Original Article

Diagnosis of COVID-19 from lower airway sampling after negative nasopharyngeal swab

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Background: False negatives in nasopharyngeal swab testing for coronavirus disease 2019 (COVID-19) are a concern due to implications of a missed diagnosis on medical decision making and transmission risk. We aimed to characterize the presentation of patients diagnosed with COVID-19 on lower airway sampling following negative nasopharyngeal swab.

Methods: We performed a retrospective observational study to identify COVID-19 patients in whom nasopharyngeal swab testing was negative. Characteristics of patients were collected including demographics, presenting symptoms, and number of false negatives prior to diagnosis.

Results: We identified 8 patients in whom COVID-19 diagnosis was assisted by lower airway sampling following between 1 and 4 negative nasopharyngeal swabs. While presenting characteristics of such patients were non-specific, we identify those with negative nasopharyngeal testing on initial presentation versus later in illness, who subsequently tested positive on lower airway sampling.

Conclusions: Presentations of patients with COVID-19 in whom nasopharyngeal swab is negative are non-specific. A high degree of clinical suspicion is required in approaching patients with persistent, unexplained respiratory symptoms given the high prevalence of COVID-19. It is imperative to correctly identify patients positive for COVID-19, and lower airway sampling with tracheal aspirate or bronchoalveolar lavage (BAL) may be of value in cases of high suspicion by expediting the diagnosis, though further study is required.

Keywords: Coronavirus; coronavirus disease 2019 (COVID-19); bronchoscopy; tracheal aspirate; nasopharyngeal swab

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Introduction

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and may lead to severe lung injury that progresses to acute respiratory distress syndrome (ARDS) (1). It was first reported in the United States in January 2020 following its discovery the month prior in Wuhan, China, where it was initially identified via negative-stained transmission electron microscopy of bronchoalveolar lavage (BAL) samples (2). In the ensuing months, the worldwide proliferation of SARS-CoV-2 caused a global pandemic.

Various diagnostic tests have been developed to aid in identification of infected patients, utilizing specimens from both the upper and lower respiratory tract. Use of a nasopharyngeal swab to obtain an upper respiratory tract sample is the most common method given its minimally invasive nature. Unfortunately, data are wide ranging on its sensitivity, and false negative results occur, although reports on their frequency are very heterogenous (3). In an early study by Zhao *et al.*, only 67% of upper respiratory samples were positive, while 93% of study subjects had antibody seroconversion, suggesting a high rate of false negatives (4).

Initial review of published literature identified few reports of patients with negative nasopharyngeal swab testing but with positive result on a lower airway sampling, as well as one report which was positive on expectorated sputum sample (5-9). An analysis of SARS-CoV-2 detection rates in samples from different parts of the body by the National Institute of Viral Disease Control and Detection in China noted a higher sensitivity on BAL, although it was based on a sample size of only 15 subjects (10). Early literature suggested that peak nasopharyngeal shedding of virus is within 24 hours of symptoms and decreases thereafter (11). This phenomenon has prompted experts to question the utility of delayed nasopharyngeal sampling in hospitalized patients (12).

As further retrospectively and prospectively collected datasets of COVID-19 patients have been examined and published, further information regarding the phenomenon of false negative initial nasopharyngeal and oropharyngeal PCR testing has become available as it relates to repeat testing (13-15). The false negative rate varied widely across studies from 2% to 60%, leading the authors of a recent meta-analysis of 34 datasets addressing false negative initial testing to conclude that the data available to date, although they are sizeable, represent a low level of evidence (3).

A study out of Stanford based on 23,126 initial SARS-CoV-2 PCR tests demonstrated a low rate of false negatives, concluding that repeat PCR testing within 7 days very rarely (3.5%) demonstrated a positive result (16). In a series of 177 asymptomatic patients (58% of whom were immunocompromised) with negative nasopharyngeal swabs, repeat testing on bronchoscopy was negative in all cases (17). It is to be noted that in symptomatic patients, less attention has been given to instances of false negative testing in which diagnosis was clarified by lower airway sampling, a topic sure to be relevant to the bronchoscopist and clinician.

Experience with false negative PCR testing in the H1N1 influenza pandemic may be instructive, as it involved a similar disease process wherein a primarily upper airway infection caused subsequent lower airway infections and manifestations. One retrospective cohort study identified 6.3% of cases of H1N1 during a 2-year period in which diagnosis was aided by lower airway sampling. Further investigation of similar findings may be instructive during the COVID-19 pandemic and applicable in future respiratory disease outbreaks (18).

We present a dedicated case series of patients in whom a lower respiratory tract sample was positive for infection after a negative nasopharyngeal swab. We examine the characteristics of these patients in order to better inform future clinical management and research studies. We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/jphe-21-7).

Methods

We performed a retrospective multicenter review of all adult patients from 3/1/2020 to 8/15/2020 who were positive for COVID-19 on any lower respiratory tract specimen. We then excluded those patients who were also positive on an upper respiratory tract specimen collected via nasopharyngeal swab. After doing so, 8 patients were identified that were only positive on a lower respiratory tract specimen.

All hospitalized adult patients (≥18 years of age) whose diagnosis of COVID-19 disease was established by lower airway sampling, including BAL or sputum, were included.

Statistical analysis

Data collected included demographics, comorbidities, nature of presentation, steroid and antibiotic use, duration of time between symptoms and negative and positive

Table 1 Patient characteristics at time of lower airway sampling

Age (years)	Sex	Race	Medical comorbidities	Presenting symptoms	Steroid use[a]	Antibiotics[b]	Duration between symptom onset and false negative (days)	Duration between symptom onset and positive (days)	Lower respiratory specimen
76	M	Caucasian	Myelodysplastic syndrome, bone marrow transplant, hypertension	Dyspnea, weakness, fever	Yes	Yes	3	10	BAL
77	M	Caucasian	Cirrhosis, hepatocellular carcinoma, hypertension, diabetes	Dyspnea, cough, anosmia, confusion	No	Yes	7	9	BAL
68	M	Caucasian	Hypertension	Cough, fever	No	Yes	32	34	Tracheal aspirate
53	M	Black	None	Dyspnea, cough, fever	No	Yes	4	5	Tracheal aspirate
34	F	Asian	None	Dyspnea, cough	No	Yes	2	3	Tracheal aspirate
45	F	Black	Diabetes, obesity	Rhinorrhea, cough, fever, diarrhea	No	Yes	33	34	Tracheal aspirate
88	M	Caucasian	Multiple myeloma, pulmonary fibrosis	Dyspnea, cough, fever, confusion	Yes	Yes	7	10	BAL
53	M	Black	None	Cough, fever, diarrhea	No	Yes	4	14	BAL

[a], steroid use was prophylactic prednisone; [b], antibiotic regimen varied. M, male; F, female; BAL, bronchoalveolar lavage.

testing, nature of testing and sample collected, clinical course, and disposition. Analysis was performed using descriptive statistics presented in table format given the small sample size of our case series.

Deidentified data were collected and stored in a secure database. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Review Board of the Medical College of Wisconsin (PRO 38240), which granted a waiver of informed consent and monitored all participating institutions through a standard reliance agreement.

Results

We identified 8 patients in which COVID-19 infection was confirmed via a lower respiratory tract sample after a negative nasopharyngeal swab. Their presenting characteristics are noted in *Table 1*.

All patients presented with non-specific symptoms of respiratory infection and were retested on lower airway sampling due to a high index of suspicion and high prevalence in the community. Four patients were identified on bronchoscopic sampling, and four were identified on tracheal aspirate. One of the eight was immunosuppressed, and all were hospitalized.

Four patients were Caucasian, three were black, and one was Asian. Time from negative symptoms to false negative nasopharyngeal swab was widely variable from days to over a month in two cases, and number of negative swabs prior to diagnosis varied between 1 and 4. Ages ranged from 34 to 88 years old, with five of eight patients being over 60 years of age. All patients received antibiotics prior to testing. Two of eight patients received steroids prior to admission.

Discussion

Our aim was to examine patient presentation, comorbidities, testing methods, outcomes, and disease course with the goal of identifying characteristics that may alert clinicians to patients in whom diagnostic evaluation beyond a simple nasopharyngeal swab may be appropriate. Given the variability in demographics and comorbidities of patients described in this retrospective cohort, the unifying characteristic was upper respiratory symptoms not well explained by alternate etiologies and a high degree of clinical suspicion during a pandemic. Immunocompetence was variable within the cohort described. Age tended toward older patients, which reflects demographic trends in

hospitalization for COVID-19 (19). Two of eight patients received steroids prior to admission. This was done in the context of treatment of hematologic disorders. Most subjects (five out of eight) were identified within the first two weeks of symptom onset via lower airway sampling following an upper airway swab.

Two patients were identified as COVID-19 positive a month after initial symptoms during a second presentation with respiratory failure. Initial nasopharyngeal swab on readmission was negative in both cases. However, given the history and characteristic presentation, positive tracheal aspirates were obtained. It is unclear whether these cases represent a false negative COVID-19 test on initial readmission swab testing or if COVID-19 remained persistently detectable despite not being the true etiologic agent in their recurrent respiratory failure. Bacterial infection is a possible alternate explanation that may have been negative on tracheal aspirate as both patients were started on antibiotics prior to culture. Another possible etiology is post-viral organizing pneumonia, which has been described in influenza and an increasingly recognized phenomenon in COVID-19 (19).

A recent editorial raised concern for an elevated rate of false negatives in COVID-19 PCR testing (20). Due to the urgent nature of the problem, the Emergency Use Act (EUA) was passed to expedite test availability with subsequent alteration in sensitivity validation on artificial samples rather than clinical samples. This change may lead to an overestimation of sensitivity due to a differing sensitivity to naturally occurring virus (21).

A great deal of concern focused on false negative initial SARS-CoV-2 PCR testing has lead to reporting of false negative rates in studied cohorts, although these have varied significantly raising concern for variability in methodology and consistency in reporting in available literature (3). Regardless, false negative testing during the COVID-19 pandemic likely represents a risk due to inappropriate treatment and precautions resulting from a failure to identify the correct diagnosis. A variety of explanations have been proposed to explain variability in false positive rates based on patient and testing conditions.

The extent to which sampling technique truly affects sensitivity remains unclear given recent efficacy reports. Instructions for use of current nasopharyngeal swabs require deep nasopharyngeal sampling, which may affect sensitivity if not performed appropriately (22). Regardless of technique utilized, within our sample of 8 patients, the number of negative nasopharyngeal swabs prior to diagnosis varied from 1 to 4. In cases where there is sufficient doubt regarding the accuracy of an initial test, early lower airway sampling may be an appropriate alternative to repeat testing.

Another proposed explanation for false negative initial nasopharyngeal swabs in our cohort of hospitalized patients relates to the temporal association of viral shedding. During the SARS pandemic of 2002–2003, a study of viral shedding and quantitative testing of viral load on respiratory samples demonstrated that unlike most viruses which peak early in the disease course, SARS levels rose within the first week and were often persistent through two weeks. This prolonged viral shedding was felt to be contributory to in-hospital transmission (23). A study examining sampling from various sources during COVID-19 differed significantly, noting that even in mildly symptomatic patients, quantitative testing on nasopharyngeal samples demonstrated peak viral shedding within 5 days of testing and may have peaked prior to testing, while viral shedding from sputum was more prolonged (11). This represents a significant departure from prior studies, raising the possibility of lower sensitivity on nasopharyngeal swabs if tested later in the course of disease, as may have been the case in our cohort of hospitalized patients. This raised the possibility of peak viral shedding early in the symptomatic phase of the illness or even before this time point. A study to further elucidate false negative rates related to temporal relation to time of symptom onset indicates lower sensitivity both very early and very late in the symptomatic phase, with lower rate of false negative testing occurring within the first week of symptoms (24).

Nosocomial transmission represents another possible explanation for an initial negative COVID-19 test followed by positive deep airway sample. Healthcare providers account for up to 16% of detected COVID-19 cases in some areas, with factors including penetration of testing strategies, adequacy of personal protective equipment, and public health strategies to control disease spread likely contributing to a variable rate (25). The infection rate reported in Long Island, New York, for example, was similar to that of the general population, while anecdotally, approximately half of emergency room workers in a single institution in Wales tested positive (26,27). Ultimately, the number of healthcare providers infected is unknown due to a lack of universal testing and reporting, but it is likely high (28). Accounts from the experience of the 2002-2003 SARS pandemic indicate that healthcare associated transmission is of significant concern, which is exacerbated

by the high rate of asymptomatic COVID-19 carriers (29). Thus, nosocomial transmission of COVID-19 is a risk inherent to in-person healthcare exposure, despite attempts to reduce this risk by PPE policies that have been adopted in many hospitals. Respiratory decompensation in patients with prior healthcare exposure should trigger evaluation for COVID-19.

Conclusions

We present a dedicated case series in which COVID-19 diagnosis was confirmed by lower airway sampling following an initial negative nasopharyngeal sample. While lacking large numbers from which to make broader inferences, a smaller study such as this has the strength of allowing for a case-by-case assessment and comparison. Within the cohort identified on our retrospective review, we have identified two groups of patients: those with early negative nasopharyngeal testing presenting with respiratory failure in whom the diagnosis of COVID-19 is made on lower airway sampling (tracheal aspirate or BAL) and those presenting after resolution of initial COVID-19 in whom lower airway sampling persistently demonstrated COVID-19 positivity. We have also provided possible explanations for both types of presentation.

Recognizing clinical situations suggestive of false negative COVID-19 testing is important for several reasons including increased risk of transmission, inadequate care, and inadequate triaging. Many hospitals have modified workflow to reduce risk of exposure of healthcare workers and other patients by recognizing early signs of decompensation from COVID-19 and providing appropriate escalation of care. Patients with COVID-19 can have variable courses, and precipitous clinical decompensation is a recognized phenomenon, which makes early identification imperative. A high degree of suspicion is warranted despite highly sensitive assays when there is high pre-test probability of a disease (high prevalence), as high pre-test probability decreases the negative predictive value of a test. Clinicians must be cognizant of the possibility of false negative nasopharyngeal swabs, and based upon our experience we suggest that they should consider lower airway sampling in patients when there remains a high clinical suspicion for COVID-19 infection.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Review Board of the Medical College of Wisconsin (PRO 38240), which granted a waiver of informed consent and monitored all participating institutions through a standard reliance agreement.

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