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High positive rate after consecutive negative tests of SARS-CoV-2

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been a global public health event since 2019. Real-time reverse transcription polymerase chain reaction (RT-PCR) assays of upper respiratory tract specimens were used as the most common method for confirmation of SARS-CoV-2. Patients diagnosed with coronavirus disease 2019 and hospitalized in Cancer Center of Wuhan Union Hospital were retrospectively enrolled. Epidemiological, clinical, and laboratory records were analyzed with highlights on the pattern of results of repeated RT-PCR tests. Nine hundred eighty-four patients admitted to hospital between February 13, 2020 to March 10, 2020 were enrolled. The median age was 62.0 years (interquartile range 49.0–68.0) and 44.5% was male. Three thousand-three hundred eleven specimens were collected for RT-PCR tests with a median of 3 tests (interquartile range 2.0–4.0) per patient. Three hundred sixty-two (36.8%) patients showed positive records from repeated RT-PCR tests. For the 362 confirmed patients, 147 cases received further RT-PCR tests after 2 consecutive negative records of SARS-CoV-2 and 38 (26%) of them obtained a positive result. Ten (23%) of 43 patients showed positive results after 3 consecutive negative tests and 4 (24%) of 17 patients were positive after 4 negative tests. Consecutive negative RT-PCR tests with respiratory specimens could not guarantee a viral clearance.

Abbreviations: COVID-19 = coronavirus disease 2019, IQR = interquartile range, RT-PCR = real-time reverse transcription polymerase chain reaction, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

Keywords: COVID-19, SARS CoV-2, RT-PCR

1. Introduction

In December 2019, a series of cases with respiratory symptoms and typical chest computed X-ray tomography features were reported in Wuhan city, Hubei province, China.[1,2] A previously unknown beta coronavirus was then discovered through use of full-genome sequencing in samples from these patients.[3,4] The new beta coronavirus was named 2019 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and formed another clade within the subgenus sarbecovirus, orthocoronavirinae subfamily. SARS-CoV-2 was believed to be the pathogen of coronavirus disease 2019 (COVID-19), which caused fever, cough, and dyspnea as common symptoms, and led to an epidemic throughout the world.

Real-time reverse transcription polymerase chain reaction (RT-PCR) assays of upper respiratory tract specimens were used as the most common method for confirmation of SARS-CoV-2. Patients infected with SARS-CoV-2 usually need quarantine and medical care in hospitals. According to the guidelines of SARS-CoV-2 infection from the National Health Commission of China,[5] a key criterion for discharge was negative RT-PCR results with respiratory tract specimens for 2 consecutive times with 24 hours apart. But, positive RT-PCR results were found among discharged patients,[6] indicating 2 consecutive negative RT-PCR tests may not guarantee a complete viral clearance. Illuminating the transformation rate of SARS-CoV-2 from negative cases to positive cases is vital for management of convalescent patients and related data is void.

In this study, we describe the results of repeated RT-PCR tests among patients with COVID-19 with highlights on patients showed consecutive negative tests. Clinical characteristics and laboratory data were further analyzed among patients with 2 consecutive negative tests. We believe our study would help improving the understanding and management of COVID-19.

2. Methods

2.1. Patients

In late December 2019, several hospitals of Wuhan reported clusters of patients with pneumonia of unknown cause, which was identified as SARS-CoV-2 soon after. The local government proclaimed a list of designated hospitals to treat patients infected with SARS-CoV-2, including the Union hospital. In this retrospective study, all consecutive patients admitted to Cancer Center of Union Hospital from February 13 to March 10 and received ribonucleic acid tests of SARS-CoV-2 during

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The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

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Table 1

Detection of SARS-CoV-2 with repeated RT-PCR tests.

	Total	Positive
Calculated by cumulative records		
Age		
<15	1/984 (0.1%)	0
15–49	250/984 (25.4%)	72/250 (28.8%)
50–69	532/984 (54.1%)	204/532 (38.3%)
≥70	201/984 (20.4%)	86/201 (42.8%)
Total	984/984 (100%)	362/984 (36.8%)
Specimens		
Throat swabs	3228/3311 (97.5%)	576/3228 (17.8%)
Sputum	78/3311 (2.4%)	33/78 (42.3%)
Others	5/3311 (0.0%)	0

Data are n/N (%), where N is the total number of patients or specimens with available data.
RT-PCR = real-time reverse transcription polymerase chain reaction, SARS-CoV-2 = 2019 severe acute respiratory syndrome coronavirus 2.

hospitalization were enrolled. All cases were diagnosed with COVID-19 based on symptoms, radiology or SARS-CoV-2 tests. Patients were divided into severe and mild cases according to the guidelines of SARS-CoV-2 infection from the National Health Commission of China.[5] This study was approved by the medical ethics committee of Union Hospital. The requirement for informed patient consent was waived by the ethics committee for this retrospective study.

2.2. RT-PCR tests

Clinical specimens were used for RT-PCR tests with SARS-CoV-2. Most patients were collected with throat swabs or sputum after 1 to 3 days after admission. Repeated collections of specimens were executed according to the course of illness. Following the recommendation of China National Center for Disease Control, 2 target genes were set as described previously,[4] including open reading frame1ab and nucleocapsid protein (N), and simultaneously amplified and tested during the real-time RT-PCR assay. Target 1 (open reading frame1ab): forward primer CCCTGTGGGTTTTACACTTAA; reverse primer ACGATTGTGCATCAGCTGA; and the probe 5'-FAM-CCGTCTGCGGTATGTGGAAAGGTTATGG-BHQ1-3'. Target 2 (N): forward primer GGGGAACTTCTCCTGCTAGAAT; reverse primer CAGACATTTTGCTCTCAAGCTG; and the probe 5'-FAM-TTGCTGCTGCTTGACAGATT-TAMRA-3'. A cycle threshold value (Ct value) <37 was defined as a positive record, and a Ct value exceeds 40 was defined as a negative test.

2.3. Data collection

Epidemiological, clinical, and laboratory records were collected from the electronic medical network of Union hospital with standardized data collection forms. To ensure the accuracy of data, 2 independent researchers were arranged to review and check the data form.

2.4. Statistical analysis

Continuous variables were reported as median (interquartile range, IQR), and compared with the Mann–Whitney *U* test. Categorical variables were reported as number and percentages, and compared by χ^2 test. Statistical analysis was performed with SPSS (version 26.0) software. A *P* value of <.05 was considered to indicate statistical significance. All probabilities are 2-tailed.

3. Results

984 patients admitted to Cancer Center of Wuhan Union Hospital between February 13, 2020 to March 10, 2020 were enrolled in this study and followed up to March 12. The median age of enrolled patients was 62.0 years (IQR 49.0–68.0) and 44.5% was male. three thousand-three hundred eleven specimens were collected from the 984 patients for RT-PCR tests with SARS-CoV-2. With cumulative records, 576 (17.8%) of 3228 throat swabs and 33 (42.3%) of 78 sputum were positive. All non-respiratory specimens were negative. In total, 362 (36.8%) patients showed positive records from repeated RT-PCR tests with SARS-CoV-2 (Table 1).

Most patients received repeated RT-PCR tests during hospitalization. The median repeated times of RT-PCR tests were 3 times (IQR 2.0–4.0) per patient. The median time interval between 2 consecutive tests was 3 days (IQR 2.0–5.0). The positive rate was 20.0% for patients receiving 1 RT-PCR test, 6.4% for receiving 2 tests, 31.5% for receiving 3 tests, and 60.8% for receiving 4 tests. All patients received more than 8 times of tests were positive with SARS-CoV-2. Generally, the positive rate improved with the increase of repeated tests. Along with the disease course, most patient transformed from positive cases to negative cases, but some patients then developed to positive again. For the 362 patients with confirmed SARS-CoV-2 infection, 324 cases received further tests of respiratory specimens after 1 negative RT-PCR result with SARS-CoV-2 and 152 (47%) of them obtained a positive record. One hundred forty-seven patients received further RT-PCR tests after 2 consecutive negative records and 38 (26%) of them obtained a positive result. Ten (23%) of 43 patients showed positive results after 3 consecutive negative records, and 4 (24%) of 17 patients were positive after 4 negative records. Three patients continued to receive RT-PCR tests after 5 consecutive negative records and showed no positive results then (Table 2).

The characteristics of the 147 patients receiving further RT-PCR test after 2 consecutive negative records of SARS-CoV-2 were analyzed. All the 147 patients were SARS-CoV-2 confirmed cases. According to RT-PCR results after the 2 consecutive negative records, the 147 patients were divided into positive group (n = 38) and negative group (n = 109). The median age was 61.50 years (IQR 45.00–71.00) for positive group and 63.00 years (IQR 52.00–68.00) for negative group. The portion of severe cases were 26.3% for positive group and 25.7% for negative group. No significant differences in sex distribution between the 2 groups were identified. Time from the onset of illness to admission were 13.50 days (IQR 9.00–21.00) for positive group, which was longer than the 10.00 days (IQR 6.00–19.00) observed in the negative group. As for the time from onset of illness to first result of consecutive negative tests, the positive group was 30.50 days (IQR 22.00–39.00) and the negative group was 30.00 days (IQR 26.00–37.00), suggesting a long-lasting presence of SARS-CoV-2 in the respiratory system. The time between first negative and a consecutive positive test for positive group was 6.00 days (IQR 4.00–10.00). As significant changes of serological indicator of patients with COVID-19 were reported previously,[7,8] serological records on admission and time of continual RT-PCR test after 2 consecutive negative records were analyzed and no significant difference between groups were found (Table 3).

4. Discussion

We described the pattern of repeated RT-PCR tests with SARS-CoV-2 among patients of COVID-19. For confirmed SARS-CoV-2 infected patients, 26% of them may transformed to positive cases after 2 consecutive negative RT-PCR tests, indicating patients need a further period of quarantine even after consecutive negative results.

A various of kinds of specimens had been used for detection of SARS-CoV-2 with RT-PCR tests. From the report of Wenling Wang, the positive rate of multiple clinical samples was 93% for bronchoalveolar lavage fluid, 72% for sputum, 63% for nasal swabs and 32% for pharyngeal swabs.[9] With the highest positive rate, bronchoalveolar lavage fluid may behave as the most accurate method for SARS-CoV-2 detection, but it was limited by complication of operations and suffering of patients from a broad use. Although sputum showed a high positive rate, but the application of sputum was hindered by the fact that most people with COVID-19 do not have sputum, especially during the convalescent period.[7] While, a method of sputum induction reported previously may solve this problem and reduce the risk of exposure of medical staff to SARS-CoV-2 when collecting respiratory specimens.[10]

Detection of SARS-CoV-2 with throat swabs were reported in a series of studies, with the positive rate ranging from 32% to 71%.[11–13] Vary of the criterion for enrolling suspected patients may account for this inconsistency. Meanwhile, time from onset of illness to collection of specimens could be another influence factor. According to the report of Yang Yang, the positive rate of throat swabs was 60.0% in 7 days after onset of illness, 50.0% during 8 to 14 days after onset, and 36.8% after 15 days from onset.[14] The viral load of SARS-CoV-2 was reported to change with time, with high viral load observed in the first week after onset of illness and low viral load after 2 weeks from onset.[15] In our work, most patients received first time of RT-PCR tests during hospitalization on more than 10 days from onset of illness, and the viral loads at that time can probably be low, which may account for the low positive rate of throat swabs. In line with previous reports,[9] we also found a lower positive rate in throat swabs than sputum did. It was reported that SARS-CoV-2 uses the same cellular receptor as severe acute respiratory syndrome coronavirus did, which targets human angiotensin-converting enzyme 2 and infects intrapulmonary epithelial cells more than cells of the upper airways, so the replication of SARS-CoV-2 is more likely to happen in the lower respiratory tract rather than the throat.[16–18] This hypothesis may be the reason for low positive rate of throat swabs for RT-PCR assays of SARS-CoV-2.

Table 2

Pattern of results of repeated RT-PCR tests with SARS-CoV-2.

	Total patients	Positive patients
Repeated times of RT-PCR tests		
1	35/984 (3.6%)	7/35 (20.0%)
2	297/984 (30.2%)	19/297 (6.4%)
3	298/984 (30.3%)	94/298 (31.5%)
4	171/984 (17.4%)	104/171 (60.8%)
5	86/984 (8.7%)	59/86 (68.6%)
6	49/984 (5.0%)	38/49 (77.6%)
7	27/984 (2.7%)	20/27 (74.1%)
8	9/984 (0.9%)	9/9 (100.0%)
9	7/984 (0.7%)	7/7 (100.0%)
10	2/984 (0.2%)	2/2 (100.0%)
11	2/984 (0.2%)	2/2 (100.0%)
14	1/984 (0.1%)	1/1 (100.0%)
After consecutive negative tests*		
After 1 negative test	324/362 (90%)	152/324 (47%)
After 2 negative tests	147/362 (41%)	38/147 (26%)
After 3 negative tests	43/362 (12%)	10/43 (23%)
After 4 negative tests	17/362 (5%)	4/17 (24%)
After 5 negative tests	3/362 (1%)	0

Data are n/N (%), where N is the total number of patients with available data. The median time interval between 2 consecutive tests was 3 days (IQR 2.0–5.0).

RT-PCR = real-time reverse transcription polymerase chain reaction, SARS-CoV-2 = 2019 severe acute respiratory syndrome coronavirus 2.

* This part shows the results after consecutive negative records of RT-PCR tests with SARS-CoV-2 among RT-PCR positive patients. The non-respiratory specimens were excluded from calculation.

Table 3

Characteristics of confirmed SARS-CoV-2 infected patients with 2 consecutive negative tests.*

	Positive	Negative	*P* value
Female sex	18/38 (47.4%)	61/109 (56.0%)	.36
Severe	10/38 (26.3%)	28/109 (25.7%)	.939
Age (yr)	61.50 (45.00–71.00)	63.00 (52.00–68.00)	.965
Characteristics on admission			
Days from onset to admission	13.50 (9.00–21.00)	10.00 (6.00–19.00)	.034
Days from onset to consecutive negative tests	30.5 (22.00–39.00)	30.00 (26.00–37.50)	.639
Days from first negative to consecutive positive tests	6.00 (4.00–10.00)	-	-
Lymphocyte count, $\times 10^9$/L	1.24 (1.00–1.55)	1.29 (0.98–1.68)	.624
Eosinophil count, $\times 10^9$/L	0.06 (0.02–0.12)	0.06 (0.01–0.11)	.445
Lactate dehydrogenase, U/L	206.50 (149.50–271.50)	200.00 (171.00–262.00)	.957
IL6, pg/mL	16.55 (6.81–25.99)	12.30 (5.13–36.25)	.941
IL10, pg/mL	3.97 (2.96–5.06)	3.24 (2.68–3.81)	.104

Data are median (IQR) or n/N (%), where N is the total number of patients with available data. *P* values are comparing the positive group and the negative group from χ^2 test or Mann–Whitney *U* test. $P < .05$ was considered statistically significant.

SARS-CoV-2 = 2019 severe acute respiratory syndrome coronavirus 2.

* This table shows characteristics of the confirmed infected patients who received more tests of SARS-CoV-2 after 2 consecutive negative results and were divided into positive and negative groups according to the test results. The non-respiratory specimens were excluded from calculation.

In this study, we found SARS-CoV-2 could exist in the respiratory system for a long time. Among the confirmed infected patients, about a quarter of them got positive results after 2 to 4 consecutive negative RT-PCR tests, indicating that consecutive negative tests are not a reasonable criterion for viral clearance. Accordingly, patients with consecutive negative tests of SARS-CoV-2 should be arranged with further medical observation rather than a certificate of recovery. In this work, the median time from onset of illness to the transformation from positive cases to negative cases was 30.50 days, indicating a long-lasting viral existence in the respiratory system of COIVD-19 patients. Prolonged presence of SARS-CoV-2 was also observed in faces. In the report of Yongjian Wu, of 41 of 74 patients with fecal samples that were positive for SARS-CoV-2 ribonucleic acid, respiratory samples remained positive for SARS-CoV-2 for a mean of 16.7 days and fecal samples remained positive for a mean of 27.9 days after first symptom onset.[19] As a whole, more diversified specimen testing and adequate observation time may be helpful in controlling this epidemic.

Our study had limitations. First, there were no specimens of bronchoalveolar lavage fluid detected, which may act as a positive control for analysis of positive rate of respiratory samples. Second, the non-respiratory specimens were too few to elucidate the influence of SARS-CoV-2 on organs apart from the respiratory system. Finally, more clinical data is needed to further illuminate the correlation between change of RT-PCR tests with symptoms and radiological characteristics.

In conclusion, we reported a high positive rate of SARS-CoV-2 for confirmed patients after consecutive negative results of RT-PCR test with upper respiratory tract specimens, with 26% after 2 consecutive negative tests, 23% after 3 consecutive negative tests, and 24% after 4 consecutive negative tests. It indicates that consecutive negative RT-PCR results of respiratory specimens may be unsuitable to act as a criterion for viral clearance. A longer time of quarantine and medical observation for convalescent patients with COVID-19 is needed.

Author contributions

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