherent challenges associated with data collection through questionnaires, the Lifelines cohort consists of a large and diverse sample from a general population, including multiple repeated measurements, making it highly representative of the general population in the Netherlands. Especially in comparison to other studies where only the relevant concerned participants were included [26–29], this analysis incorporated a broad group of individuals, making it possible to compare COVID-19 positive cases with COVID-19 negative cases. Remarkably, a large group of people with non-COVID-19 related smell or taste loss was discovered in this study. These patients have been under the radar for a long time. Before the pandemic, no questions regarding smell or taste were included in any Dutch cohort [14–17,30–35]. Thanks to the impact of COVID-19, the senses of smell and taste have now gained attention, as well as the unfortunate consequences associated with their loss. It is important to use this momentum to focus on patients suffering from this invalidating loss, no matter the cause.

Conclusions

The incidence of smell or taste loss is higher and more severe when induced by COVID-19 in comparison to non-COVID-19 related smell or taste loss, but the duration is longer in non-COVID-19 related causes.

Appendix A

Table A1 shows the 27 COVID-19 questionnaires (COVQ) used for this study, with the time period of assessment and their response rates [17]. Questionnaire 10 did not contain questions about smell/taste or COVID-19 and is therefore not included. Table A1. The 27 COVID-19 questionnaires (COVQ) used for this study.

COVQ	Date	Response	Response Rate
1	30 March to 23 April 2020	~53,000	41%
2	2 April to 6 May 2020	~51,000	39%
3	12 April to 6 May 2020	~50,000	38%
4	16 April to 13 May 2020	~47,000	36%
5	19 April to 20 May 2020	~45,000	35%
6	28 April to 27 May 2020	~43,000	33%
7	15 May to 29 May 2020	~45,000	34%
8	23 May to 24 June 2020	~38,000	34%
9	11 June to 29 June 2020	~37,000	33%
10	7 July to 29 July 2020	~33,000	30%
11	10 July to 5 August 2020	~35,000	32%
12	24 July to 2 September 2020	~36,000	32%
13	08 September to 30 September 2020	~35,000	32%
14	13 October to 4 November 2020	~34,000	31%
15	02 November to 26 November 2020	~34,000	31%
15 b	15 November to 10 December 2020	~33,000	30%
16	2 December to 21 December 2020	~31,000	28%
16 b	8 December to 5 January 2021	~32,000	29%
17	5 January to 8 February2021	~34,000	31%
18	25 February to 25 March 2021	~32,000	49%
19	29 March to 22 April 2021	~29,000	45%
20	26 April to 20 May 2021	~29,000	44%
21	25 May to 18 June 2021	~29,000	45%
22	4 July to 30 July 2021	~24,000	36%
23	11 October to 4 November2021	~23,000	37%
24	20 December 2021 to 12 January 2022	~25,000	48%
25	28 February to 24 March 2022	~20,000	38%
26	11 April to 4 May 2022	~20,000	40%

Supplemetary Material

Lifelines Corona Research Initiative:

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8

Summary & General Discussion

Summary

Our sense of smell is often underestimated, yet it plays a crucial role in our lives, influencing overall well-being, eating behavior, social interactions, emotions, and memories. The COVID-19 pandemic has brought attention to its significance, with the elevated occurrence of affected individuals suffering from smell loss. Health care providers face a challenge with this burden and are unprepared to provide comprehensive assistance to these patients. This thesis provides a comprehensive insight into the diagnosis, therapy, epidemiology, and clinical course of COVID-19-induced smell loss, shedding light on this important sense that has gained newfound attention in recent times. We examined the following research questions:

I) Is it possible to detect smell loss caused by COVID-19, using a screening olfactory test?

II) Is there an effective treatment for COVID-19 induced olfactory disorders?

III) What is the incidence, severity, and course of COVID-19 induced olfactory disorders, and how do they compare to olfactory disorders unrelated to COVID-19?

Part I Diagnostics

In **Chapter 2** we assessed the diagnostic accuracy of the screenings version of the validated Sniffin' Sticks Test (SST-12) in patients with olfactory disorders caused by COVID-19.

While the validated extended olfactory tests like the Sniffin' Sticks Test (SST) are well-established for evaluating COVID-19 induced olfactory function, their comprehensive time-consuming nature limits their suitability for routine clinical use. To address this limitation, we considered the potential reliability of the SST-12 as a shorter screening tool. The SST-12 serves as a diagnostic tool for screening olfaction in causes unrelated to COVID-19. As COVID-19 affects olfaction differently than most other causes, we explored its diagnostic accuracy in detecting smell loss in COVID-19 patients. We conducted a diagnostic accuracy study with a cohort of patients previously diagnosed with COVID-19. Approximately six months after their initial diagnosis, we used the outcome SST as the reference standard and the SST-12 as the index test. We assessed the diagnostic accuracy of the SST-12 using various diagnostic accuracy measures, including sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV). We found a high sensitivity (93.4%) and PPV (92.4%), and a moderately high specificity (68.2%) and NPV (71.4%). This suggests that the SST-12 holds promise as a valuable screening tool, especially for diagnosing individuals with loss of smell, also when induced by COVID-19. Nonetheless, it is necessary for healthcare professionals to have a good understanding of the interpretation of the results of the SST-12 when considering its implementation in clinical practice.

Part II Therapy

Our research was guided by the prevailing hypothesis which posited that COVID-19 triggered inflammation in the vicinity of the olfactory nerve, resulting in smell disorders. Previous studies indicated that corticosteroids, such as prednisolone, might reduce the impact of this inflammation, showing promising outcomes, especially in the early stages of olfactory disorders induced by COVID-19. However, these studies consisted of several limitations, and therefore the discussion whether or not to prescribe prednisolone remained. To establish the highest level of evidence for our research question—whether corticosteroids really have a positive effect on COVID-19 induced olfactory disorders- we conducted a randomized, double-blind, placebo-controlled trial with a carefully calculated sample size to ensure statistical rigor. This trial was called the COCOS trial: COrtisteroids for COvid-19 induced loss of Smell.

In **Chapter 4** we show the results of the randomized-controlled trial of which the protocol is provided in **Chapter 3**. We conducted a randomized, double-blind, placebo-controlled trial in the Netherlands. We included 115 eligible participants, aged >18 years, experiencing persistent (>4 weeks) olfactory disorders within 12 weeks after a confirmed COVID-19 diagnosis. The treatment group received oral prednisolone 40mg once daily for ten days and the placebo group received matching placebo. The allocation of participants into the prednisolone or placebo group was concealed from both researchers and patients. Additionally, all participants were instructed to engaged in olfactory training, with the aim to stimulate the regeneration of the olfactory nerve. During olfactory training, a patient sniffs a set of four known odors twice daily for a period of 3 months. Our primary outcome was the objective olfactory function, measured by the Threshold-Discrimination-Identification (TDI) score assessed with the Sniffin' Sticks Test (SST) 12 weeks after prednisolone treatment. Secondary outcomes included objective gustatory function, evaluated via the Taste Strip Test (TST), and self-reported olfactory and gustatory function, quality of life, and nasal symptoms. Our findings showed no difference in all these outcomes between the prednisolone and placebo group. We concluded that a prednisolone treatment does not improve olfactory function after COVID-19. Therefore, we recommend not to prescribe prednisolone for patients with persistent olfactory disorders after COVID-19. We did see, however, that the median TDI score improved over time in both groups.

The effectiveness of olfactory training in addressing post viral and posttraumatic smell disorders is well-established, yet its impact on COVID-19 induced smell disorders remains uncertain. We aimed to explore the potential benefits of olfactory training in patients experiencing COVID-19 induced smell loss. In **Chapter 5** we describe a case-control study for which we included two comparable cohorts. One group (n=111) performed olfactory training twice daily for a time-span of 12 weeks, with strict monitoring of therapy compliance.

They were provided with the olfactory training kit by us, along with detailed instructions, and were encouraged to perform the training. Conversely, the other cohort (n=50) did not perform olfactory training. To evaluate participants' olfactory function objectively, we employed the SST after the 12-week period. The main outcome was the TDI score obtained from the SST. Our findings revealed a statistically significant difference in olfactory function between patients who underwent the 12-week olfactory training and those who did not, suggesting beneficial outcomes of performing olfactory training. This difference was not clinically relevant. Intriguingly, we did not found any association between the frequency of performing smell training and olfactory function. To obtain more definitive and validated results, we recommend the pursuit of a randomized controlled trial, ideally with an extended follow-up period.

Part III Epidemiology and clinical course

Previous research primarily focused on the acute phase of these disorders, leading to inconsistent prognostic insights. In **Chapter 6** we investigated the persistence of olfactory disorders in COVID-19 patients one year after diagnosis. We performed a prospective cohort study with 77 patients who initially experienced COVID-19-induced olfactory disorders and participated in the COCOS-trial. Objective assessments were conducted one year post-diagnosis, with earlier measurements at three and six months as part of the COCOS trial, described in **Chapter 4**. The primary outcome was the TDI score obtained from the SST. Secondary measures included gustatory function on TST, self-reported olfactory and gustatory function, quality of life, and nasal symptoms. Our findings indicate that one year after COVID-19 diagnosis, improvement on the median TDI score continued, reaching a level categorized as normosmia. As well as the gustatory function on TST, self-reported olfactory and gustatory function, along with quality of life, and nasal symptoms continued to improve. This study demonstrates continuous improvement in both objective and self-reported olfactory and gustatory function in patients one year after COVID-19 diagnosis. The achievement of a normosmic TDI score is a positive outcome, though the rate of improvement reduces as time progresses.

In **Chapter 7** we performed a comparative analysis of the incidence, severity and duration of smell and taste loss in COVID-19 cases versus non-COVID-19 cases in a longitudinal cohort study. The research spanned over a two-year period from March 2020 until May 2022, and involved a large cohort consisting of 235,722 participants from the Dutch biobank Lifelines. We computed descriptive data and the incidence of smell and taste loss for both COVID-19 and non-COVID-19 related cases. To visualize the proportion of severity rates of symptoms, we created a heatmap for both groups. We conducted a survival analysis and presented this in a reversed Kaplan-Meier curve to show the probability of having persisting smell loss in both groups. This study revealed a higher incidence and severity rate of smell and taste loss

in individuals with COVID-19 compared to non-COVID-19 related cases. However, non-COVID-19 related smell and taste loss tended to have a longer duration. These patients have remained relatively unnoticed for an extended period. The COVID-19 pandemic has brought awareness to the significance of the sense of smell and taste, along with the unfortunate inconvenience coming with its impairment. It is crucial to raise awareness and provide support to patients suffering from this debilitating condition, regardless of its origin.

General discussion

Addressing the research gap

In 2020, the World Health Organization declared a global pandemic: COVID-19. Marked by over 700 million confirmed cases [1], with as most commonly reported symptom olfactory disorders [2], occurring in 40-50% of cases [3,4]. Unfortunately, the majority of studies investigating olfactory loss were deemed to have low methodological quality [5]. Many existing studies were either cross-sectional, posing a high risk of selection bias through non-consecutive sample enrollment or exclusion of individuals during the study. Even after two years since the onset of the pandemic, there remained a scarcity of well-designed studies incorporating longitudinal data [6] with objective measurements [5]. Therefore we have conducted well-established research **(Chapter 3)**, specifically employing a well-designed randomized controlled trial (RCT) and leveraging a high-quality cohort, with the aim to make a conclusion of the persisting discussion whether or not to prescribe steroids as therapy for COVID-19 induced smell disorders **(Chapter 4)**. The limited treatment options for COVID-19 induced smell loss forced us to conduct a case-control study in order to evaluate the effect of olfactory training **(Chapter 5)**. We also evaluate the diagnostic accuracy of a feasible practical, and easily implementable screenings test which afford us insights for research and patient counseling **(Chapter 2)** and we shed light on the true course **(Chapter 6)**, incidence, severity, and duration of olfactory disorders in COVID-19 cases in comparison with non-COVID-19 related cases **(Chapter 7).** This endeavor was imperative due to the less substantial body of existing evidence, coupled with the assertions in both mainstream media and specialized publications. Above assertions, even hazardous treatment recommendations were advanced, with patients having no alternative but to rely on restricted justified research for guidance [7,8]. Therefore, it became essential to reassess the scientific landscape regarding olfaction.

Treatment and therapy

Our randomized, double-blind, placebo-controlled trial did not provide support for the widely held assumption regarding the impact of prednisolone for COVID-19 induced smell disorders, as was observed in several other studies [9–11]. We obtained no beneficial effects of prednisolone in comparison to placebo on objective smell- and taste function, quality of life, and other self-reported outcomes **(Chapter 4)**. This result prevents patients from the potential unnecessary side-effects of systemic corticosteroids. In our opinion, future research for the development of treatments should not emphasize on adjusting or optimizing a steroid treatment, as we have reservations whether the ultimate solution lies in that approach. We suggest prioritizing psychosocial research as we believe that this is most effective on the short term. A collaboration with multiple healthcare professionals is essential to address the various facets of the burden and to provide patients with strategies

to cope with their impairment. This should include dieticians for assistance with dietary habits and psychologists for mental well-being [12–19]. Besides psychosocial research, fundamental research capable of revealing where in the olfactory pathway the issue is exactly rooted, could be more beneficial in advancing our understanding and thereby lead to treatment solutions [20,21]. Neuro-imaging, such as (functional) MRI or PET scans could help us to understand the possible pathways of olfactory disorders in COVID-19 [22,23]. By analyzing variations in the activation of brain regions following odor administration and examining alterations in olfactory bulb volume and other regions associated with olfaction, we can contribute to enhancing guidance, and refining treatment approaches [22,23].

Several studies examined the effect of smell training for patients with COVID-19 [24–33], though these studies come with several limitations [32]. Our case-control study **(Chapter 5)** did also not adhere to the most optimal study design for evaluating this particular case. Nonetheless, we used a large sample size, comparing two cohorts who shared the same inclusion and exclusion criteria, the same recruitment and testing period, and the same virus variants and vaccines available. Our findings imply a possible advantage of smell training for COVID-19 patients. To validate our findings, we recommend further investigation in the form of a randomized controlled trial with an extended follow-up period, objective measurements and even more adequate monitoring to assess our limitations, as this is never performed [32]. For now we do advise olfactory training in COVID-19 cases.

Measuring olfaction

There is a compelling need for objective evaluation of olfactory disorders following COVID-19, as it is a relatively new problem with infrequently objective measurements in the existing literature. Objective methods are more sensitive in identifying smell loss, whereas subjective measures tend to underestimate the true prevalence [34,35]. The absence of a precise and efficient assessment of olfaction leads to a notable lack in reliable data and restricts opportunities for replicability of trials. The widely used objective Sniffin' Sticks test® (SST) is time-consuming for clinicians and patients [36]. The simpler and quicker SST-12 is designed for screening non-COVID-19 smell loss [37–41], but its accuracy in COVID-19-related smell loss was uncertain due to the virus's unique pathophysiology of smell disorders. In our diagnostic accuracy study **(Chapter 2)** of this SST-12, we obtained a high positive predictive value (PPV) of 92.4%, and a moderate negative predictive value (NPV) of 71.4%. This moderate NPV suggests the potential for missing a diagnosis. As a result, we encourage healthcare workers to offer guidance for patients to manage their symptoms, also when the test result is negative. On the other hand, the high PPV indicates a strong likelihood of having the condition when the test result is positive. In combination with its high sensitivity, the SST-12 test proves to be particularly useful in identifying individuals with smell loss, also when induced by COVID-19. As patients may struggle to

objectively discern subtle improvements in their sense of smell, the SST-12 can provide guidance during patients recovery with ongoing monitoring. Clinicians should take into account our determined diagnostic accuracy values to interpret the results.

Reassurance & Awareness

Our study illustrated a sustained recovery process in individuals with COVID-19-induced olfactory disorders, even one year post-diagnosis **(Chapter 6)**. The median objective smell function exceeded the normosmia threshold. In terms of clinical implications, our findings offer reassurance to patients by suggesting a sustained recovery of olfactory function over the course of a year, albeit at a progressively slower rate. What is particularly encouraging is that this improvement is not limited to objective measures; it also translates into better quality of life and self-reported improvements in the sense of smell and taste. As well in **Chapter 7** we determined that most COVID-19 patients report olfactory disorders in the first weeks after diagnosis and that after a few weeks, the symptoms resolve or significantly improve. At the onset of the pandemic, losing the sense of smell and taste was one of the most common symptoms of COVID-19 [4]. When testing for the virus was not accessible, we relied on these symptoms to identify infected individuals [42]. As of 2021, with different SARS-CoV-2 variants being prominent, the risk of losing the sense of smell was lower than what was observed at the beginning of the pandemic. In 2022 and early 2023, when the Omicron variants were prevalent, the risk of losing your sense of smell or taste after SARS-CoV-2 infection was only 6-7% of what it was in 2020. This decrease in smell loss risk is thought to be linked to increased immunity, either through vaccination or prior infection [43]. Although the occurrence of smell loss is much lower [44,45], and tends to have a faster recovery trajectory than during the early stages of COVID-19 [46], prevalence still remains above 30% in Omicron variant cases [45]. Given the number of COVID-19 cases globally, there are potentially millions living with olfactory disorders, and the available treatment options are limited [47]. While the pandemic is fortunately behind us, awareness is still vital as COVID-19 and other viruses persist. New studies on treatments are promising, but they are still not sufficient to adequately help these individuals [48–53]. Recent research on long-term outcomes, revealed that after mild COVID-19, 5% had lasting smell or taste changes over three years. Despite many recovering, the prevalence of persistent smell loss following COVID-19 remains high [54]. As unveiled and discussed in **Chapter 7**, the severity rate of COVID-19-induced smell disorders is higher than in non-COVID-19 related cases, particularly in the initial phase, most likely due to the rapid onset of symptoms. This emphasizes the impact of recognizing the significance of smell in its absence. Not only are smell disorders common in COVID-19 cases, but they occur in various other underlying causes as well, such as allergies, nasal polyps, (rhino)sinusitis, aging, and in the field of oncology [55–61]. Notably, in **Chapter 7** we observed that non-COVID-19 related smell or taste loss cases are estimated to have a higher likelihood of prolonged symptoms when

compared to cases related to COVID-19. We brought attention to this population experiencing a loss of smell unrelated to COVID-19. These individuals have gone unnoticed for an extended period. It is crucial to capitalize on this newfound attention to acknowledge individuals afflicted by this debilitating loss, regardless of its origin. The data utilized in this study originates from Lifelines, a Dutch biobank [62–64]. This unique dataset is available for researchers worldwide and includes a wide range of topics, such as education, work, health and lifestyle/environmental factors. It is remarkable to know that before March 2020, all the large general prospective Dutch cohort studies, including Lifelines, did not include any questions related to smell or taste. Therefore, we had no comparative data before the pandemic. It is thus once again evident, that olfactory disorders received limited attention in both clinical practice and research prior to the emergence of COVID-19. We suggest that in the future, various extended questions about smell, taste, appetite, parosmia, and phantosmia should be incorporated into every (inter)national biobank. This will enable us to address the current deficiency in information and enhance our understanding in the forthcoming years.

Future perspectives

It is of crucial importance that the outcomes of this thesis will be implemented globally and bring together different fields of expertise to address olfactory disorders post-COVID-19. With all the available evidence to us know, there is in our opinion no place for prednisolone in the treatment of these patients. General practitioners, ENT surgeons, and other clinicians can utilize our research to offer patients the highest level of evidence, thus avoiding the prescription of prednisolone in these cases.

Despite the valuable studies conducted, there is a noticeable gap between this knowledge and its application in clinical practice and in the field of research. This is comprehensible, as clinicians cannot keep up with every scientific discovery. Therefore, we should aim on reaching the right audience, including reputable medical journals and events reaching a wide range of healthcare providers, patient forums, and (inter)national conferences. The guidelines for long-COVID, utilized by general practitioners, are set to undergo an update that will incorporate the findings from our studies. During the onset of the pandemic, we confronted a challenge where reviews were being updated too slowly, resulting in a loss of time, particularly in such a significant global issue that was rapidly evolving. As a consequence, the valuable findings from well-conducted studies could not be utilized, and researchers were unable to anticipate upon them. There should be a more timely adequate update of reviews or visibility into protocols for all ongoing trials to prevent similar delays.

Hopefully, ENT surgeons, general practitioners, and other healthcare providers, such as dieticians or trauma care physicians, who come across patients with potential smell disorders, will proactively inquire about their olfactory function. Or even better, they

implement the affordable, feasible, quick and simple screenings test (SST-12) in their daily routine. It is noteworthy that the inquiry of olfaction, let alone its measurement, is not universally integrated as a standard examination in patients, for example in those with head trauma. The evaluation of olfaction is prompted only in response to patient complaints, in contrast to our routine examination of other sensory modalities such as hearing and vision. We should sustain in this heightened awareness of the significance of smell.
Clinicians should also engage in testing for research purposes and patient guidance, otherwise, we will never achieve consensus and comparable reliable outcomes. Insufficient focus has been given previously to the selection of outcomes in clinical trials, leading to heterogeneity in outcomes of trials. To standardize clinical trials a valuable tool could be using a core outcome set [65]. The establishment of core outcome sets- standardized sets of outcomes that should be measured -, would address this issue and optimize future meta-analyses and systematic reviews. Recently, the outcome of the Sniffin' Sticks Test is in included in a developed core outcome set for olfactory disorders. This has emphasized the necessity of incorporating this test in clinical practice [66].

The lesson learned is that taking action in times of uncertainty and basing decisions on real data is crucial. In next pandemics every (inter)national research group should collaborate and invest in longitudinal studies as we learned that most symptoms will recover and treatment assumptions do not prove to be effective. This leads to unnecessary over treatment with all consequences. We have to measure, in order to understand and before we translate our assumptions into clinical actions.

In the midst of a pandemic marked by numerous uncertainties, many questions, justifiable concerns, media assertions and even affirmations in specialized journals, the absence of rigorous research became a significant issue. We conducted a well-designed study with a representative cohort. This undertaking was crucial because of the limited body of existing evidence leaving patients and clinicians with no choice but to depend on limited and justified research for guidance. Also claims made in mainstream media sometimes led to misinformation and potentially harmful treatment suggestions. We endorse the importance of thorough research, provided with well-considered study designs, especially in times of crisis were the need for well-substantiated information is high. With the current impact of (social) media, where all sorts of unfounded assumptions are being made, we urge policymakers and the mainstream media to uphold scientific integrity and provide nuance in claims. We now anticipate that the pandemic has brought to the forefront the significance of our most primitive and useful sense, along with the inconvenience and challenges that come with its impairment. So, a positive outcome of the COVID-19 pandemic – if any- has been the increased awareness in the clinical field and of the necessity for scientific

exploration into diagnosing smell disorders, understanding their underlying mechanisms and course, and developing treatment options.

Appendix

Nederlandse samenvatting

De waarde van ons reukvermogen wordt vaak onderschat, maar het speelt een cruciale rol in ons leven en beïnvloedt ons algeheel welzijn, eetgedrag, sociale interacties, emoties en herinneringen. Door de grote stijging van het aantal patiënten met reukverlies tijdens de COVID-19 pandemie, is het belang van onze reukfunctie nadrukkelijk aan het licht gebracht. Onze gezondheidszorg was echter onvoldoende voorbereid om deze nieuwe patiëntenpopulatie adequate hulp te bieden. Dit proefschrift biedt een uitgebreid inzicht in de diagnose, therapie, epidemiologie en het klinische verloop van COVID-19-geïnduceerd reukverlies en benadrukt de relevantie van dit belangrijke zintuig dat de laatste tijd zo nadrukkelijk onder de aandacht is komen te staan.
We onderzochten de volgende onderzoeksvragen:
I) Is het mogelijk om COVID-19 geïnduceerd reukverlies op te sporen met behulp van een olfactorische screeningstest?
II) Bestaat er een effectieve behandeling voor COVID-19 geïnduceerde reukstoornissen?
III) Wat is de incidentie, de ernst en het verloop van door COVID-19 geïnduceerde reukstoornissen en hoe verhouden deze zich tot reukstoornissen die niet gerelateerd zijn aan COVID-19?

Deel I Diagnose

In **Hoofdstuk 2** hebben we de diagnostische nauwkeurigheid geanalyseerd van de gevalideerde screeningsversie van de Sniffin' Sticks Test (SST-12) bij reukstoornissen na COVID-19. Hoewel de uitgebreide Sniffin' Sticks Test (SST) gevalideerd is voor het beoordelen van de reukfunctie, is deze tijdrovend voor zowel arts als patiënt, waardoor deze test minder geschikt is voor routinematig klinisch gebruik. De verkorte versie, de SST-12, wordt gebruikt als diagnostisch hulpmiddel voor het screenen van reukverlies dat veroorzaakt wordt door niet-COVID-19 gerelateerde aandoeningen. Omdat COVID-19 de reukzin op een andere manier aantast in vergelijking met de meeste andere oorzaken van reukverlies, hebben we onderzocht of de SST-12 ook effectief is in het identificeren van reukverlies wanneer het wordt veroorzaakt door COVID-19. We hebben een diagnostische nauwkeurigheidsstudie uitgevoerd bij een cohort van patiënten met een bevestigde COVID-19 diagnose. Ongeveer zes maanden na hun COVID-19 diagnose vergeleken we de uitkomsten van de SST als referentiestandaard en de SST-12 als indextest. We beoordeelden de uitkomsten van de SST-12 aan de hand van verschillende diagnostische nauwkeurigheidsmaten, waaronder de sensitiviteit, de specificiteit, de negatief voorspellende waarde (NPV) en de positief voorspellende waarde (PPV). We vonden een hoge sensitiviteit (93.4%) en PPV (92.4%) en een matige specificiteit (68.2%) en NPV (71.4%). De bevindingen van deze studie suggereren dat de SST-12 ook veelbelovend is om reukverlies aan te tonen wanneer dat veroorzaakt

wordt door COVID-19. Hierbij is het wel noodzakelijk voor zorgmedewerkers om de uitkomsten van de SST-12 goed te kunnen interpreteren in de klinische praktijk.

Deel II Therapie

Ons onderzoek is gebaseerd op de hypothese dat COVID-19 een ontsteking veroorzaakt in de buurt van de reukzenuw en de reukbanen naar de hersenen, wat leidt tot een verstoorde reukfunctie. Eerdere studies toonden aan dat corticosteroïden, zoals prednisolon, de impact van deze ontsteking zouden kunnen verminderen, met name in de vroege stadia van reukstoornissen. Deze studies hebben echter verschillende beperkingen waardoor de discussie over het al dan niet voorschrijven van prednisolon bleef bestaan. Met als doel het hoogst mogelijke niveau van bewijs voor onze onderzoeksvraag te verkrijgen - namelijk of corticosteroïden daadwerkelijk een positief effect hebben op reukstoornissen – hebben we een gerandomiseerde, dubbel geblindeerde, placebogecontroleerde studie uitgevoerd met een zorgvuldig berekende steekproefomvang om statistische betrouwbaarheid te garanderen. Dit onderzoek heet de COCOS trial; COrticosteroids for COvid-19 induced loss of Smell.

De uitkomsten van de COCOS trial waarvan het protocol is beschreven in **Hoofdstuk 3**, staan in **Hoofstuk 4**. We voerden een gerandomiseerde, dubbel geblindeerde, placebogecontroleerde studie uit. We includeerden 115 geschikte deelnemers ouder dan 18 jaar, met aanhoudende (>4 weken) reukstoornissen, binnen 12 weken na een bevestigde COVID-19 diagnose. De behandelgroep slikte gedurende tien dagen eenmaal daags 40mg prednisolon en de placebogroep slikte tien dagen eenmaal daags een placebo. Zowel onderzoekers als patiënt wisten niet of zij in de prednisolon- of placebogroup waren gerandomiseerd. Daarnaast deden alle deelnemers aan reuktraining. Bij reuktraining ruikt een patiënt voor een periode van 3 maanden tweemaal daags bewust aan een set van vier bekende geuren, met als doel de werking van de reukzenuw te stimuleren. Onze primaire uitkomstmaat was de objectieve reukfunctie 12 weken na behandeling. De reukfunctie werd uitgedrukt in de Drempel-Discriminatie-Identificatie (TDI) score, gemeten met de Sniffin' Sticks Test (SST). Secundaire uitkomsten waren onder andere de objectieve smaakfunctie, gemeten met de Taste Strip Test (TST), en uitkomsten van de zelf-gerapporteerde reuk- en smaakfunctie, levenskwaliteit en nasale symptomen via afgenomen vragenlijsten. Onze bevindingen toonden geen verschil in alle uitkomstmaten tussen de prednisolon- en de placebogroep. We concludeerden dat prednisolon de reukfunctie na COVID-19 niet verbetert. Daarom adviseren wij om geen prednisolon voor te schrijven aan patiënten met reukstoornissen na COVID-19. We zagen echter wel dat de mediane TDI score over tijd evenveel verbeterde in beide groepen.

De effectiviteit van reuktraining bij de behandeling van postvirale en posttraumatische reukstoornissen is bewezen, maar het effect ervan op COVID-19 geïnduceerde reukstoornissen blijft onzeker. Ons doel was om het potentiële positieve effect van reuktraining te onderzoeken bij patiënten met COVID-19-gerelateerd reukverlies. In **Hoofdstuk 5** beschrijven we een case-control studie waarvoor we twee vergelijkbare cohorten hebben geïncludeerd. De ene groep (n=111) deed tweemaal daags reuktraining gedurende een periode van 12 weken, met controle van de therapietrouwheid via een dagboek. Zij kregen de reuktrainingset van ons mee met uitgebreide instructies en werden gemotiveerd om de training te doen. Het andere cohort (n=50) deed daarentegen geen reuktraining. Om de reukfunctie van de deelnemers objectief te evalueren, testten we deze met de SST na de periode van 12 weken. Onze bevindingen toonden een statistisch significant verschil in reukfunctie tussen patiënten die de 12 weken durende reuktraining ondergingen en degenen die dat niet deden, wat suggereert dat reuktraining een positief effect heeft op het herstel van de reuk. Het verschil was echter niet klinisch relevant. Opvallend genoeg zagen we geen associatie tussen de therapietrouwheid van de reuktraining en de verbetering van de reukfunctie. Voor meer betrouwbare resultaten adviseren we om een gerandomiseerde gecontroleerde trial uit te voeren, idealiter met een langere follow-up periode.

Deel III Epidemiologie en klinisch beloop

Eerder onderzoek richtte zich voornamelijk op de acute fase van reukstoornissen na COVID-19, wat leidde tot inconsistente prognostische inzichten. In **Hoofdstuk 6** onderzochten we hoe de reuk zich ontwikkelt één jaar na COVID-19. We voerden een prospectieve cohortstudie uit met 77 patiënten die aanvankelijk COVID-19-geïnduceerde reukstoornissen hadden en deelnamen aan de COCOS trial. De primaire uitkomstmaat was de TDI score verkregen met de SST. Deze werd een jaar na de diagnose uitgevoerd, en vergeleken met eerdere metingen op drie en zes maanden tijdens COCOS-trial, beschreven in **Hoofdstuk 4**. Secundaire metingen waren de smaakfunctie gemeten met de TST, de zelf-gerapporteerde reuk- en smaakfunctie, levenskwaliteit en nasale symptomen op afgenomen vragenlijsten. Wij zagen dat één jaar na COVID-19, de verbetering van de mediane TDI score doorzette en een niveau bereikte dat werd gecategoriseerd als normosmie (normale reukfunctie). Deze blijvende verbetering zagen we ook bij de smaakfunctie op de TST, de zelf-gerapporteerde reuk- en smaakfunctie, levenskwaliteit en nasale symptomen op de vragenlijsten. Deze studie toont een aanhoudend herstel aan van zowel de objectieve als de zelf-gerapporteerde reuk- en smaakfunctie bij patiënten één jaar na de diagnose COVID-19. Het bereiken van een normosmische TDI-score is een positief resultaat, hoewel de mate van verbetering afneemt in de loop van de tijd.

In **Hoofdstuk 7** hebben we een vergelijkende analyse uitgevoerd van de incidentie, ernst en duur van reuk- en smaakverlies in COVID-19-gevallen versus niet-COVID-19-gevallen. We voerden hiervoor een longitudinale cohortstudie uit. Het onderzoek besloeg een periode van twee jaar, van maart 2020 tot en met mei 2022 en betrof een groot cohort bestaande uit 235.722 deelnemers van de Nederlandse biobank Lifelines. We berekenden beschrijvende gegevens en de incidentie van reuk- en smaakverlies voor zowel COVID-19 als niet-COVID-19 gerelateerde gevallen. We maakten een heatmap voor beide groepen om de verhouding in ernst van de symptomen te visualiseren. Om de kans op aanhoudend reukverlies in beide groepen weer te geven over de tijd, presenteerde we in een omgekeerde Kaplan-Meier curve een overlevingsanalyse. Deze studie onthulde een hogere incidentie en hogere mate van ernst van reuk- en smaakverlies bij personen met COVID-19 in vergelijking met niet-COVID-19 gerelateerde gevallen. De duur van reukverlies is echter langer bij patiënten met reukverlies veroorzaakt door andere oorzaken dan COVID-19. Deze patiënten zijn een lange periode relatief onopgemerkt gebleven en kregen vanuit de klinische praktijk en de onderzoekswereld weinig aandacht. De COVID-19 pandemie heeft het belang van reuk en smaak onder de aandacht gebracht, net als het ongemak dat gepaard gaat met de aantasting ervan. Het is belangrijk om hier bewust van te zijn en steun te bieden aan patiënten die lijden aan reuk- en of smaakstoornissen, ongeacht de oorsprong ervan.

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Abbreviations list

COVID-19 Coronavirus Disease 2019
SARS-CoV-2 Severe Acute Respiratory Syndrome Coronavirus 2
COCOS COrticosteroids for COVID-19 induced Smell loss
COVORTS COVid-19 cohORT for Smell loss
CONSORT Consolidated Standards of Reporting Trials
SPIRIT Standard Protocol Items; Recommendations for Interventional Trials
GDPR General Data Protection Regulation
ISO International Organization for Standardization
GMP Good Manufacturing Practice
ISRTCN International Standard Randomised Controlled Trial Number
ICHGCP International Conference on Harmonisation Good Clinical Practice
GGD Dutch Public Healthcare Service
WMO Medical Research Involving Human Subjects Act
SAE Serious Adverse Event
METC Medical Ethical Assessment Committee
WHO World Health Organization
ENT Ear, Nose and Throat
PCR Polymerase Chain Reaction
RCT Randomized Controlled Trial
eCRF electronic Case Report Form
EDC Electronic Data Capture
IBM SPSS IBM software for Statistical Package for the Social Sciences
SD Standard deviation
SE Standard Error
IQR Interquartile Range
CI Confidence Interval
PPV Positive predictive value
NPV Negative predictive value
SNOT-22 Sino-Nasal Outcome Test -22
VAS Visual-Analogue-Scale
ODQ Olfactory Disorders Questionnaire
TDI Threshold-Discrimination-Identification
SST Sniffin' Sticks Test
TST Taste Strip Test
CN Cranial Nerve
ACE Angiotensin Converting Enzyme
OR Odorant Receptors

ORN Olfactory Receptor Neuron
OSN Olfactory Sensory Neuron
GPCR Guanine Nucleotide Protein Coupled Receptor

PhD Portfolio

PhD Training	European Credits	Year
Introduction to Brain Center	0.5 EC	2020
Presentation at Brain Center Day 2022	0.3 EC	2020
Attendance Summer School	0.5 EC	2023
Workshop Tutoring master students (summerschool 2023)	0.5 EC	2023
Workshop Effective meetings (summerschool 2023)	0.5 EC	2023
Responsible Conduct of Research 1	0.15 EC	2020
Responsible Conduct of Research 2	0.15 EC	2021
Introduction into R and data 2022	0.3 EC	2022
Workshop Systematically Searching Literature (including reviews) 2022		2022
Academic Writing in English	1.8 EC	2022
Adobe Illustrator Course 2023	0.6 EC	2023
Adobe Illustrator, infographics and Artwork	1.2 EC	2023
BROK cursus	1.0 EC	2020
Presentations and conferences		
Dutch Neuroscience Meeting, Tiel- 'Overview of the current knowledge about (treatment of) COVID-19-induced loss of smell.'	0.3 EC	2022
ORL-HNS conference, Milaan- 'Corticosteroids for COVID-19 induced loss of smell; a randomized, double-blind, placebo-controlled trial.'	1.2 EC	2022
Nationale patiënten dag voor reuk en smaakstoornissen- 'Resultaten COCOS-trial.'	0.3 EC	2022
European Rhinology Society Congress, Sofia- 'The effect of smell training on COVID-19 induced smell loss', 'Prednisolone does not improve olfactory function after COVID-19', Winnaar van Dragon's Den session; Q&A van vijf-ledig rhinologisch panel over de COCOS-trial.	1.2 EC	2023
KNO-vergadering, paneldiscussie kerngroep rhinologie	0.3 EC	2023
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Teaching		
Teaching medical ENT subjects to medical students		2020-2023
Supervising thesis of research master student		2021-2022
Work group supervision and assessment of masterstudents		2022
In training of obtaining the Basis Kwalificatie Onderwijs (BKO)	5.0 EC	2023-2024

About the author

Emma Josephine Adriana was born on December 31th 1993 in Laren, The Netherlands. She grew up with her brother Gianni and her parents. In 2013 she obtained her VWO diploma from the Willem de Zwijger College te Bussum.
Afterwards she moved to Groningen to study Medicine. She joined a student team at the Department of Hepato-Pancreato-Biliary Surgery at the University Medical Center Groningen, where she assisted in the operating room during liver transplants. She also conducted her scientific internship in this department under the supervision of Dr. C.I. Buis and Dr. M.T. de Boer. During her study she worked for a couple of months in the North of Mozambique, in a small local village called Nanatha. She taught the locals about the importance of hygiene and how to ensure it. She is still involved in the projects of Mozambique by being a board member of Anan clinica, a NGO to increase medical knowledge of the local population through the establishment of programs for education, agriculture, water management, health care and finance. In her final years of study, she completed internships at Isala Hospital in Zwolle, Erasmus Medical Center in Rotterdam, and the Antoni van Leeuwenhoek Cancer Institute in Amsterdam. After obtaining her master's degree in 2020, she began working at the Department of Otorhinolaryngology in the Tergooi Medical Center and frequently worked at the COVID-19 cohort department. Following working for several months at the emergency department at the Flevoziekenhuis in Almere, she began her role as a PhD student at the Department of Otorhinolaryngology and Head and Neck Surgery at UMC Utrecht (under the supervision of Dr. D.M.A. Kamalski, Dr. I. Stegeman, Prof. Dr. R.J. Stokroos, and Dr. S. Boesveldt). The results of her research are presented in this thesis.

Emma lives together with Mathijs Derksen in Amsterdam, the Netherlands.