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Low Utility of Repeat Real-Time PCR Testing for SARS-CoV-2 in Clinical Specimens

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Abstract

In a multicenter cohort of 22,315 patients tested for COVID-19, 1676 (7.5%) had repeat testing via real-time polymerase chain reaction following an initial negative test. Of those retested within 7 days of their first negative test, only 2.0% had a positive result. This suggests that repeat testing from the same source is unlikely to provide additional information.

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In response to the coronavirus disease-2019 (COVID-19) pandemic, there was rapid development and implementation of wide-scale testing using real-time reverse transcriptase polymerase chain reaction (RT-PCR) assays to detect severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). However, little is known about the clinical performance characteristics of these tests. Early reports suggested a clinical sensitivity of 70% in symptomatic patients.[1,2] There are substantial risks and public health implications associated with COVID-19 false negative results.[3] In an effort to improve the sensitivity of PCR testing, many providers will often opt to repeat the test in situations when the suspicion for COVID-19 remains high following an initial negative PCR test. There are no recommendations to date about the utility of serial testing in patients with an initial negative PCR. In this retrospective large multicenter observational study, we aim to describe the clinical utility of serial PCR testing in a cohort of patients receiving a PCR assay for suspected COVID-19.

There are several clinical factors that may affect the performance characteristics of molecular testing, which make repeat testing a clinically useful strategy. During a SARS-CoV-2 infection, the viral load appears to peak in the upper respiratory tract approximately 24 hours before the onset of symptoms and then decrease over the next 5 to 7 days.[4] This suggests that the best time to obtain an upper respiratory specimen is very early in the disease course, when the viral replication is at its highest.[5] The variation in viral load is also confounded by the site from which the specimen is taken. For example, early in the illness, a nasopharyngeal (NP) or oropharyngeal specimen may be more sensitive than a lower respiratory tract specimen (eg, sputum, bronchoalveolar lavage [BAL] fluid). The opposite may be true later in the illness.[6] The severity of the COVID-19 illness also plays a major role, as severe cases of COVID-19 have significantly higher viral loads compared with mild cases.[7] In addition to these factors, inconsistencies in specimen collection, handling, and processing may affect the accuracy of PCR testing. Considering these limitations, we set out to examine and describe the cohort of patients who received serial NP swab testing for COVID-19 at Mayo Clinic.

METHODS

This study was performed at Mayo Clinic, an academic medical center with campuses in Minnesota, Arizona, Florida, and Wisconsin. We collected the results of all patients (n=22,315) in our electronic health record (EHR) who underwent PCR testing for COVID-19 between March 10, and April 13, 2020. Testing across Mayo Clinic sites

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TABLE. Characteristics of Patients Tested for COVID-19 via Real-Time PCR at Mayo Clinic Between March 10, 2020, and April 13, 2020

Patients	All patients (n=22,315)	Patients with > 1 test (n=1870)	Patients with >1 test AND test 1 was negative (n=1676)	Patients with >1 test within 7 days of initial negative test results (n=1113)
Number of tests (average tests/patient)	24,517 (1.1)	4072 (2.2)	3530 (2.1)	2262 (2.0)
Positive	720 (3.0%)	421 (10.3%)	44 (1.2%)	22 (1%)
Negative	23,797 (97.0%)	3651 (89.6%)	3486 (98.8%)	2240 (99%)
Inpatient collection	4634 (18.9%)	1091 (26.8%)	988 (28.0%)	575 (25.4%)
Outpatient collection	19,883 (81.1%)	2981 (73.2%)	2542 (72.0%)	1687 (74.6%)
Age, median (IQR)	46 (32-62)	50 (35-63)	49 (35-63)	48 (35-61)
Male	8697 (39%)	729 (39%)	644 (38.4%)	441 (39.6%)
State of residence				
Minnesota	12,169 (54.6%)	827 (44.2%)	746 (44.5%)	430 (38.6%)
Wisconsin	4023 (18.0%)	143 (7.7%)	130 (7.8%)	65 (5.8%)
Arizona	2509 (11.3%)	185 (9.9%)	141 (8.4%)	87 (7.8%)
Florida	2968 (11.3%)	643 (34.4%)	595 (35.5%)	493 (44.3%)
First test positive	493 (2.2%)	194 (10.4%)	N/A	N/A
Any test positive	525 (2.4%)	226 (12.0%)	32 (1.9%)	22 (2.0%)

IQR = interquartile range.

was completed using various Emergency Use Authorized (EUA) assays, with labs in Minnesota and Florida using the Roche cobas SARS-CoV-2 test (Roche Diagnostics, Basel, Switzerland) and a laboratory-developed test (LDT)[8] and the laboratory in Arizona performing testing by the Abbott RealTime SARS-CoV-2 assay on the m2000 instrument (Abbott Molecular, Abbott Park, Illinois). All results through April 13, 2020 were included in this analysis. In addition to the test result, we also collected the test order date, collection date, result date, specimen type, and collection location. Patient-specific information—including age, sex, county, and state of residence—were also collected and matched with the corresponding tests. Patients who had not previously provided authorization for research were excluded. We also excluded patients who were tested for SARS-CoV-2 as part of a screening program for urgent and semi-urgent surgeries. We used JMP Version 14 (SAS Institute Inc., Cary, North Carolina) for statistical analysis. We first examined the entire cohort of patients, then specifically investigated patients with multiple tests and patients with multiple tests after the first test results were negative. We also evaluated tests performed within 7 days of the first test. This was to reduce error from patients who may have become infected between the first and second tests. This study received institutional review board approval.

RESULTS

This study included 24,517 test results from 22,315 unique patients. Of these, 1870 patients were tested more than once (Table). The median age of the cohort was 46 years, 39% percent of the patients were men, and the majority had their primary residence listed in the state of Minnesota. Of the 24,517 SARS-CoV-2 PCR tests performed, there were 720 positive results (2.9%) on 525 distinct patients. Among tests performed on an initial sample collection, 2.2% results were positive (Arizona: 3.1%; Florida: 2.3%, Minnesota: 2.2%; Wisconsin: 1.5%).

There were 1870 patients (8.4%) who were tested more than once. In those retested, the median time to a second test was 5 days (interquartile range [IQR]: 3 to 7). Of these patients, 1676 (90%) had initially negative test results. In general, these patients were similar in age and sex

to the entire cohort of tested patients. Compared with patients from other states, patients from Florida were more likely to be tested multiple times (odds ratio [OR], 3.96; 95% confidence interval [CI], 3.56 to 4.38), even if their first test result was negative (OR, 4.14; 95% CI, 3.71 to 4.61). Among the 1676 patients with negative first test results, 1113 (66.4%) had repeat testing within 7 days (median time to second test 7 days, IQR: 4 to 10 days). Of the patients who underwent repeat testing within 7 days after a first negative test, only 22 (2.0%) had subsequent positive test results. Only 5 of these 22 were tested using an assay different from the assay used for the initial test. In these 22 cases, both specimens were obtained from nasopharyngeal swabs. The median time from an initial negative test result to a positive test result was 4 days (IQR: 3 to 9 days). Single variable logistic regression models suggested that neither sex, age, obtaining the repeat test within 7 days, nor repeat test collection location (inpatient vs outpatient) were predictive of a positive test result after an initial negative test result.

All but 13 of the specimens came from upper respiratory tract swabs (ie, oropharynx, nasopharynx, or both). Most of these specimens were collected in the outpatient setting (82%), with a smaller percentage collected in the emergency department (13%) or inpatient setting (5%). Repeat collections were more common in the inpatient setting. Only 3.7% of first specimens and 14.9% of second specimens were collected in the inpatient setting. During the study period, 123 patients died (0.5%), and 11 of these tested positive for SARS-CoV-2. Their cause of death was not examined in this study but presumed to be unrelated to COVID-19. None of the 22 patients with delayed positive tests within 7 days had died at the time of this analysis.

DISCUSSION

In this large cohort of patients tested for SARS-CoV-2 (COVID-19), repeat testing was frequent but rarely resulted in a positive result after an initial negative test result. This observation remained true among patients who had repeat testing performed in $\leq$7 days. Possible explanations for disparate results include the first test being a false negative (ie, because of the timing of testing, inadequate sample collection, or laboratory error) or the possibility that the patient contracted the viral infection following the initial test. Also possible, but less likely, is a false positive second test result. The observation that short-interval repeat testing rarely led to positive results suggests that the decision to repeat testing must be more nuanced. The decision to repeat a COVID-19 PCR should involve the estimated revised probability of COVID-19 based on the initial negative test, an assessment of whether an alternate specimen type (such as a lower respiratory-tract specimen) may provide a higher diagnostic yield, and the impact on the patient's management if a laboratory diagnosis is not secured. Ongoing studies to characterize the clinical sensitivity and specificity of COVID-19 PCR assays will provide important information for clinicians who will be able to determine the positive and negative predictive values depending on disease burden in their area.

This study has a number of limitations. It is retrospective, and there is a possibility of selection bias. Overall, patients undergoing retesting had higher initial positivity rates than the entire cohort, suggesting that some retesting was being performed for confirmatory purposes (ie, for clinical trial enrollment). This is further illustrated by the higher rate of repeat testing in the inpatient setting compared with the outpatient setting. Higher rates of retesting in Florida may have reflected a lower threshold for retesting in a region that was experiencing a higher prevalence of COVID-19 disease at the time of this study. However, clinical reasoning behind repeat testing was not formally examined. This study also considered the multiple COVID-19 assays performed at our sites as identical, and they may have different performance characteristics. Also, as previously discussed, there are several other patient and pathogen features that may affect the ability of a laboratory assay to detect this virus. Keeping in mind these limitations, we believe that determining the frequency of conversion from negative to positive PCR results in this

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large cohort is helpful for clinical decision making and resource allocation.

CONCLUSION

Among 1113 patients with initial negative test results, and who underwent repeat testing within 7 days, 22 (2.0%) patients had subsequent positive PCR test results during that time. This suggests that, in most cases, repeat testing will not result in a positive test result, especially when prevalence of disease is low. In these cases, in which clinical suspicion of COVID-19 is high but initial testing results are negative, clinicians should consider collecting a lower respiratory tract sample (eg, sputum, BAL fluid)[9] and treating patients as if they have COVID-19 while searching for alternative diagnoses. A repeat test from the same source in this situation is unlikely to yield additional clinical utility.

Abbreviations and Acronyms: **COVID-19** = coronavirus disease 2019; **IQR** = interquartile range; **RT-PCR** = reverse transcriptase polymerase chain reaction; **SARS-CoV-2** = severe acute respiratory syndrome coronavirus 2

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