JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Coronavirus Pandemic

Effects of serial swabs on the nasopharyngeal mucosa: our experience in SARS-CoV2 screening

Giovanni Dell'Aversana Orabona[1], Vincenzo Abbate[1], Gianluca R De Fazio[1], Carlo Calvanese[1], Luigi Vaira[2], Paola Bonavolontà[1], Antonio Romano[1], Giovanni Improta[3], Pasquale Piombino[1], Luigi Califano[1]

[1] Maxillofacial Surgery Unit, Department of Neurosciences, Reproductive and Odontostomatological Sciences, University Federic II, Via Pansini 5, 80100 Naples, Italy
[2] Maxillofacial Surgery Operative Unit, University Hospital of Sassari, Viale San Pietro 43/B, 07100 Sassari, Italy
[3] Department of Public Health, University of Naples "Federico II", Naples, Italy

Abstract

Introduction: The purpose of the study was to analyze the effect of swabs on nasal mucosa.
Methodology: Since May 2020, our department was responsible for screening coronavirus disease 2019 (COVID-19) among the employees of a company that continued its activity during the pandemic. The screening protocol consisted of two swabs per week. The samples were analyzed through objective endoscopic and subjective clinical evaluations with sino-nasal outcome test (SNOT Test) at three time points (T0, T1 - three months, T2 - six months).
Results: 23.76% of patients showed an increase in the SNOT score at T1, and the score decreased at T2. This could be due to the phenomenon of "adaptation" of the nasal mucosa. Endoscopic control showed that at T1, secretion, hyperemia, and edema are the most common signs. At T2, however, the crusts accounted for 52.94% of all damage. It is evident that at T1 the endoscopically detected signs of "acute" damage were more represented than at T2, while the signs of "chronic" damage increased as the number of swabs increased.
Conclusions: We demonstrated that mucosal damage and perceived symptoms were absolutely acceptable compared to the diagnostic advantage obtained with serial screening.

Key words: COVID-19; SARS CoV-2; nasal swab; swab effect; COVID-19 screening; nasal mucosa.

J Infect Dev Ctries 2024; 18(7):987-992. doi:10.3855/jidc.17957

(Received 18 January 2023 – Accepted 31 July 2023)

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an RNA virus [1] that appeared in Wuhan, one of the largest Chinese metropolitan center, in December 2019. In a few months, the epidemic spread to all continents, and the World Health Organization (WHO) declared it a global pandemic on 11 March 2020.

Coronavirus disease 2019 (COVID-19) infection, frequently, causes symptoms such as fever, cough, and asthenia; and, in some cases, it evolves into pneumonia, putting the patient's life at risk. In the beginning of the pandemic, diagnostic methods were few and difficult to find. Consequently, the detection of SARS-CoV2 infection was often late; the disease reached advanced stages at the time of diagnosis and spread among the population. The disease took all national health systems by surprise and there were various treatment guidelines.

At the beginning of March 2020, about 4 million cases worldwide had already been confirmed by molecular testing and more than 250,000 deaths were registered globally [2]. The scientific community faced many diagnostic and therapeutic issues. The current diagnostic methods [3] that allow identification SARS-CoV-2 are based on the study of the viral genome [4,5]. The virus is detected by oropharyngeal swabs [5,6], throat swabs [4,7], sputum analysis [4,7,8], bronchoalveolar lavage fluid (BALF) analysis [9–11], whole blood tests [6], serum analysis [6], stool analysis [12,13,16], urine analysis [13,14], saliva swabs [15–17], rectal swabs [13,18] and conjunctival swabs [19,20]. The swab works by taking cells and any virions present in the respiratory nasopharyngeal epithelium that break off with brushing, thereby making them particularly suited to mass screening.

The rapid spread of the epidemic and its harmful effects on health led national governments to shut down several businesses. Some companies started SARS-CoV-2 diagnostic screening programs. The Department of Maxillofacial Surgery of the Federico II University Hospital of Naples, Italy was in charge of screening the employees of one of these companies since the

beginning of May 2020. The screening protocol was implemented by the Federation of which the Company was a part and to which it was accountable. This screening required each employee to undergo two oral-nasopharyngeal swabs per week. As a result, newly infected employees were immediately identified and removed from the workplace, thus avoiding spread of the infection.

Although the diagnostic accuracy of these tests based on mucosal brushing is well recognized and documented in the literature, the effects that frequent swabs (> 2 times a week) can have on nasal tissues are not well documented. The aim of our study was to identify any collateral damage caused by the high frequency of oral-nasal-pharyngeal swabs.

Methodology

We conducted this study at the Maxillo-Facial Surgery Unit of the University Hospital of Naples Federico II on a sample of 110 employees who underwent the screening procedure with two oral-nasopharyngeal swabs per week. The study lasted six months and a total of 48 swabs per patient were used. Heavy smokers (more than 20 cigarettes a day) and all those who already presented basic functional endonasal pathologies (chronic rhinosinusitis, turbinate hypertrophy, septal deformity, etc.) were excluded because they could not be evaluated based on existing chronic damage.

Eighty-eight patients satisfied the inclusion criteria and were enrolled in the study. The diagnostic procedure was always carried out by the same team. The swab was used to collect the sample first from the oropharyngeal wall (through the oral cavity) and then from the nasopharyngeal wall (through both nasal cavities). Each swab collection was performed according to the methods reported by Marty *et al.* [21] COPAN FLOQswabs (COPAN ITALIA, Brescia, Italy) were used for the procedure. After the swab samples were collected, they were analyzed on the same day in the Laboratory of Molecular Virology of the Federico II University Hospital of Naples. The swabs were analyzed for the presence of SARS-CoV-2 RNA using Abbott Real Time PCR SARS-CoV-2 assay (Abbott, Abbott Park, Illinois, USA).

The real-time polymerase chain reaction (PCR) assay was used which detects a positive sample by the accumulation of fluorescent signal indicating amplification of the target sequence. The cycle threshold (Ct) is defined by the number of cycles required for the fluorescent signal to cross the background level (threshold). Ct levels are inversely proportional to the amount of target nucleic acid in the sample; the lower the Ct number, the greater the amount of target sequence. In particular, the Abbott Real Time PCR SARS-CoV-2 assay (Abbott, Abbott Park, Illinois, USA) is a qualitative assay, CE-IVD (European certificate — In Vitro Diagnostics) marked and Food and Drug Administration (FDA) approved, and it detects dual targets: *RdRp* and *N* genes. This assay has a limit of detection (LoD) of 100 copies/mL and its sensitivity and specificity are 93% and 100%, respectively. The samples were stored at -80 °C for further assays.

The follow-up included three clinical evaluation time points: T0, before the beginning of the screening for SARS-CoV2; T1, three months after the beginning of the screening; T2, six months after the beginning of the screening. All the follow-ups were carried out at the Department of Maxillofacial Surgery of the Federico II University Hospital of Naples, Italy, staggered at various times of the day in order to avoid crowds and possible sharing of information among patients.

The patients were clinically and endoscopically assessed at each time point. Nasal fibro endoscopy was used to identify different symptoms such as nasal dryness, mucosal hyperemia, mucosal edema, secretions, crusting, traces of blood, and synechia. These observations were recorded according to a dichotomous scale (presence/absence). A self-assessment questionnaire, the Sino-Nasal Outcome Test (SNOT-22), was filled out by the patients to report a subjective assessment of the quality of life. The questionnaire was used to determine whether the patient was suffering from one or more of the 22 symptoms (need to blow nose, sneezing, runny nose, cough, dripping at the back of the nose, thick nasal discharge, ear fullness, dizziness, ear pain/pressure, facial pain/pressure, difficulty falling asleep, waking up at night, lack of a good night's sleep, waking up tired, fatigue during the day, reduced productivity, reduced concentration,
frustrated/restless/irritable/sad/embarrassed, sense of taste/smell, blockage/congestion of nose) and the severity of the problem from 0 (no problem) to 5 (as bad as it can be).

All the included patients signed an informed consent approved by the local ethics committee in compliance with World Medical Association (WMA) Helsinki Declaration.

Statistical analysis

Each SNOT was assigned a value based on the formula below:

$$Value = S(1 + (\frac{n}{10}))$$

where S = sum of the scores obtained from the individual symptoms detected, and n = number of symptoms detected. Thus, the SNOTs with more symptoms had greater relevance than those which had fewer symptoms.

The T1 and T2 values of the endoscopic signs were calculated with the following formula:

$$Value = \frac{(n1\ x\ 1.2) + (n2\ x\ 1.4) + (n3\ x\ 1.6) + (n4\ x\ 1.8) + (n5\ x\ 2)}{5}$$

where n1 was the number of secretions, n2 the number of hyperemia, n3 the number of edemas, n4 the number of crusts, and n5 the number of traces of blood.

A data distribution test was performed. The non-parametric Mann-Whitney U test (group comparison) was used to examine the data for signs of significant differences. A p value of < 0.05 was considered as statistically significant.

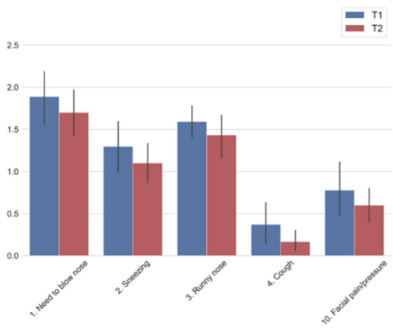
No statistically significant differences ($p \leq 0.05$) were found between T0-T1 and T1-T2, indicating that the swab procedure did not directly cause any symptom.

Results

The sample included 88 patients (64 males and 24 females) of ages between 28 years and 60 years.

At T0, all the 88 patients evaluated had no underlying functional pathologies with normochromic endonasal mucosa and did not show any pathological endoscopic signs (Figure 1).

Figure 1. Average values of the 5 symptoms detected at time T1 (blue) and at time T2 (red).



Symptoms		
1. Need to blow nose	1.888889	T1
10. Facial pain/pressure	0.777778	T1
2. Sneezing	1.296296	T1
3. Runny nose	1.592593	T1
4. Cough	0.370370	T1
1. Need to blow nose	1.700000	T2
10. Facial pain/pressure	0.600000	T2
2. Sneezing	1.100000	T2
3. Runny nose	1.433333	T2
4. Cough	0.166667	T2

At T1, 27 of 88 (23.76 %) patients showed an increase in SNOT score. The average SNOT score of the 27 symptomatic patients was 6 with values ranging between 3 and 11 (Figures 1–4). At the time of endoscopic control, 6 patients (6.8%) had nasal crusting, 12 patients (13.6%) had hyperemia, 9 patients (10.2%) had edema, 16 patients (18.2%) had secretions and 3 patients (3.4%) had blood traces (Figure 5).

At T2, 30 (34%) patients were symptomatic with mean SNOT of 5 and values ranging between 2 and 9 (Figures 1, 3, and 4). At the time of endoscopic control, 27 patients (30.7%) had nasal crusting, 9 patients (10.2%) had hyperemia, 7 patients (7.9%) had edema, 6 patients (6.8%) had secretions, and 2 patients (2.3%) had traces of blood.

Figure 2. Heatmap showing distribution and occurrences intensity of SNOT values for T1. The color varies from white (no symptoms) to black (persistent and/or recurrent symptoms), thus showing which symptoms are more frequent and which ones occur less often.

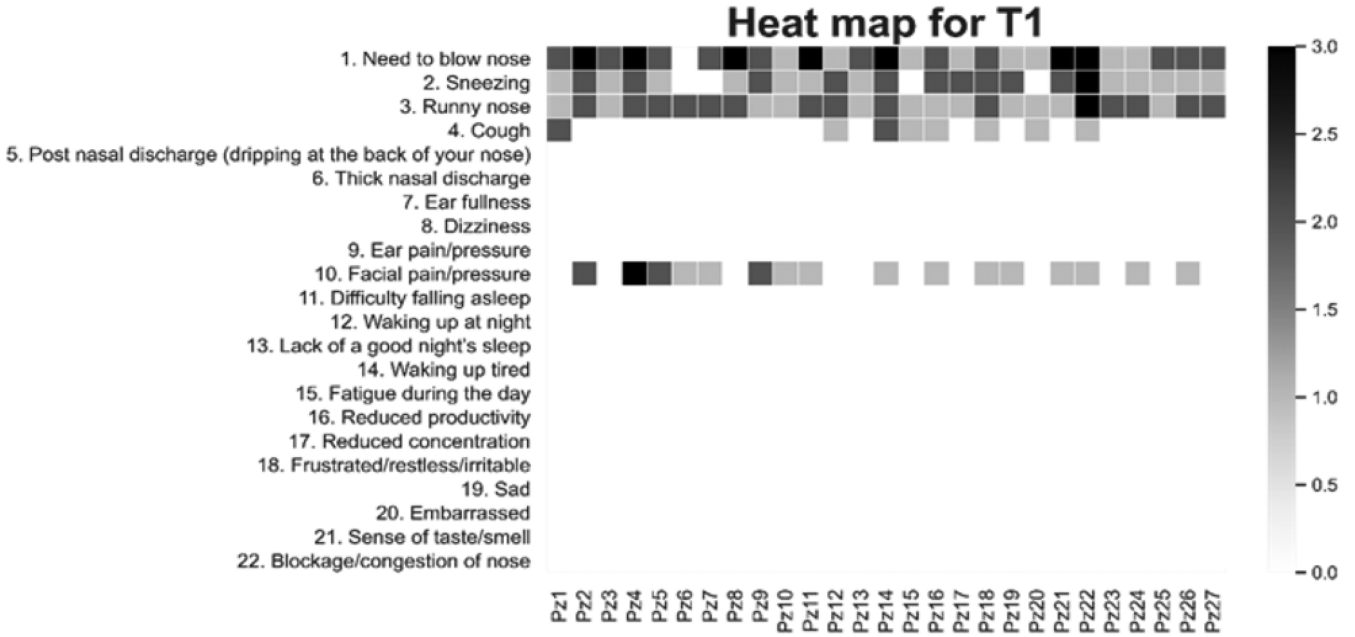


Figure 3. Heatmap showing distribution and occurrences intensity of SNOT values for T2. The color varies from white (no symptoms) to black (persistent and/or recurrent symptoms). In this way can be seen which symptoms are more frequent and which ones occur less often.

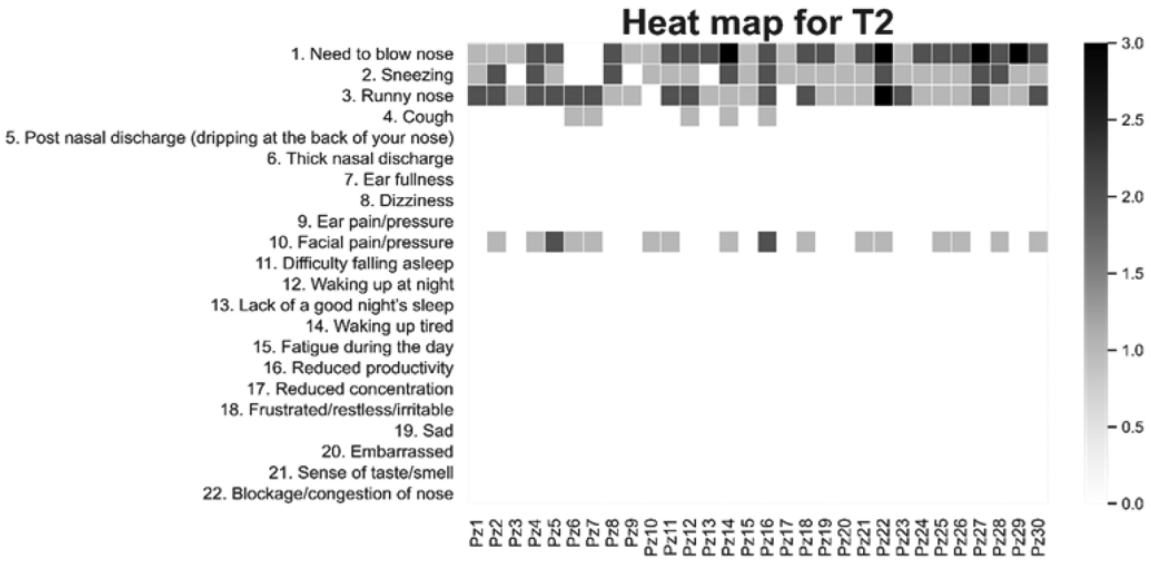
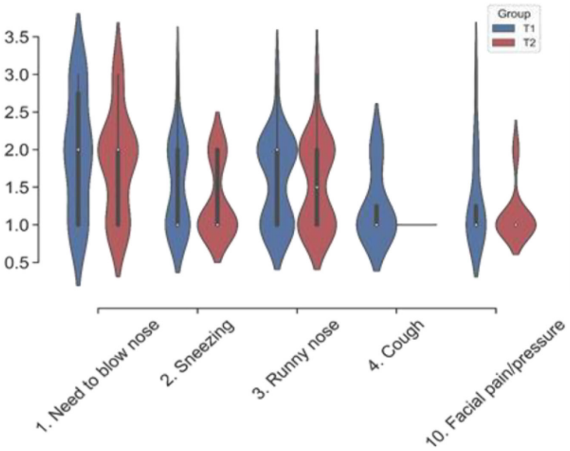


Figure 4. Violin plot showing mean values and standard deviation of each score of SNOT22 test for T1 and T2.



	Need to blow nose	*Sneezing*	*Runny nose*	*Cough*	*Facial pain/pressure*
T1	1.962 ± 0.759	1.522 ± 0.580	1.593 ± 0.562	1.250 ± 0.433	1.313 ± 0.583
T2	1.821 ± 0.658	1.320 ± 0.466	1.536 ± 0.566	1.000 ± 0.000	1.125 ± 0.331

Figure 5. The graph shows the number of patients with each symptom endoscopically detected at time T1 (blue) and at time T2 (red).

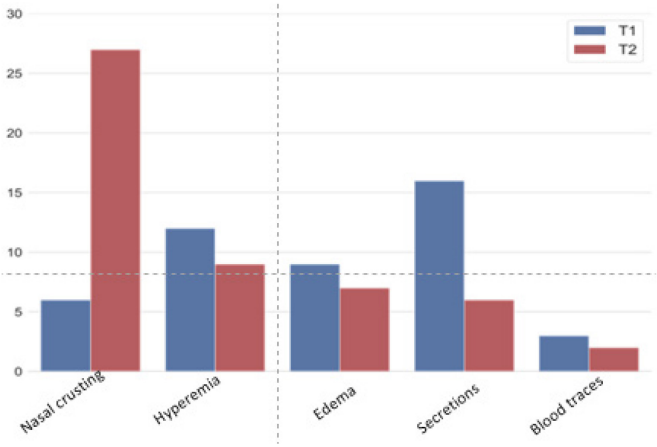
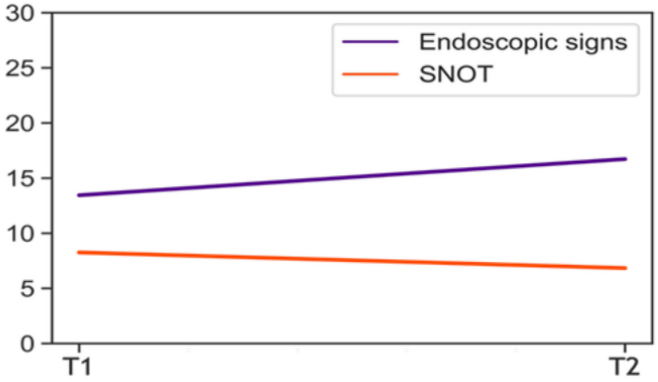


Figure 6. The trend of the average data relating to SNOT (purple) and endoscopic symptoms (orange) from T1 to T2.



	Endoscopic signs	**SNOT**
T1	13.44	8.25
T2	16.72	6.83

Discussion

SARS CoV2 replicates in the upper respiratory tract, and the viral load peaks about five days after the time of infection [22]. Oropharynx and nasopharynx are the best anatomical sites for sample collection. The inferior meatus, delimited superiorly by the inferior turbinate and inferiorly by the nasal floor, represents the meatus with the largest volume and with the greatest airflow. The innervation of the nasal cavities is not only provided by the olfactory nerve, but it also consists of tactile and thermal receptors that derive from the ophthalmic and maxillary branches of the trigeminal nerve. The nasal mucosa is therefore extremely sensitive to nociceptive stimuli. There are vascular structures on the medial wall, consisting of anastomosed vessels that collect the plexus of Kiesselbach anteriorly and the plexus of Woodruff posteriorly. The mucous layer is thicker and rich in blood vessels over the conchae and over the nasal septum, while it reaches the minimum thickness on the floor of the cavity.

Life cycle of the SARS CoV-2 virus begins when respiratory droplets containing the virions are inhaled by the host. Once they come into contact with the upper and lower respiratory tract cells, they attach with spike proteins, present on the virion envelope, to the angiotensin-converting enzyme 2 (ACE2) receptors present on the respiratory cell membrane. From this point, efficient host cell entry depends on (i) cleavage of the S1/S2 site by the surface transmembrane protease serine 2 (TMPRSS2); and/or (ii) endolysosomal cathepsin L, which mediates virus–cell membrane fusion at the cell surface and endosomal compartments, respectively. Through either entry mechanism, the RNA genome is released into the cytosol, where it is translated into the replicase proteins (open reading frame 1a/b: ORF1a/b) [23].

As reported by Mawaddah *et al*. [22], the FDA has been issuing guidelines for the detection of SARS-CoV2 since February 2020. The detection method is based on reverse transcription polymerase chain reaction (RT-PCR) analysis of viral nucleic acid that may be present in the sample taken from the respiratory tract [24]. These samples may be collected from both the upper (nasopharyngeal and oropharyngeal swabs) and lower (bronchoscopic brushing, bronchial-alveolar lavage, and sputum) respiratory tract [25]. Sampling from the upper airways is much more practicable, particularly for screening (> 2 swabs per week), such as the one we analyzed for our study. There are therefore several articles in the literature regarding the detection of SARS-CoV2, but to the best of our knowledge, there are no studies regarding the possible damages and side effects caused by serial swabs.

Our goal was to identify the possible harmful effects of repeated nasopharyngeal swabs in a sample of 88 patients who were being screened for SARS-CoV2 with two swabs per week, by objectively analyzing their tolerability and nasal signs. The study is based on three endoscopic checks carried out at T0, T1, and T2, where T0 indicated time 0 without any swab test, and T1 and T2 indicated three and six months after the start of regular swab testing, respectively. At each endoscopic follow up an informed consent and a questionnaire which requested data on 22 possible side effects due to repeated swabs according to the SNOT-22 score, was filled out by the patients (Figure 6).

Conclusions

Based on our findings, we can state that the screening for SARS-CoV-2 by nasopharyngeal swabs twice a week is an effective method for the early detection of infection and is overall well-tolerated by patients and free from clinically relevant sequelae. We have demonstrated that after the screening for SARS-CoV-2 through several nasopharyngeal swabs performed on employees of a company, the mucosal damage and perceived nasal symptoms are absolutely acceptable compared to the diagnostic advantages obtained with serial screening.

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Corresponding author

Carlo Calvanese, MD.
Maxillofacial Surgery Unit, Department of Neuroscience, Reproductive and Odontostomatological Sciences,
School of Medicine, University of Naples "Federico II", Via Sergio Pansini, 5 Naples, Italy 80131
Tel: 0039 081 746 2175 / +393398695959
Fax: 0817462175
Email: carlo.calvanese@libero.it

Conflict of interests: No conflict of interests is declared.