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BRIEF REPORT

Yield of Severe Acute Respiratory Syndrome Coronavirus 2 Lower Respiratory Tract Testing After a Negative Nasopharyngeal Test Among Hospitalized Persons Under Investigation for Coronavirus Disease 2019

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Among hospitalized persons under investigation for coronavirus disease 2019 (COVID-19), more repeated severe acute respiratory syndrome coronavirus 2 nucleic acid amplification tests (NAATs) after a negative NAAT were positive from lower than from upper respiratory tract specimens (1.9% vs 1.0%, $P = .033$). Lower respiratory testing should be prioritized among patients displaying respiratory symptoms with moderate-to-high suspicion for COVID-19 after 1 negative upper respiratory NAAT.

Keywords. COVID-19 testing; coronavirus; lower respiratory tract.

Accurate diagnosis of coronavirus disease 2019 (COVID-19) among hospitalized persons under investigation (PUI) for COVID-19 is vital to ensure appropriate use of transmission-based precautions and initiation of therapy for infected individuals [1]. Nucleic acid amplification tests (NAATs) are the gold standard for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) detection and are most commonly performed on upper respiratory tract (URT; eg, nasal, nasopharyngeal, and saliva) specimens due to ease of collection and wide availability of validated testing platforms [1]. URT NAAT sensitivity varies from 70% to 95% based on time from symptom onset and other factors, raising concerns for false-negative tests [2–4].

NAAT sensitivity is higher using lower respiratory tract (LRT; eg, sputum, tracheal aspirate, and bronchoalveolar lavage [BAL]) specimens compared with URT specimens [5–8]. The Infectious Diseases Society of America (IDSA) recommends repeated SARS-CoV-2 testing from LRT rather than URT specimens when suspicion for COVID-19 remains high despite a negative initial test [1]. However, data on the real-world yield of repeated NAATs using LRT specimens are lacking. We aimed to evaluate the yield of LRT NAATs among hospitalized PUI with moderate-to-high suspicion for COVID-19 despite an initial negative test and describe the characteristics of individuals diagnosed using LRT NAATs.

MATERIALS AND METHODS

We conducted a retrospective study of adults >18 years old who underwent a SARS-CoV-2 NAAT for initial COVID-19 diagnosis between 1 March and 31 December 2020 at Massachusetts General Hospital (MGH). Subjects were identified through electronic health records. We included PUI without prior diagnosis of COVID-19 who were hospitalized at MGH for ≥24 hours. From 1 March to 7 April 2020, the PUI definition was limited to patients experiencing symptoms consistent with COVID-19 [1]. As of 8 April 2020, the PUI definition was broadened to include patients with an epidemiologic risk factor (eg, persons experiencing homelessness, exposed to a confirmed COVID-19 case, or residing in congregate settings), irrespective of symptoms. We excluded individuals tested while hospitalized but not meeting PUI criteria.

Repeated NAATs for diagnosis were recommended either by infectious diseases physicians (through 20 May 2020) or by the CORAL (COvid Risk cALculator) diagnostic algorithm (21 May 2020 onward) [9]. Sputum induction was not recommended. URT NAATs were almost exclusively performed on nasopharyngeal specimens using US Food and Drug Administration (FDA) emergency use authorization (EUA) assays [9, 10]. LRT NAATs were performed at the Massachusetts state laboratory, or with an internally validated protocol using the Cepheid Xpert Xpress SARS-CoV-2 assay.

Demographic characteristics of patients who did and did not undergo LRT testing were compared using χ^2 tests for proportions and Mann-Whitney U tests for continuous variables (Stata version 15.1). We considered a P value $<.05$ to be statistically significant. We grouped NAATs performed within a

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hospitalization into categories based on chronologic order, that is, first tests, second tests, and all third and subsequent tests combined. We compared URT and LRT NAAT positivity within each group using χ^2 tests. We then conducted medical records review to examine the clinical and radiologic characteristics of patients diagnosed with a positive LRT NAAT after an initial negative URT NAAT during the same hospitalization.

Ethical Considerations

The study was approved by the Massachusetts General Brigham Institutional Review Board with a waiver of written informed consent.

RESULTS

A total of 18 379 SARS-CoV-2 NAATs were performed in 9925 hospitalized PUI. Demographic characteristics were similar between patients who did and did not have LRT testing performed, including sex (male: 59.7% vs 55.8%, $P = .055$), age (median [interquartile range], 61 [51–71] vs 63 [48–75] years, $P = .052$), and race (nonwhite: 23.9% vs 27.1%, $P = .099$).

Of the 18 379 NAATs examined, 17 682 (96.2%) were performed on URT specimens and 697 (3.8%) were performed on LRT specimens (Table 1). Subjects in the intensive care unit had a higher proportion of NAATs performed on LRT specimens than subjects not in intensive care (39.3% vs 8.9%, $P < .01$). Among LRT NAATs, 56 of 697 (8.0%) were performed on BAL specimens; no BAL NAATs were positive. Among 641 of 697 (92.0%) LRT NAATs that were performed on sputum or tracheal aspirates, 15 (2.3%) were positive.

Among first NAATs performed during the hospitalization, 1209 of 11 198 (10.8%) URT NAATs were positive, while 2 of 28 (7.1%) of LRT NAATs were positive ($P = .534$). Among second NAATs, 58 of 5593 (1.0%) URT NAATs were positive compared with 6 of 114 (5.3%) LRT NAATs ($P < .001$). Positivity on third or later NAATs was similar between specimen types (URT: 9/891 [1.0%] vs LRT: 7/555 [1.3%], $P = .657$). Considering all repeated tests after the first negative NAAT, a lower proportion of URT NAATs were positive compared to LRT NAATs (67/6484 [1.0%] vs 13/669 [1.9%], $P = .033$).

Among the 13 subjects with COVID-19 diagnosed on LRT specimens after an initial negative URT NAAT, ages ranged from 22 to 82 years, 8 (61.5%) were male, and 6 (46.2%) self-identified as non-Hispanic white (Table 2). All subjects had symptoms consistent with COVID-19 and abnormal chest radiographs; 9 of 12 (75.0%) subjects had chest computed tomographic findings typical of COVID-19 [11]. One subject with a chronic tracheostomy was diagnosed using a tracheal aspirate; all others were diagnosed using expectorated sputum. Subjects required up to 4 NAATs for diagnosis. Average time from symptom onset to first NAAT performed was 10 days (range, 0–21 days). Average time from symptom onset to diagnosis was 15 days (range, 2–26 days). Among subjects who underwent repeated NAATs within 14 days after diagnosis, 4 of 8 (50%) had positive tests (URT: 2/7 [28.6%]; LRT: 2/2 [100%]). Only 2 of 4 (50%) subjects with SARS-CoV-2 serologies performed had positive serologies. All subjects survived until hospital discharge and remained on transmission-based precautions for at least 10 days following diagnosis.

DISCUSSION

We found that the yield of repeated SARS-CoV-2 NAATs among hospitalized persons under investigation for COVID-19 was higher when the repeated test was performed on LRT compared with URT specimens. The greatest difference in test positivity between specimen types was observed on the second NAAT (URT: 1.0%, LRT: 5.3%). All subjects diagnosed using LRT NAATs displayed symptoms and/or imaging findings highly concerning for COVID-19. However, most subjects diagnosed using a LRT NAAT had their initial negative URT NAAT performed >7 days after symptom onset and were diagnosed with COVID-19 ≥14 days after symptom onset,

Table 1. Severe Acute Respiratory Syndrome Coronavirus 2 Nucleic Acid Amplification Test Percentage Positivity by Specimen Type and Number of Tests in Chronologic Order During the Same Hospitalization

	Specimen Type			
Test Result	Total	URT	LRT	*P* Value
First NAAT				
Positive	1211 (10.8)	1209 (10.8)	2 (7.1)	.534
Negative	10 015 (89.2)	9989 (89.2)	26 (92.9)	
Second NAAT				
Positive	64 (1.1)	58 (1.0)	6 (5.3)	<.001
Negative	5643 (98.9)	5535 (99.0)	108 (94.7)	
Third and subsequent NAATs				
Positive	16 (1.1)	9 (1.0)	7 (1.3)	.657
Negative	1430 (98.9)	882 (98.9)	548 (98.7)	

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: LRT, lower respiratory tract; NAAT, nucleic acid amplification test; URT, upper respiratory tract.

Table 2. Characteristics of Subjects With Coronavirus Disease 2019 Diagnosed on Lower Respiratory Tract Specimens After a First Negative Upper Respiratory Tract Nucleic Acid Amplification Test

Patient No.	Age/ Sex	Race/ Ethnicity	Presenting Symptoms	CT Chest Findings[a]	No. of NAATs for Diagnosis	Symptom Onset to First Negative NAAT, d	Symptom Onset to Diagnosis, d	Serology Result	Required Intensive Care
1[b]	32/F	Black, non-Hispanic	Fever, hypoxia, vomiting	NA	2	NA	NA	NA	No
2	63/F	White, non-Hispanic	Fever, cough, nausea, vomiting	Indeterminate	3	16	19	Negative 32 d after symptom onset[c]	No
3	82/F	White, non-Hispanic	Cough, dyspnea, rhinorrhea, sore throat, anosmia, fatigue	Typical	3	21	26	NA	No
4	53/M	White, non-Hispanic	Fever, cough, dyspnea, myalgias, fatigue, anorexia, diarrhea	Typical	4	10	18	NA	Yes, intubated after diagnosis, with *Streptococcus pneumoniae* pneumonia
5	22/M	Latinx/ Hispanic	Fever, cough, dyspnea, myalgias, nosebleeds	Typical	2	7	13	NA	No
6	55/M	Latinx/ Hispanic	Fever, cough, dyspnea, myalgias, diarrhea	Typical	3	13	18	Negative 14 d after symptom onset	No
7	62/M	Black, non-Hispanic	Fever, cough, fatigue	Indeterminate	3	1	6	NA	No
8	53/F	Black, non-Hispanic	Headache (in the setting of trauma)	Typical	2	0	9	NA	Yes, related to trauma
9	38/F	Latinx/ Hispanic	Fever, cough, nausea, vomiting, diarrhea, anorexia	Typical	2	9	14	NA	No
10	57/M	Latinx/ Hispanic	Fever, dyspnea, chest pain, myalgias	Typical	2	16	22	Positive IgM/IgG 22 d after symptom onset	No
11	53/M	White, non-Hispanic	Dyspnea, sore throat	Typical	2	0	2	NA	No
12[b]	36/M	White, non-Hispanic	Fever, hypoxia, vomiting	Indeterminate	3	NA	NA	NA	No
13	57/M	White, non-Hispanic	Fever, cough, dyspnea, myalgias, nausea	Typical	2	13	18	Positive IgM/IgG 16 d after symptom onset	No

Abbreviations: CT, computed tomography; F, female; IgG, immunoglobulin G; IgM, immunoglobulin M; M, male; NA, not available; NAAT, nucleic acid amplification test.

[a]All patients had abnormal chest radiographs. Chest CT findings were reported in accordance with the Radiological Society of North America criteria for findings related to coronavirus disease 2019 [11].

[b]Subject was unable to provide history directly, so information on presenting symptoms was obtained from a surrogate.

[c]Subject was immunosuppressed on rituximab; repeat severe acute respiratory syndrome coronavirus 2 serologies 1 month later also remained negative.

suggesting that LRT NAAT may be most useful for PUI presenting late in disease.

Our findings are consistent with other studies reporting higher sensitivity of NAATs from LRT compared with URT specimens [7, 12]. A meta-analysis involving 3442 NAATs found that sputum NAAT sensitivity (71% [95% confidence interval {CI}, 61%–80%]) was higher than nasopharyngeal NAAT sensitivity (54% [95% CI, 41%–67%]) [8]. Reported BAL NAAT sensitivity is even higher at >85% [7, 13]; however, no subjects were diagnosed with COVID-19 by BAL in our study, potentially reflecting sampling bias. Furthermore, other than 1 patient with a chronic tracheostomy, no subjects diagnosed on LRT NAAT were intubated when the diagnostic specimen was obtained. We hypothesize that the vast majority of individuals with sufficiently severe COVID-19 to require intubation have high enough viral burden upon admission to detect SARS-CoV-2 on URT NAAT [14].

Most patients diagnosed by LRT NAAT in our study were initially tested by URT late in disease, when URT viral load may be below the limit of detection of URT testing [3, 4] but high viral burden in the lungs may persist [13, 14]. Patients diagnosed ≥14 days into illness may no longer have transmissible disease and thus may not require transmission-based precautions [15]. However, it is still critical to confirm the diagnosis of COVID-19 to guide targeted treatment [16], initiate contact tracing, and establish the 90-day recovery period during which reinfection is unlikely [15]. Screening of donor lungs prior to transplantation also necessitates the exclusion of SARS-CoV-2 infection by LRT testing [17].

Operationally, the benefit of improved sensitivity with LRT NAATs must be weighed against the challenges of obtaining LRT testing. Less than a third of patients presenting with COVID-19 endorse sputum production, and many cannot provide expectorated sputum [18, 19]. BAL and sputum induction are additional means of LRT sampling. However, these procedures are considered aerosol generating and BAL is invasive, so they should be avoided unless clinically indicated [15]. Additionally, LRT NAAT turnaround time is often longer than for URT specimens, as most available FDA EUA testing platforms are not authorized for use with LRT specimens, requiring their referral to laboratories with internally validated SARS-CoV-2 LRT testing [10]. Longer test turnaround time leads to increased duration of transmission-based precautions in patients who ultimately test negative and greater use of personal protective equipment, with potential impact on hospital capacity. Validation and FDA authorization of LRT specimen types would help improve LRT NAAT availability and test turnaround time.

This analysis has several important limitations. First, it is a single-site study and may not reflect URT and LRT testing practices in other populations. Second, our study was nonrandomized; patients with COVID-19 may be more likely to have lower respiratory symptoms and produce sputum than PUI without COVID-19 infection [20], so LRT NAAT may have been more likely to be performed on PUI with COVID-19. Last, we were unable assess the relative value of expectorated sputum and tracheal aspirate specimens in our full study population; however, all but 1 subject diagnosed on LRT NAAT had testing performed on expectorated sputum.

CONCLUSIONS

Our findings support the IDSA recommendation to perform repeated SARS-CoV-2 testing on LRT rather than URT specimens, when needed for diagnosis among patients with lower respiratory symptoms. Validation of SARS-CoV-2 tests on LRT specimen types should be prioritized to increase access to LRT testing.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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