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SHORT COMMUNICATION

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Repeat virological and serological profiles in hospitalized patients initially tested by nasopharyngeal RT-PCR for SARS-CoV-2

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Abstract

Real-time polymerase chain reaction (PCR) for SARS-CoV-2 is the mainstay of COVID-19 diagnosis, yet there are conflicting reports on its diagnostic performance. Wide ranges of false-negative PCR tests have been reported depending on clinical presentation, the timing of testing, specimens tested, testing method, and reference standard used. We aimed to estimate the frequency of discordance between initial nasopharyngeal (NP) PCR and repeat NP sampling PCR and serology in acutely ill patients admitted to the hospital. Panel diagnosis of COVID-19 infection is further utilized in discordance analysis. Included in the study were 160 patients initially tested by NP PCR with repeat NP sampling PCR and/or serology performed. The percent agreement between initial and repeat PCR was 96.7%, while the percent agreement between initial PCR and serology was 98.9%. There were 5 (3.1%) cases with discordance on repeat testing. After discordance analysis, 2 (1.4%) true cases tested negative on initial PCR. Using available diagnostic methods, discordance on repeat NP sampling PCR and/or serology is a rare occurrence.

KEYWORDS

Coronavirus, COVID-19, diagnostic accuracy, discordance, RT-PCR, SARS-CoV-2, serology

1 | INTRODUCTION

Molecular methods such as real-time polymerase chain reaction (PCR) are the mainstay for the diagnosis of COVID-19 in hospitalized patients. Although there are differences in molecular assays, analytical sensitivity for the detection of SARS-CoV-2 is high.[1] Most conflicting reports are on the clinical performance of PCR. Reports of a high rate of initial false-negative SARS-CoV-2 results by PCR, later diagnosed with COVID-19 by chest CT and repeat PCR, have emerged and continue to be reported.[2–7] As SARS-CoV-2 serological testing has become available, this has presented another testing modality to retrospectively adjudicate suspected cases with negative PCR and a way to ascertain true cases of COVID-19.[8]

The aim of our study is to estimate the frequency of discordance between initial nasopharyngeal (NP) PCR and repeat NP sampling PCR and/or serology in acutely ill patients admitted to the hospital. We further aim to use a panel diagnosis of COVID-19 in discordance resolution.

2 | METHODS

2.1 | Participant selection

All adult patients aged ≥18 years admitted to an acute care hospital for >24 h who had NP swabs tested by PCR for SARS-CoV-2 from March 13 to April 17, 2020, were initially evaluated. Patients with repeat NP sampling PCR or serology performed were included in the study. Patients were excluded from the study if they had testing performed >40 days after symptom onset.[9] This study was approved by the Research Ethics Board, Providence Health Care/University of British Columbia.

2.2 | Diagnostic testing

NP swabs were collected as per local guidelines.[10] Testing for viral RNA consisted of one of two commercial methods: LightMix® Real-Time PCR COVID-19 assay for the Envelope E-gene (TIB Molbiol) with amplification on the Roche LightCycler® 480, or cobas® SARS-CoV-2 Qualitative Assay on the cobas® 6800 System (Roche Molecular Diagnostics). Antibody testing was performed on patients with serum collected ≥1 week and <4 months after initial PCR test or symptom onset. Elecsys® Anti-SARS-CoV-2 assay (Roche) using recombinant protein representing the nucleocapsid (N) antigen for determination of total antibodies was performed on the Roche cobas® e601. The test result is given as a cut-off index (COI) with COI ≥1.0 considered "reactive" and COI <1.0 as "nonreactive" as per package insert.[11]

2.3 | Panel diagnosis of COVID-19

Patients were classified as having a low, moderate, or high likelihood of COVID-19 based on chart review and clinical assessment.[12,13] Low probability cases had a clear alternative diagnosis explaining the clinical presentation and/or clinical features inconsistent with a COVID-19 infection; moderate probability cases had compatible clinical features and/or radiology findings but a presumptive alternative diagnosis; and high probability cases presented with compatible clinical features, radiological findings, no alternative diagnosis and/or an epidemiologic link to a known COVID-19 case. Patients deemed moderate to high risk for COVID-19 on initial assessment underwent further review by two internal medicine specialists caring for patients on dedicated COVID-19 hospital units. Any discordance in assessment was reviewed by an infectious disease specialist. Reviewers were not blinded to the PCR test results as these were reported in the electronic medical chart but were blinded to the serological results. Interobserver reliability of clinical likelihood was evaluated using Cohen's kappa calculation.[14]

2.4 | Discordance analysis

Discordant cases were resolved using panel diagnosis to mirror the practical approach to testing in a clinical context. For example, discordant cases which tested negative on initial PCR and positive on repeat PCR, for which clinical likelihood for COVID-19 was high were deemed false negative by initial PCR. On the other hand, cases that tested positive on initial PCR and negative on repeat PCR, and had high likelihood clinical assessment were deemed true positive cases.

3 | RESULTS

During the study period, 323 patients had NP swabs tested by PCR, of these 290 (89.8%) initially tested negative and 33 (10.2%) tested positive. Included in the study were 160 patients who underwent additional testing by either repeat NP sampling PCR and/or serology. The median time since onset of symptoms to initial test was 3 days (range: 0–31). Repeat NP sampling PCR was performed on 123 patients for a total of 232 repeated tests (187 NP swabs, 18 sputa, 10 tracheal aspirates, 6 bronchoscopy specimens, 5 saliva, 2 nares, and 1 each of rectal swab, throat, saline gargle, and mouth rinse). The median number of repeat PCR tests was 2 (range: 1–8) per patient. None of the cases testing negative on initial NP PCR tested positive by an alternative sampling method. Serology was available for 91 patients, of which 18 tested "reactive" and 73 tested "nonreactive."

The percent agreement between included patients' initial and repeat PCR was 96.7% (119/123), while the percent agreement between initial PCR and serology was 98.9% (90/91), with one PCR positive case testing nonreactive on serology (COI = 0.73). Serology was done at a mean of 69 days (range: 14–138 days) from symptom onset. In cases of discrepancy between initial PCR and repeat PCR or serology, panel diagnosis was used in discordance resolution.

Discordance was observed in 5 (3.1%) of 160 patients, of which 2 had initial PCR positive followed by a negative PCR, 2 had initial PCR negative followed by positive PCR and 1 had positive initial and

TABLE 1 Discordant case analysis of initial PCR and additional testing (repeat NP sampling PCR/serology) resolved by panel diagnosis

Case	Initial PCR	Repeat PCR	Time between initial PCR and repeat PCR (days)	Serology	Time between initial PCR and serology (days)	Panel diagnosis
1.	Negative	Positive Ct = 19.06/19.36	8	nd	n/a	High likelihood of COVID-19
2.	Negative	Positive Ct = 34.99/37.14	4	Negative (COI = 0.099)	13	High likelihood of COVID-19
3.	Positive Ct = 23.72/25.91	Negative (saliva)	7	nd	n/a	High likelihood of COVID-19
4.	Positive Ct = 32.34/35.39	Negative (NP)	5	nd	n/a	High likelihood of COVID-19
5.	Positive Ct = 23.43/23.88	Positive (sputum) Ct = 20.92/20.91	6	Negative (COI = 0.73)	73	High likelihood of COVID-19

Note: Cases 1 and 2 deemed false negative on initial PCR based on discordance analysis.
Abbreviations: COI, cut-off index; n/a, not applicable; nd, not done; PCR, polymerase chain reaction.

repeat PCR but was "nonreactive" on serology (COI = 0.73) (Table 1). Based on panel diagnosis, all five were deemed to be true cases with a high likelihood of COVID-19. Good agreement between a panel of reviewers was observed with a kappa of 68.4%. Of the cases that tested negative on initial PCR, there were 2 (1.4%) cases with repeat positive PCR tests occurring during the same clinical episode (within the 40 days).

4 | DISCUSSION

Recent systematic reviews illustrate high heterogeneity and variability in estimated false-negative rates of NP swab PCR in the range of 1.8%–33%.[5,6] The wide range of clinical diagnostic accuracy estimates are influenced by the timing of presentation, clinical syndrome, anatomical site of testing, quality of specimen collection, and reference standard used.[6,15,16] In our study initial testing was performed on NP swabs of patients requiring admission to hospital. Most patients were tested at 3 days since symptom onset and had on average of two repeat tests. In addition to repeat NP sampling PCR, we used serology as an independent diagnostic method. Panel diagnosis, consisting of two separate assessors with a good inter-rater agreement, was used in discordance analysis.

We estimated percent agreement between patients' initial and repeat PCR of 96.7%, and initial PCR and subsequent serology of 98.9%. We were unable to calculate true sensitivity and specificity due to the lack of a true gold standard for COVID-19 diagnosis, a limited number of patients, and as additional testing was only done on a subset of the total cases. We identified 5 (3.1%) discordant cases. Two cases tested positive by initial PCR and negative by subsequent PCR, both were deemed high likelihood on panel diagnosis and PCR reversion part of the natural progression of the disease. One case with concordant initial and repeat positive PCR, tested negative on serology (COI = 0.73) was deemed to be a true case of COVID-19. This patient's immunosuppression as a result of heart transplantation may have been responsible for a blunted serological response.[17] Only 2 (1.4%) of initially negative PCR tests converted to positive. Similarly, a large study by Long et al.[18] found a low rate of discordance of initially negative PCR with subsequent PCR positivity at 3.5% but did not include serology in subsequent testing. In our first false-negative case the initial negative NP swab was taken 24 h after symptom onset and became positive 8 days later. PCR testing earlier than 48 h of symptom onset can lead to false-negative results as viral shedding can be below the level of detection.[6] The second false-negative case occurred in an elderly patient with severe viral pneumonia and a high clinical likelihood of COVID-19. The patient's first three NP swabs were negative until the fourth one tested positive 18 days after symptom onset. In severe infection viral load tends to be higher and peaks later; in this patient, it is possible that a lower respiratory specimen would have yielded better viral RNA recovery.[19] Difficulties with sputum production and concerns of aerosol generation with bronchoscopy present practical challenges in collecting lower respiratory tract specimens. In our

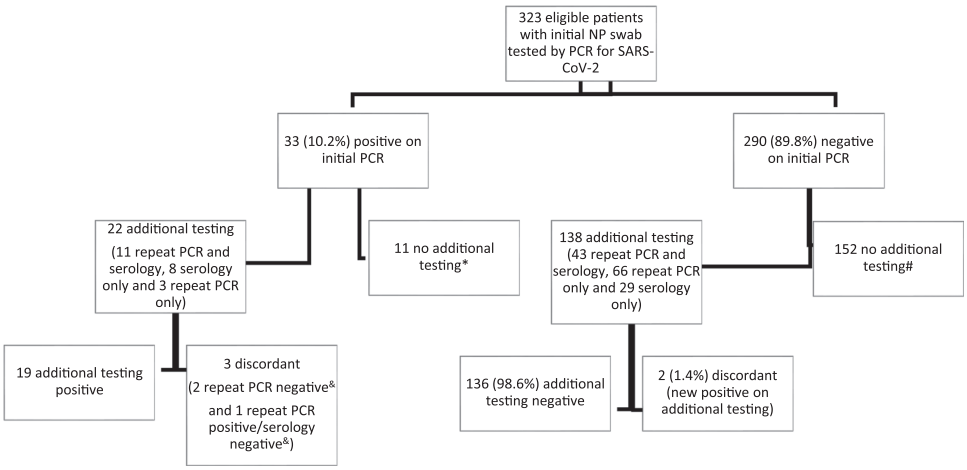

FIGURE 1 Flow diagram of patients undergoing additional testing (repeat NP sampling PCR and/or serology). Among patients testing negative on initial RT-PCR, 98.6% remained negative on additional testing and 1.4% converted to positive.*90.9% High clinical likelihood for COVID-19, #95.4% low clinical likelihood for COVID-19, &true positive cases on panel diagnosis. NP, nasopharyngeal

study, only 14% of included patients had lower respiratory specimens collected and no cases had PCR conversion to positive on lower respiratory specimens. Encouragingly in most cases, NP swabs performed well with a low rate of discordance, minimizing the need for more invasive specimen collection. Overall, the findings of our study support the use of NP PCR as the recommended specimen for the diagnosis of COVID-19 and demonstrate that follow-up testing (subsequent NP swabs and serology) in hospitalized patients are highly concordant with initial NP PCR.

The limitations of our study are the retrospective design and selection bias due to a hospital setting. Our data applies to sicker, hospitalized patients who tend to have greater viral shedding, possibly leading to improved rates of PCR detection.[19] Due to the small number of patients as only a subset of patients had repeat PCR or serology, we were limited in inferences to false-negative rates. We were also unable to determine the status of cases with no repeat testing performed but 95.4% of cases with initial negative PCR were deemed low clinical likelihood likely not warranting further testing. As our patient cohort had initial NP PCR testing on average 3 days since symptom onset, we could not comment on the potential for false-negative results at the time before or at symptom onset, though the rate of false negatives in this setting has been reported to be higher.[6] False-negative serology could have underestimated percent discordance, but in our dataset, this is a rare occurrence that primarily applies to highly immunocompromised individuals.[17] Last, clinical assessment of COVID-19 likelihood was not blinded to PCR results subject to incorporation bias, but at the same time reducing the risk of case misclassification (Figure 1).

5 | CONCLUSION

Molecular testing on NP swabs has a high correlation with repeat NP sampling PCR and serology and discordance remain a rare phenomenon. As recommended by IDSA guidelines, cases with a high clinical likelihood of COVID-19 and repeatedly negative NP swab PCR should undergo testing with serology to further enhance diagnostic yield, and a single PCR result cannot be interpreted in isolation without full clinical assessment of the case.[20]

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CONFLICT OF INTERESTS

Roche Diagnostics provided Elecsys® Anti-SARS-CoV-2 serology test kits for the study. Roche had no involvement in the design or conduct of this study, namely collection, analysis, and interpretation of the data; preparation, and decision to submit the manuscript.

AUTHOR CONTRIBUTIONS

Study conceptualization and design: Aleksandra Stefanovic, Noah Reich, Christopher F. Lowe, David Puddicombe, Nancy Matic, Nick Myles, and Jesse Greiner. *Data acquisition*: Noah Reich, Aleksandra Stefanovic, Nancy Matic, Christopher F. Lowe, Jesse Greiner, Laura Burns, Victor Leung, Terry Chu, Hiten Naik, Janet Simons, Kent Dooley, Inna Sekirov, and Gordon Ritchie. *Manuscript write-up*: Aleksandra Stefanovic, Noah Reich, Christopher F. Lowe, and David Puddicombe. Revision and approval of the final draft by all authors.

DATA AVAILABILITY STATEMENT

Data presented in the manuscript has not been made available but can be shared on request.

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