Comparing Nasopharyngeal and Mid-Turbinate Nasal Swab Testing for the Identification of SARS-CoV-2

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Abstract:

Testing of paired mid-turbinate (MT) nasal and nasopharyngeal (NP) swabs, collected by trained personnel from 40 patients with COVID-19 showed more NP (76/95, 80%) than MT swabs tested positive (61/95, 64%; p=0.02). Among samples collected a week after study enrollment, fewer MT than NP samples were positive (45% vs 76%; p=0.001).

Keywords: COVID-19, nasopharyngeal, mid-turbinate swab, viral load

Initial reports of pneumonia caused by a novel coronavirus, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), and the infection referred to as COVID-19, were reported from Wuhan, China in December 2019[1, 2]. COVID-19 has since been declared a pandemic and has been reported in almost all regions across the globe, contributing to significant mortality, morbidity, and economic losses.

Use of nasopharyngeal samples for the detection of respiratory viruses is considered the standard of care. However, the Centers for Disease Control and Prevention (CDC)[3] and the Infectious Disease Society of America (IDSA)[4] currently recommend testing of NP, mid-turbinate nasal (MT), anterior nares, oropharyngeal and saliva swabs or washes from upper or lower respiratory tract for SARS-CoV-2 by reverse transcription (RT)-PCR based on limited data [5-7]. Moreover, MT swab testing is gaining wider acceptance due to the ease of sample collection, ability to self-collect and personal protective equipment may not be needed[8]. However, few studies have directly compared the reliability of NP and MT swabs for the detection of SARS-CoV-2 RNA[9]. The objective of this study is to determine whether MT swabs are comparable to NP swabs in detecting SARS-CoV-2 by RT-PCR in patients with confirmed COVID-19.

Methods

Subjects and Specimens: All hospitalized patients with confirmed COVID-19 infection were eligible for participation in the study and 40 hospitalized patients with COVID-19 were enrolled in a prospective study between April 5 and May 16, 2020, from whom serial NP and MT nasal swabs were collected weekly and analyzed by RT-PCR. Both swabs were collected by the same medical provider from both nares. Demographic data and clinical characteristics for the study participants were collected from electronic medical records. The research

protocol was approved by the Institutional Review Board for Human Use and an informed consent was obtained from all study participants or their legally authorized representatives.

Specimen collection and processing: Paired MT and NP swabs were collected at enrollment and weekly thereafter by trained bedside nursing staff, placed in transport media, and stored at -80^{o}C until processed. For the current study, paired samples collected at enrollment and a week after hospitalization were compared. RNA was extracted using commercial spin column kits (QAmp viral RNA mini kit, Qiagen, Inc., Valencia, CA), and stored at -80^{o}C.

Real-time Reverse Transcription PCR (RT-PCR): Our laboratory developed a real-time RT-PCR assay for the detection of SARS-CoV-2 RNA is based on the CDC protocol (https://www.cdc.gov/coronavirus/2019-nCoV/lab/index.html). The protocol is described in detail in the supplementary information and summarized here. The RT-PCR reaction mix includes 5 µL of extracted RNA, 1.5 µL primer/probe mix, and 5 µL of TaqPath 1-step RT-qPCR Master Mix (Thermofisher, Waltham, MA). Samples were run in duplicate and each run included a no-target control and a synthetic RNA standard (SeraCare Life Sciences, Milford, MA) as a positive control. A specimen was considered positive if one or more copies/reaction were detected in both wells before 40 cycles. The detection limit of our real-time RT-PCR assay was between 100 and 200 copies/mL. The cycle threshold (CT) value was used to estimate viral load.

Statistical analysis: The difference in the proportion of positive NP and MT swabs for SARS-CoV-2 RNA was assessed using the Fisher's exact test. To determine if MT swabs are comparable to NP samples in detecting SARS-CoV-2 at higher CT values (lower viral load), MT swabs were categorized into two groups based on NP swab CT values of ≤30 and >30. The correlation between NP and MT CT values was determined by Spearman rank correlation test and represented graphically with linear regression.

Sample quality: The quality of NP and MT samples was determined by PCR amplification of RNase P gene for all discordant samples (Table S2 of the supplementary information).

Results

The demographic and clinical characteristics are provided in Table S1 (supplementary information). Briefly, 12.5% (5/40) were ≤ 18 years, 47.5% were between the ages of 19-64 years, while 40% (16/20) were ≥ 65 years of age. The majority of study participants were men (60%, 24/40) and African American (52.5%, 21/40). 17.5% were residents of long-term care facilities and nearly half the cohort (47.5%, 19/20) had a known or possible COVID-19 exposure. Presenting complaints included dyspnea (62.5%), fever (52.5%), cough (45%), myalgias (15%), gastrointestinal symptoms (15%) and chest pain (12.5%). Only 12.5% of the cohort lacked any co-morbidities with the most frequent co-morbid conditions reported including hypertension (60%), diabetes (45%) and chronic kidney disease (10%). While 75% of this cohort required care in the ICU, 65% required mechanical ventilation. At the time of submission of this report, 7.5% of the cohort remain hospitalized and 15% died with an overall mortality rate per 1000 per year of 0.41. The average time to collection of the first paired sample after hospital admission was 4.2±3.8 days and the 2nd sample was collected at 8.9±3.2 days.

Of the 95 paired NP and MT swabs tested for SARS-CoV-2 from 40 patients, 76 (80%) NP and 61 (64%) MT swabs were positive (p = 0.02, Figure 1A). While most initial NP (34/40, 85%) and the corresponding MT swabs (29/40, 73%) (sampling time point 1; Fig 1A) were positive (p=0.53), significantly fewer MT swabs were positive (24/29, 82% NP vs 13/29, 45% nasal; p=0.001) about a week later (sampling time point 2; Fig 1A).

The relationship between the CT value and the RT-PCR results of NP and MT swabs is shown in Figure 1B and depicts the results of testing from longitudinal samples from a single patient. As can be seen, while both swabs collected at the first 3 time points were positive

when CT values were low, the MT swab at the 4th sampling time point was negative when the NP swab remained positive but with a CT value of 35. To further examine the association between the specimen type and viral load, RT-PCR positive NP swabs were divided into 2 groups based on CT values ≤ 30 or >30 and the results of corresponding MT swabs were compared. Of those with positive NP samples with CT ≤ 30, most corresponding MT swabs (50/54, 94%) were also positive. In contrast, only 9/22 (41%) MT samples from patients with positive NP swabs with CT values >30 were positive ($p<0.0001$; Fig 1C). A modest correlation between NP and nasal swabs for SARS-CoV-2 RNA was observed only at CT values ≤30 ($r=0.51$) (Figure 1C).

RNAse P PCR of discordant NP and MT samples showed similar amplification plots without significant differences in CT values (Table S2).

Discussion

Our study of paired NP and MT swabs from a cohort of hospitalized COVID-19 patients demonstrates that while both NP and MT swabs are reliable for diagnosis early during hospitalization, NP swabs are more reliable later in disease course (Figures 1B and 1C). Both the CDC and the IDSA currently endorse testing of NP or MT samples for SARS-CoV-2 with limited data. By analyzing serial samples from 40 patients, we examined the reliability of MT swabs in the setting of varying SARS-CoV-2 viral load levels. Our findings suggest that patients whose NP swabs are PCR-positive but have a lower viral load as suggested by high CT values (>30), often test negative by MT swab.

To date, only two peer reviewed publications have directly compared NP and MT swabs in patients with suspected COVID-19[9, 10]. Péré et al. tested NP and MT swabs from 44 patients and found that NP and MT swabs were positive in 37 and 33 patients, respectively. We report similar findings but only for samples obtained earlier during hospitalization. However, Péré et al did not examine samples obtained at later time points. The study by Tu et

al., compared self-collected nasal, tongue, and MT swabs with provider-collected NP samples in 504 individuals. NP swabs were positive in 52 patients and of those, 50 were also positive by MT swabs however, samples were not collected at later time points. Our findings show that the concordance between these sample types decreases at later time points since only 40% of MT swabs tested positive when compared to corresponding NP swabs.

Limitations of our study include a small sample size and inclusion predominantly of patients with severe COVID-19. We estimated viral load based on the CT values, similar to other investigators who have employed this strategy[7, 11]. Testing of paired NP and MT swabs early and later during hospitalization has allowed us to compare the reliability of MT swabs in the context of decreasing SARS-CoV-2 viral loads in the upper respiratory tract. While sample quality might explain this discrepancy, RNase P PCR performed on discordant samples showed similar CT values for both NP and MT swabs (Table S2).

Our findings demonstrate that MT swabs are less reliable in detecting SARS-CoV-2 in individuals with lower viral loads in the upper respiratory tract. Therefore, MT swab testing for SARS-CoV-2 may not be reliable for making infection prevention decisions. In conclusion, both NP and MT swabs identify individuals with COVID-19 early in the course of the illness, but NP swabs are more reliable later in disease course.

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Figure Legends

Figure 1: Comparison of nasopharyngeal (NP) and mid-turbinate (MT) nasal swabs for the detection of SARS-CoV-2 and the relationship between CT values of both swabs.

A) Proportion of positive tests for paired MT nasal and NP swabs for all tests, those at the first sample timepoint and those at a second, later timepoint. Significance determined by fisher's exact test. B) CT values by MT or NP swab over sequential sampling in one individual is shown to illustrate the relationship between the CT values and the results of RT-PCR for the detection of SARS-CoV-2 RNA. Dotted line represents the RT-PCR limit of detection. C) Relationship between CT values of MT and NP swabs, compared between NP swabs with a CT value ≤ 30 or >30. R and p values determined by spearman correlation and graphically represented by linear regression. Red dashed line of y = x.

Figure 1