Profile of RT-PCR for SARS-CoV-2: a preliminary study from 56 COVID-19 patients

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Abstract

A novel coronavirus (COVID-19) pandemic threatens the world. Here, we first studied the dynamics profile of SARS-CoV-2 from 56 recovered COVID-19 patients. We found virus shedding was up to 6 weeks after onset of symptoms. Prolonged observation period is necessary for older patients.

Key words

COVID-19; SARS-CoV-2; Dynamics profile
Introduction

To date, an outbreak of infectious diseases--coronavirus disease 2019 (COVID-19) associated with Severe Acute Respiratory Syndrome Coronavirus -2 (SARS-CoV-2) continues in Wuhan, China and threatens countries such as Korea, Italy, Iraq and Japan, etc. [1-2] Over 50 countries are fighting against the disease. As of March 4th, 2020, more than 85,000 cases were diagnosed worldwide with a death rate around 2%.

Before this study, some studies reported cases of viral detection by RT-PCR at different timepoints throughout the disease course. [3-5] However, these reports monitored SARS-CoV-2 in the acute phase of infection. Currently no study reported the viral dynamics of SARS-CoV-2 infection in a long observation period. Understandings the profile of virus in patients’ respiratory specimens can assist diagnosis and reflect the disease course. Therefore, we collected clinical specimens from 56 COVID-19 patients and reported the results of SARS-CoV-2 detection during the disease course.

Methods

A total of 56 hospitalized patients (admission date from Jan 21st to Feb 12th, 2020) with confirmed SARS-CoV-2 infection in three branches (Hankou, Sino-French new city and Optical Valley) of Tongji Hospital at Tongji Hospital of Huazhong University of Science and Technology in Wuhan, China were included in this study. All enrolled patients were confirmed diagnosed of COVID-19 according to the diagnosis and treatment guideline for SARS-CoV-2 from Chinese National Health Committee (Version 5) and the interim guidance from Centers for Disease Control and Prevention. [6-7] Throat swab samples or deep nasal cavity swab samples were collected from patients on different dates after the onset of symptoms. SARS-CoV-2 were detected by real-time reverse transcription polymerase chain reaction (RT-PCR) assay using a COVID-19 nucleic acid detection kit according to the manufacturer’s protocol (Shanghai Huirui Biotechnology Co., Ltd). Specifically, two target genes, including open
reading frame 1ab (ORF1ab) and nucleocapsid protein (N), were tested during the real-time RT-PCR assay. If two consecutive negative results were achieved, the period between symptoms onset and the date of first negative RT-PCR test result was defined as viral nucleic acid conversion time. All data (test dates and results of RT-PCR assay) were collected up to the final follow-up date (March 3rd, 2020).

Results

A total of 56 patients diagnosed as COVID-19 were included in this study. According to the guideline, all included patients were mild to moderate [7]. No patient was transferred to ICU. The median age was 55 years (interquartile range, IQR, 42-68; range, 25-83), comprising 34 (60.7%) men and 22 (39.3%) women. At the end of follow-up, all patients recovered and were discharged from hospital.

The total number of SARS-CoV-2 RT-PCR assay from 56 patients was 299, with 5 tests per patient. The longest duration between RT-PCR test for SARS-CoV-2 was 42 days after onset of symptoms. The median duration between onset of symptom to nucleic acid conversion was 24 days (IQR, 18-31). The details of demographic characteristics and RT-PCR test results were shown in supplemental Table 1.

The number of positive and negative results of RT-PCR tests were shown in Figure 1A. In first 3 weeks after symptoms onset, majority results of RT-PCR for SARS-CoV-2 were positive. From week 3 after symptoms onset, number of negative RT-PCR results increased. All results of RT-PCR tests were negative in week 6 after onset. (Supplemental Table 2) The positive rate of RT-PCR test results was highest at week 1 (100%), followed by 89.3%, 66.1%, 32.1%, 5.4% and 0% at week 2, week 3, week 4, week 5 and week 6 respectively. (Figure 1B)

We divided patients into non-prolonged and prolonged shedding group according to nucleic acid conversion time (≤24 days or >24 days). As shown in supplemental table 3, patients with prolonged viral shedding tend to be older (p=0.011) and were more likely to have comorbidities as diabetes (p=0.016) and hypertension (p=0.006).
Discussion

This study is the first case series from 56 COVID-19 patients with 299 samples of RT-PCR tests for SARS-CoV-2 detection. Our preliminary results are notable for providing evidence of SARS-CoV-2 dynamic profile in patients infected.

Genomic studies have shown that SARS-CoV-2 shared around 80% identity sequencing with SARS-CoV, which caused a global epidemic with 8096 confirmed cases worldwide in 2002-2003. [8] Presumed person-to-person transmission of SARS-CoV-2 was suggested based on epidemiology and clinical evidences. [9, 10] Although SARS-CoV-2 share similar sequencing characteristics with SARS-CoV and MERS-CoV, study of case series suggested the viral nucleic acid shedding pattern of patients infected with SARS-CoV-2 is different from SARS-CoV, which had a modest viral loads in the early stage and peaked approximately 10 days after symptoms onset. [11]

Our study collected series RT-PCR test results from 56 recovered COVID-19 patients and investigate the dynamic profile during the disease course. We showed majority of patients got positive results of RT-PCR test for SARS-CoV-2 within 3 weeks after the onset of symptoms. The negative results of RT-PCR test for SARS-CoV-2 began dominant from week 4 after onset of symptoms and by the end of follow-up (6 weeks), all results of RT-PCR test were negative. The positive rate of RT-PCR test results kept declining in 6 weeks. (Figure 1) The above findings suggested that SARS-CoV-2 viral replication has a relatively long period in infected patients.

Our study attempted to explore the correlation between clinical characteristics and viral shedding in COVID-19 patients. We found that patients with prolonged viral nucleic acid conversion tend to be older and with more comorbidities. Previous studies suggested that coronavirus is more likely to infect older individuals, for whom the immunopathogenesis and induction of a proinflammatory cytokine storm might be the culprit. [12] Older patients with impaired immune function might have a prolonged period of viral elimination.
As a result of errors in sampling and testing, false negative result of RT-PCR for SARS-CoV-2 is very common in clinical settings. Meanwhile, it is recommended by the current diagnosis and treatment guideline for SARS-CoV-2 from Chinese National Health Committee that the criteria to discharge a patient included the relief of symptoms, improvement in radiography and two consecutive negative RT-PCR results for SARS-CoV-2. [7] In our study we found two consecutive negative RT-PCR test results followed by a positive result in 4 patients (patient number 20, 24, 37 and 56). *(Supplemental table 1).* Recent report by Lan et al found positive RT-PCR test results in cases of recovered COVID-19 patients. [13] These infected patients could be the source of transmission. The above findings questioned the current criteria of discharge.

Evidence suggested that the outbreaks of COVID-19 may be correlated to its rapid person-to-person transmission ability. [2] Since specific treatment had not been validated for COVID-19, traditional public health tactics— isolation, quarantine and community containment are critical to control the spread. [14-16] This preliminary study has found evidence of the dynamic profile of SARS-CoV-2 in non-ICU COVID-19 patients during disease course. According to the results in our study, we suggested prolonged observation and repeat confirmation of RT-PCR test from respiratory specimens for safe discharges and discontinuation of quarantine.
NOTES

Author Contributions

All authors participated in the study design. ATX and SZ conceived the study, analyzed the data and drafted the manuscript. YXT helped critically revise the manuscript and collected data. All authors have agreed on the final version and meet the major criteria recommended by the ICMJE (http://www.icmje.org/)

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We thank Ms. Cheng Chen for English grammatic correction of this manuscript. This study was approved by the Ethics committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. All procedures followed in this study were in accordance with the 1964 Helsinki Declaration and later versions. Oral consent was obtained from patients involved before enrollment when data were collected retrospectively. The database used and/or analyzed during the current study is not publicly available (to maintain privacy) but can be available from the corresponding author on reasonable request.

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Conflict of Interest All authors declare that there are no conflicts of interest.
Reference


Figure Captions

**Figure 1.** Dynamic Profile of RT-PCR for SARS-CoV-2.

**(A)** Dynamic Profile of SARS-CoV-2 Detected by RT-PCR from 56 COVID-19 Patients (N=299). Numbers of the positive (red bar) and negative (blue bar) results of SARS-CoV-2 RT-PCR were sum on weeks after the onset of symptoms. **(B)** Positive rate of SARS-CoV-2 Detected by RT-PCR from 56 COVID-19 Patients (N=299). Percentage of positive results of SARS-CoV-2 RT-PCR were calculated on weeks after the onset of symptoms.
Figure 1

A

Number of HIV P24 Tests

Week 1  Week 2  Week 3  Week 4  Week 5  Week 6
Weeks after onset of symptoms

Negative  Positive

B

Positive rate

Week 1  Week 2  Week 3  Week 4  Week 5  Week 6
Weeks after onset of symptoms