Short communication

When should clinicians repeat SARS-CoV-2 RT-PCR?: Repeat PCR testing targeting patients with pulmonary CT findings suggestive of COVID-19.

Running head: Repeated RT-PCR testing for SARS-CoV-2

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Summary

Real-time reverse transcription polymerase chain reaction (RT-PCR) testing for SARS-CoV-2 is sometimes repeated when clinicians suspect a false-negative result, but the conditions under which repeated RT-PCR testing is warranted remain unclear. We evaluated the practice of repeat RT-PCR testing for SARS-CoV-2 in 45 patients who retested after an initial negative PCR test. Of these, the diagnosis of coronavirus disease (COVID-19) was confirmed in four patients with typical chest computed tomography (CT) findings, and one patient without typical CT findings in whom the test result was strongly suspected to be false positive. We recommend repeat RT-PCR only for patients with typical CT findings of COVID-19.
Main Text

Since December 2019, the novel coronavirus infectious disease (COVID-19) has become a pandemic. As of 11 June 2020, more than 7.2 million confirmed COVID-19 cases and 413,000 deaths have been reported in over 200 countries (1). The acute phase of COVID-19 can mostly be confirmed by a nucleic acid amplification test such as real-time reverse transcription polymerase chain reaction (rRT-PCR) for SARS-CoV-2. From March 9, 2020, when the cost of COVID-19 screening was covered by health insurance in Japan, to April 24, 2020, the central laboratory of our hospital in Tokyo and outsourced laboratories performed over 2,000 rRT-PCR tests for COVID-19. Although there were variations depending on the reports, the initial nasopharyngeal rRT-PCR sensitivity for COVID-19 diagnosis was 89% according to a meta-analysis report (2). rRT-PCR sometimes yields false-positive results, as noted in the Infectious Diseases Society of America (IDSA) guidelines for diagnosing COVID-19 (3), and may also yield false-negative results, so a single rRT-PCR test is not enough to rule out SARS-CoV-2 infection. It has been proposed that repeat testing be performed for individuals with suspected COVID-19 who test negative on the initial test (4), but it is unclear which individuals require repeat rRT-PCR. This study aimed to evaluate cases of repeated rRT-PCR and make recommendations regarding indications for testing.

The single-center retrospective observational study was approved by the Research
Ethics Committee of the National Center for Global Health and Medicine

(NCGM-G-003565-00). We reviewed test results and medical records of 1,803 patients who underwent rRT-PCR tests at our institution from March 9 to April 24, 2020. While there were no strict criteria for the PCR test, we generally performed the tests for patients with symptoms, including fever, cough, other respiratory tract symptoms, taste disturbances, and smell disturbances and for those with symptoms occurring within a few days of presentation. Furthermore, rRT-PCR was performed for asymptomatic patients who had contact with COVID-19 patients. Of them, 364 (20%) patients were confirmed as SARS-CoV-2-positive by rRT-PCR. rRT-PCR was performed according to the method recommended by the National Institute of Infectious Diseases, Japan (5). A nasopharyngeal swab was used as the primary sample type. The swabs were stored in universal transport media (1-3 mL) after collection, and 140 µL of the media was used for testing purposes. Sputum specimens were processed using 10% dithiothreitol in phosphate-buffered saline, as recommended by the National Institute of Infectious Diseases, Japan (5). Patients who underwent at least two rRT-PCR tests at our hospital were included. Those who took the tests for different symptoms or separate clinical episodes were excluded.

Sixty-six patients had 2 or more rRT-PCR tests, of whom 45 met the inclusion criteria. Their median age was 51.1 (interquartile range: 33.5–73.8) years. Three patients had contact with someone confirmed to have COVID-19, and 13 patients had a high-risk...
exposure history (defined as working as a healthcare worker or talking to a non-family member in an enclosed space for more than 1 hour). Three individuals had 3 or more RT-PCR tests, and the remaining 42 had only 2 tests. Chest computer tomography (CT) was performed on 36 patients (80%), with multiple typical ground glass opacities, typical of COVID-19, noted in 16 patients. Atypical CT findings for COVID-19 were noted in a further 14 patients, and 6 patients had no abnormalities detected in their lungs. The main indication for more than one rRT-PCR test was typical CT findings in 16 patients, followed by persistent symptoms in 13 patients. Three patients were re-tested because of indeterminate results or false-positive results due to cross-contamination in the laboratory (Table 1). Of 346 patients with confirmed COVID-19, 99% (95% CI: 97–100%) of cases were positive on initial rRT-PCR, and only 5 were positive on the second or subsequent tests. Of 4 patients who were re-tested because of typical CT findings, two were positive on second rRT-PCR test of a nasopharyngeal swab (Table 1, Cases 2 and 3), while two were negative on the second nasopharyngeal swab (Table 1, Cases 15 and 16), who were confirmed positive on rRT-PCR for sputum and induced sputum, respectively. One patient, re-examined due to prolonged symptoms, was positive on second rRT-PCR of nasopharyngeal swab (Table 1, Case 19). However, because her clinical course was typical of pleural tuberculosis, 3 further tests were performed immediately after obtaining a positive rRT-PCR result. All 3 tests were negative, strongly suggesting that the second rRT-PCR test was a false-positive result.
In this study, most patients with confirmed as SARS-CoV-2-positive using rRT-PCR were positive on the initial test, and only 5 (0.3%) were diagnosed on a second or subsequent rRT-PCR test. In 4 of the 5 patients who tested positive by rRT-PCR on the second or subsequent rRT-PCR test, typical pulmonary CT findings were observed. In an earlier report, sensitivity of COVID-19 for initial rRT-PCR was reported to be approximately 70% (6,7). However, a study of more than 1,000 patients reported that the sensitivity was higher (8).

Further, a meta-analysis found the sensitivity of the initial rRT-PCR to be 89% (95% CI: 81–94%, $I^2=90\%$) (2). In the same meta-analysis, the diagnostic accuracy of typical chest CT findings was 93% sensitivity and 35% specificity. Chest CT findings are useful but non-specific. The Society of Thoracic Radiology does not recommend the use of pulmonary CT as a routine screening test for COVID-19 (9). However, for patients with persistent symptoms, coupled with a negative initial rRT-PCR result, pulmonary CT may be a more useful test than a repeat rRT-PCR to exclude COVID-19. The proportion of patients with no abnormal findings on initial chest CT was only 2% in the past report (8). Moreover, pulmonary findings on CT may appear later in such cases (6,7). Conversely, it has been reported that two rRT-PCR negatives do not rule out the possibility of COVID-19, as noted in Cases 15 and 16 of this study (4). If patient experience severe symptoms, including increased oxygen demand, collecting a specimen from the lower respiratory tract to diagnose COVID-19.
Although SARS-CoV-2 is abundant in lower airway specimens from patients with COVID-19 (10), good quality specimens from lower respiratory tract are often difficult to collect. The IDSA guidelines recommend using lower respiratory tract specimens (excluding induced sputum) if possible (3). The positive rate of sputum specimens is generally higher than that of nasopharyngeal swab specimens (10), and the detection rate for SARS-CoV-2 in the same patient was also higher than that of pharyngeal swab (11). If a patient has prolonged symptoms with typical pulmonary CT findings, a lower respiratory tract sample should be collected after two negative nasopharyngeal swab results. Conversely, multiple rRT-PCR test repetitions or rRT-PCR of sputum should not be routinely performed to exclude COVID-19 in patients without typical CT findings. The gratuitous routine practice of repeat rRT-PCR to rule out COVID-19 may lead to questionable cases with false-negative rRT-PCR results such as Case 19 in this study.

In conclusion, we recommend that CT should be performed for patients with COVID-19 symptoms or signs and a negative first rRT-PCR result and that repeated rRT-PCR testing for confirmation should only be performed in those with CT findings suggesting COVID-19.

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**Conflicts of interest**

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Table 1. Characteristics of patients with suspected COVID-19 and two or more real-time RT-PCR tests for SARS-CoV-2

| No. | Age, Sex | Underlying disease | Contact with COVID-19 patient | International travel | High risk exposure | Other pathogens | CT findings | Symptom onset to initial test (days) | Initial test to final test* (days) | Total no. of tests | Result | Sample type in final test |
|-----|----------|--------------------|-------------------------------|----------------------|-------------------|-----------------|-------------|------------------------------------|-----------------------------------|------------------|--------|-------------------------|
| 1   | 86 M     | None               | No                            | None                 | No                | NA              | Typical     | UN                                 | 7                                 | 2                 | Negative     | NS                      |
| 2   | 51 M     | None               | Yes                           | None                 | Yes               | NA              | Typical     | 4                                  | 6                                 | 2                 | Positive     | NS                      |
| 3   | 33 M     | None               | No                            | None                 | No                | NA              | Typical     | 8                                  | 2                                 | 2                 | Positive     | NS                      |
| 4   | 49 F     | None               | No                            | None                 | No                | NA              | Typical     | 1                                  | 3                                 | 2                 | Negative     | NS                      |
| 5   | 44 M     | None               | No                            | None                 | Yes               | NA              | Typical     | 3                                  | 1                                 | 2                 | Negative     | SP                      |
| 6   | 87 F     | dementia           | No                            | None                 | No                | ND              | Typical     | 3                                  | 1                                 | 2                 | Negative     | NS                      |
| 7   | 24 M     | None               | No                            | None                 | No                | ND              | Typical     | AS                                 | 1                                 | 2                 | Negative     | NS                      |
| 8   | 24 F     | UC                 | No                            | Yes; Europe          | No                | ND              | Typical     | 8                                  | 3                                 | 2                 | Negative     | NS                      |
| No. | Age, Sex | Underlying disease | Contact with COVID-19 patient | International travel | High risk exposure | Other pathogens | CT findings | Symptom onset to initial test (days) | Initial test to final test* (days) | Total no. of tests | Result | Sample type in final test |
|-----|----------|---------------------|-------------------------------|----------------------|-----------------|----------------|-------------|------------------------|------------------------|-----------------|--------|----------------------|
| 9   | 34 M     | DM                  | No                            | None                 | Yes             | ND             | Typical     | 1                       | 1                      | 2                | Negative | NS                   |
| 10  | 88 M     | MI, Intracranial hematoma | No                            | None                 | No              | ND             | Typical     | 3                       | 1                      | 2                | Negative | NS                   |
| 11  | 61 M     | Rectal cancer       | No                            | None                 | No              | ND             | Typical     | AS                      | 2                      | 2                | Negative | NS                   |
| 12  | 81 F     | DM                  | No                            | None                 | No              | ND             | Typical     | 1                       | 1                      | 2                | Negative | NS                   |
| 13  | 58 M     | DM                  | Yes                           | None                 | Yes             | ND             | Typical     | 7                       | 7                      | 2                | Negative | NS                   |
| 14  | 27 F     | BA                  | No                            | None                 | No              | ND             | Typical     | 11                      | 1                      | 2                | Negative | NS                   |
| 15  | 74 M     | None                | No                            | None                 | No              | ND             | Typical     | 2                       | 11                     | 4                | Positive | SP                   |
| 16  | 45 M     | DM, CKD, HT, depression | Yes                           | None                 | No              | NA             | Typical     | 2                       | 8                      | 3                | Positive | SP                   |

Persistent symptoms (N=13)
| No. | Age, Sex | Underlying disease | Contact with COVID-19 patient | International travel | High risk exposure | Other pathogens | CT findings | Symptom onset to initial test (days) | Initial test to final test* (days) | Total no. of tests | Result | Sample type in final test |
|-----|----------|--------------------|-----------------------------|----------------------|-------------------|----------------|-------------|----------------------------------|-------------------------------|------------------|--------|--------------------------|
| 17  | 47 M     | None               | No                          | Yes; Europe          | Yes               | NA             | Atypical    | 2                                | 5                            | 2                 | Negative | NS                       |
| 18  | 40 M     | HIV                | No                          | None                | Yes               | NA             | Atypical    | 4                                | 11                           | 2                 | Negative | NS                       |
| 19  | 33 F     | None               | No                          | None                | No                | NA             | Atypical    | 2                                | 5                            | 2                 | Positive** | NS                       |
| 20  | 28 F     | None               | No                          | None                | Yes               | NA             | None        | 14                               | 27                           | 2                 | Negative | NS                       |
| 21  | 31 M     | None               | No                          | Yes; Europe         | Yes               | NA             | None        | 9                                | 6                            | 2                 | Negative | NS                       |
| 22  | 36 M     | None               | No                          | None                | Yes               | NA             | None        | 3                                | 7                            | 2                 | Negative | NS                       |
| 23  | 51 F     | None               | No                          | None                | Yes               | NA             | NA          | 1                                | 5                            | 2                 | Negative | NS                       |
| 24  | 24 M     | None               | No                          | None                | No                | NA             | NA          | 10                               | 5                            | 2                 | Negative | NS                       |
| 25  | 29 F     | None               | No                          | None                | No                | NA             | NA          | 4                                | 13                           | 2                 | Negative | NS                       |
| 26  | 59 F     | None               | No                          | None                | No                | NA             | NA          | 5                                | 15                           | 2                 | Negative | NS                       |
| No. | Age, Sex | Underlying disease | Contact with COVID-19 patient | International travel | High risk exposure | Other pathogens | CT findings | Symptom onset to initial test (days) | Initial test to final test* (days) | Total no. of tests | Result | Sample type in final test |
|-----|----------|-------------------|-------------------------------|---------------------|-------------------|----------------|------------|------------------------------------|-------------------------------|-----------------|--------|------------------------|
| 27  | 22 M     | None              | No                            | None                | Yes               | NA             | NA         | 1                                  | 7                            | 2               | Negative           | NS               |
| 28  | 26 F     | None              | No                            | None                | No                | NA             | NA         | 3                                  | 6                            | 2               | Negative           | NS               |
| 29  | 34 F     | None              | No                            | None                | No                | NA             | NA         | 5                                  | 8                            | 2               | Negative           | NS               |
|     | Others (including unknown reason) (N=13) | | | | | | | | | | |
| 30  | 77 M     | brain stroke, aortic dissection | No                            | None                | No                | NA             | Atypical   | UN                                 | 1                            | 2               | Negative           | NS               |
| 31  | 70 F     | BE                | No                            | None                | No                | NA             | Atypical   | 4                                  | 1                            | 2               | Negative           | NS               |
| 32  | 56 M     | COPD, BA          | No                            | None                | No                | NA             | Atypical   | 2                                  | 1                            | 2               | Negative           | NS               |
| 33  | 40 M     | HIV, CKD, DM      | No                            | None                | No                | ND             | Atypical   | 8                                  | 1                            | 2               | Negative           | NS               |
| 34  | 75 M     | schizophrenia     | No                            | None                | No                | ND             | Atypical   | 3                                  | 1                            | 2               | Negative           | NS               |
| 35  | 88 M     | MI, CKD           | No                            | None                | No                | ND             | Atypical   | UN                                 | 8                            | 3               | Negative           | NS               |
| No. | Age, Sex | Underlying disease | Contact with COVID-19 patient | International travel | High risk exposure | Other pathogens | CT findings | Symptom onset to initial test (days) | Initial test to final test* (days) | Total no. of tests | Result | Sample type in final test |
|-----|----------|--------------------|-------------------------------|----------------------|-------------------|----------------|-------------|--------------------------|-----------------------------|----------------|--------|----------------------|
| 36  | 74 M     | pulmonary fibrosis, DM | No                           | None                 | No                | ND             | Atypical    | 7                        | 1                           | 2                | Negative | NS                  |
| 37  | 78 M     | HC, empyema         | No                           | None                 | No                | ND             | Atypical    | 3                        | 7                           | 2                | Negative | NS                  |
| 38  | 73 M     | Parkinson disease, ovarian cancer, SBO | No                           | None                 | No                | ND             | Atypical    | 6                        | 0                           | 2                | Negative | NS                  |
| 39  | 66 M     | epidural hematoma   | No                           | None                 | Yes               | NA             | None        | UN                       | 1                           | 2                | Negative | NS                  |
| 40  | 51 M     | None               | No                           | None                 | Yes               | ND             | None        | 5                        | 1                           | 2                | Negative | NS                  |
| 41  | 17 F     | pneumothorax        | No                           | None                 | No                | ND             | None        | 1                        | 2                           | 2                | Negative | NS                  |
| 42  | 38 F     | pregnancy           | No                           | None                 | No                | NA             | NA          | 3                        | 1                           | 2                | Negative | NS                  |

**Laboratory problem (suspected of cross contamination, indeterminate result) (N=3)**

| No. | Age, Sex | Underlying disease | Contact with COVID-19 patient | International travel | High risk exposure | Other pathogens | CT findings | Symptom onset to initial test (days) | Initial test to final test* (days) | Total no. of tests | Result | Sample type in final test |
|-----|----------|--------------------|-------------------------------|----------------------|-------------------|----------------|-------------|--------------------------|-----------------------------|----------------|--------|----------------------|
| 43  | 86 M     | lung cancer, LC    | No                           | None                 | No                | ND             | Atypical    | 4                        | 5                           | 2                | Negative | NS                  |
| No. | Age, Sex | Underlying disease | Contact with COVID-19 patient | International travel | High risk exposure | Other pathogens | CT findings | Symptom onset to initial test (days) | Initial test to final test* (days) | Total no. of tests | Result | Sample type in final test |
|-----|----------|--------------------|--------------------------------|----------------------|-------------------|----------------|-------------|-----------------------------------|-----------------------------------|------------------|---------|-----------------------|
| 44  | 70 M     | Colon cancer       | No                             | None                 | No                | ND             | Atypical    | 0                                 | 4                   | 2                | Negative | NS                   |
| 45  | 52 M     | SAH                | No                             | None                 | No                | NA             | NA          | UN                  | 1                   | 2                | Negative | NS                   |

Abbreviations: AS, asymptomatic; BA, bronchial asthma; BE, bronchiectasis; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; CT, computed tomography; DM, diabetes mellitus; F, female; HC, hepatitis C; HT, hypertension; LC, liver cirrhosis; M, male; MI, myocardial infarction; NA, not applicable; ND, not detected; NS, nasopharyngeal swab; SAH, subarachnoid hemorrhage; SBO, small bowel obstruction; SP, sputum; UC, ulcerative colitis; UN, unknown.

Typical CT findings were defined as a multiple ground-glass opacity. High-risk exposure was defined as individuals who had spent over 1 hour with an unrelated individual in a closed space, and healthcare workers. Other pathogens were determined using a FilmArray respiratory panel.

* The time between the initial PCR test and final tests performed during the study period that lasted from March 8 to April 24, 2020

**Suspected case of false positive: a positive result was obtained only once out of a total of five rRT-PCR tests performed within 7 days. Thereafter,
the patient was diagnosed with pleural tuberculosis.