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Resurgence of Positive qRT-PCR Test Results in Patients Recovered from COVID-19: Case Reports

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ABSTRACT

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a newly emerging coronavirus. This virus poses a great threat to human society and has been marked as the third introduction of a highly pathogenic coronavirus into the human population. This is following severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) in the 21st-century. While China has achieved initial success in controlling the spread of COVID-19 and treating those infected with SARS-CoV-2, up to 14% of COVID-19 convalescents can still be detected with virus nucleic acid. Thus, there is an urgent need for more information to understand this new virus. Here we report the detailed clinical characteristics of three cases of COVID-19 convalescents that had repeated positive quantitative reverse transcription-polymerase chain reaction (qRT-PCR) test results for over three months. This may arouse concerns regarding the present quarantine protocol after convalescence and provide a reference for governments to consider when to reopen the community.

Key Indexing Terms: COVID-19; SARS-CoV-2; Computed tomography; Isolation.

INTRODUCTION

The emergence of the novel 2019 coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first reported in Wuhan, China. In just three months, it swept the globe, with more than three million confirmed cases and over 210,000 deaths reported.¹ While it is pleasing that over one million patients with COVID-19 have recovered, there is evidence indicating that some recovered SARS-CoV-2 patients who have been discharged subsequently return positive tests and some patients with resolved infection will exhibit prolonged viral detection.²-⁴ This raises concerns regarding the present standard for discharge and quarantine time for these convalescents.⁵-⁶ We found that three patients had repeated positive results in viral nucleic acid tests over more than three months, where the possibility of reinfection was ruled out in the diagnosis and treatment of patients with COVID-19 pneumonia. We report these cases below. This study was approved by the Institutional Review Board of the Wenzhou Central Hospital, and the requirement of informed consent was waived.

CASE 1

A 55-year-old man, who had a partial pancreatectomy four years prior due to pancreatic cancer, now has complications of cirrhosis and ascites. He presented with a fever for 13 days (peak body temperature: 38.5 °C) and was admitted to the Fever Clinic of the Sixth People’s Hospital of Wenzhou City. This patient was doing business in Wuhan city. He came back to his hometown of Wenzhou city two days before. Upon admission, a physical examination showed a fever with a body temperature of 38.7 °C. His-clinical symptoms included dry throat, paroxysmal cough, and purulent sputum; no rhinitis was observed. Influenza A and B testing were negative. Chest high-resolution computed tomography (HRCT) showed scattered infections with patchy shadowing in the middle and lower lobes of both lungs. A sputum specimen tested by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) for SARS-CoV-2 RNA showed positive. COVID-19 pneumonia was, thus, confirmed. The date of the first positive SARS-CoV-2 test result was defined as d1(24/01/2020), and the qRT-PCR test was repeated at 1- to 14-day intervals. Sputum specimen tests were negative on d40 and d41. The man met the criteria for hospital discharge.
and was discharged on d43 and medically observed in isolation. Two weeks later (d57), the man had an occasional cough with a small amount of white phlegm and no fever. Nonetheless, the test for viral nucleic acid was positive again. He was re-hospitalized, and the qRT-PCR tests repeated at 3- to 5-day intervals. On d97 (29/04/2020), the test for SARS-CoV-2 RNA was still positive, and ELISA assays for anti-SARS-CoV-2 antibodies IgG and IgM were positive and negative, respectively Fig. 1.

**CASE 2**

A 57-year-old female was living with hypertension and coronary heart disease for more than two years. She developed a fever (38.6 °C) accompanied by chills during a medical observation period one day prior. Her husband had been confirmed as having COVID-19 pneumonia five days prior. The chest HRCT demonstrated multiple ground-glass opacities in the right upper lobe subpleural area, and the sputum viral nucleic acid was positive on 01/02/2020 (defined as d1). Following admission, the viral nucleic acid tests showed negative on d26 and d27, and she was discharged on d28. Upon the outpatient follow-up on d34, she was asymptomatic and her nasopharyngeal (NP) swab specimens for viral nucleic acid detection were positive, and she was re-admitted for treatment. The next test on d37 was positive. However, the d39 and d40 tests were negative. Nebulized sputum specimens from the lower respiratory tract were tested by qRT-PCR on d41 and were negative. She was diagnosed as cured and discharged for medical observation. However, only ten days later, on d51, the NP specimen test for SARS-CoV-2 RNA was positive again. During the ensuing hospitalization period, she was very nervous and had severe insomnia. She developed an upper respiratory tract infection on d82. On d91 (01/05/2020), the viral nucleic acid test was still positive, and the viral antibodies IgG and IgM were positive and negative, respectively Fig. 2.

**CASE 3**

A 40-year-old female employed as a clothing salesperson in a mall contracted COVID-19 during communication with a customer from Wuhan city one week prior. This lady was later confirmed with the disease. Four days prior, she experienced fatigue and mild chest pain, but no fever. The coronavirus nucleic test result was positive on 25/01/2020 (defined as d1). She was confirmed as having COVID-19 and, thus, admitted to the Sixth People’s Hospital of Wenzhou City. Upon admission, the chest HRCT showed bilateral diffused ground-glass opacities, mainly focused in the right lower lobe subpleural area. Her first negative qRT-PCR result was on d27. However, she subsequently tested positive on d28, d30, and d32. Following this, two consecutive negative results were obtained on d34 and d35, and she was discharged.

**FIGURE 1.** Chest CT findings of Case 1: Dynamic changes of typical chest HRCT layers over time (in the same part of lung). A (d4, 27/01/2020): The chest CT shows consolidation in the right lower lobe subpleural area, multiple ground-glass opacities and fibrous stripes in the bilateral lower lobes; B (d33, 25/02/2020): Follow-up HRCT shows the infiltrates in bilateral lower lobe which have significantly resolved; C (d77, 09/04/2020): Infiltrates almost entirely cleared. Abbreviations: CT, computed tomography; HRCT, high-resolution computed tomography.

**FIGURE 2.** Chest CT findings of Case 2: A (d4, 04/02/2020) shows: Multiple ground-glass opacities in the right upper lobe subpleural area; B (d28, 28/02/2020) shows: The ground-glass opacities in the right upper lobe which have significantly resolved; C (d77, 17/04/2020) shows: Both lungs show no obvious abnormalities. Abbreviation: CT, computed tomography.
on d36 per the discharge criteria. The follow-up test for viral nucleic acid was positive on d43, and she was rehospitalized. On d44 and d46, the tests were positive. However, three consecutive negative results were obtained on d48, d49, and d50. She was discharged again. Only eight days later, on d58, the NP specimen tested by qRT-PCR was positive again. During her third hospitalization period, the qRT-PCR tests were repeated at 3- to 5-day intervals and were all positive. She continued to be asymptomatic by clinical examination, and chest HRCT findings yielded normal results. On d97 (30/4/2020), the qRT-PCR test for SARS-CoV-2 RNA was still positive, and ELISA assays for anti-SARS-CoV-2 antibodies IgG and IgM were positive and negative, respectively Fig. 3.

**TREATMENT METHODS AND DISCHARGE CRITERIA FOR COVID-19**

The treatment and medications followed the Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia issued by the Chinese National Health Commission (trial version 7). All three cases belonged to the moderate severity type. Antiviral treatment (interferon-alpha inhalation every 12 h, 500 mg of lopinavir/ritonavir taken orally every 12 h and 200 mg of Arbidol taken orally every 12 h), combined with traditional Chinese medicines (Lianhua Qingwen capsules and Shufeng Jiedu capsules) were provided for the three patients. Antibiotics were used for preventing secondary infection. For case one, polynye phosphatidylcholine capsules, human serum albumin, and thymalfasin injection were used as supporting therapy. Further, chemotherapy with gimeracil-oteracil potassium capsules orally every 12 h, and esomeprazole magnesium enteric-coated tablets were provided to protect the stomach.

The discharge criteria were as follows: normal temperature for more than three days; significant improvement in respiratory symptoms; significant improvement in acute exudative lesions by pulmonary imaging; and two consecutive respiratory tract samples testing negative in viral nucleic acid tests with a sampling time interval of at least 24 h. Each time the patients were discharged from hospital, they were asked to continue the quarantine protocol at home for two weeks. All discharged patients were scheduled for an outpatient review on around d7, d14, and d28 after discharge. This included viral nucleic acid tests, chest CT scanning, and routine blood tests. These three patients did not report being in contact with any person with respiratory symptoms. No family member was infected.

**DYNAMIC CHANGES OF SARS-COV-2 VIRAL LOAD DETECTION IN RESPIRATORY SPECIMENS**

Specimens for qRT-PCR were collected based on each patient’s symptoms: if the patient had a cough with sputum, we obtained the sputum; if not, we obtained NP swab specimens. Patient of case two was asymptomatic during her second hospitalization and was tested negatively on d39 and d40. Patient of case three was tested negatively on d48 and d49; thus, we performed a third test using nebulized sputum specimens from the lower respiratory tract of the two patients to rule out the possibility of false negative results. Figure 4 shows the viral loads (threshold cycle (Ct) values) detected by qRT-PCR of the three patients over time: Ct value $>38$ refers to negative; Ct value $\leq 35$ refers to positive; $35 < Ct \leq 38$ refers to weakly positive ($\pm$), which should be retested. Viral load in these three patients showed a slight downward trend.

Laboratory findings (Table 1): Data were obtained within three days after admission or before discharge or the latest laboratory date. Abnormal findings in case one on admission were decreased blood leukocytes count $\leq (2.2 \times 10^9/L)$ and lymphocytes count $\geq (0.6 \times 10^9/L)$, increased serum alanine aminotransferase (81 U/L) and aspartate aminotransferase (128 U/L). In case three only the lymphocytes count decreased $\leq (0.7 \times 10^9/L)$. Case two developed an upper respiratory tract infection on d82. The serum test for CRP levels slightly increased on d85, but it dropped quickly after antibiotic prophylaxis.

**DISCUSSION**

Consistent with the study of He et al., viral loads of the three patients with COVID-19 gradually decreased towards the detection limit at around d30. The clinicians determined that they met the criteria for hospital discharge according to the national guidelines. However, they all had positive qRT-PCR test results 6 to 14 days.
later and were re-hospitalized. Two patients had negative virus tests 5 days after the second admission. After three consecutive negative qRT-PCR test results, they were discharged. However, both tested positive for viral nucleic acids 8 to 10 days after discharge and thus, were re-hospitalized for a third time. As of the submission of this article, the three patients are still in the hospital. Notably, stool specimens from the three patients for qRT-PCR were only positive within the first 34 days of hospitalization period and remained undetectable ever since.8

Case three had negative qRT-PCR test results on d27. However, the following three repeated tests were positive. Therefore, we speculate that the d27 result was a false negative.9-10 False-negative testing ranges of 17%–63% for NP RT-PCR for SARS-CoV-2 have been reported in many studies,9-12 which is a clinically relevant problem not limited to a single platform with current testing strategies.1 In the case of three consecutive negative viral nucleic acid tests, we believe that the chance of false-negative qRT-PCR test results will be excluded.

We know from previous observations13 and the reports of other scholars6,14 that it takes about 6 to 24 days for the viral nucleic acid test to be negative. The duration of RNA detection may relate to host cell immunity,14 host status, and virologic factors.15 These three patients had positive qRT-PCR results for over three months, despite the short interval of negative qRT-PCR results during the disease. These cases indicate a greater likelihood of the virus remaining in certain cells to be reactivated, rather than the reoccurrence of infection.15 This is because the patients retested positive a relatively short time after being released from quarantine. During the short interval of discharge time, they had no contact with anyone displaying respiratory symptoms. The ELISA assays for anti-SARS-CoV-2 antibodies IgG and IgM were positive and negative, respectively. This further confirms the absence of a new infection.16

The three patients were relatively young and healthy, showed no obvious disease progression or infectivity when readmitted to the hospital.4,15 The three cases

| Case1 | 24/01/2020 | d1 | 2.2 | 0.6 | 120 | 66 | 81 | 128 | 54 | 310 | 9.0 | + | + | N |
| Case2 | 01/02/2020 | d1 | 5.3 | 1.0 | 126 | 196 | 50 | 47 | 60 | 206 | 1.1 | + | N | N |
| Case3 | 25/01/2020 | d1 | 3.7 | 0.7 | 126 | 188 | 11 | 21 | 53 | 121 | 2.2 | + | + | N |

We know from previous observations and the reports of other scholars that it takes about 6 to 24 days for the viral nucleic acid test to be negative. The duration of RNA detection may relate to host cell immunity, host status, and virologic factors. These three patients had positive qRT-PCR results for over three months, despite the short interval of negative qRT-PCR results during the disease. These cases indicate a greater likelihood of the virus remaining in certain cells to be reactivated, rather than the reoccurrence of infection. This is because the patients retested positive a relatively short time after being released from quarantine. During the short interval of discharge time, they had no contact with anyone displaying respiratory symptoms. The ELISA assays for anti-SARS-CoV-2 antibodies IgG and IgM were positive and negative, respectively. This further confirms the absence of a new infection. The three patients were relatively young and healthy, showed no obvious disease progression or infectivity when readmitted to the hospital.

![FIGURE 4. Dynamic changes of SARS-CoV-2 viral load detection in respiratory specimens.](image-url)
reported had few respiratory symptoms and no fever. Further, the CT indicated that lung inflammation had significantly subsided or disappeared. For patients of case two and case three, a second peak (around 50–60 days after disease onset) in viral load was detected; the viral loads then slightly decreased but were still relatively high over time. Our findings suggest that, consistent with other studies,2–4 the present symptom-based strategy which allows for discontinuation of isolation 10 days after symptom onset should not be used to all patients,5 because at least a small proportion of recovered patients may still be asymptomatic virus carriers and the virus may rebound after shedding.7 Case two became very anxious and developed severe insomnia, which may have been due to the long-term isolation from relatives and uncertainty relating to the course of the disease.17 The patient was given psychological counselling and Estazolam tablets at night to improve her sleep. It remains unclear how long it will take for the qRT-PCR test results of these three patients to become negative. At present, the combined Chinese and Western medication regimes proposed in the protocol appeared ineffective at achieving persistent viral clearance.

Our case series is limited by the small number of patients. Although our patients exhibited prolonged periods of viral detection, there is no evidence that they were infectious during their follow-up periods.

In conclusion, we confirmed that a prolonged viral detection exists in COVID-19 convalescents, despite an improvement in their clinical symptoms. This prolonged viral shedding of SARS-CoV-2 should be further studied to inform public health guidelines. In China, it is recommended that patients are followed up at 2 weeks and 4 weeks post-discharge.5 It may be wise to shorten the first follow-up period and extend the outpatient follow-up time to monitor for potential healthy carriers.

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