

Cases Report of Cured Novel Coronavirus-infected Pneumonia Patients with Viral Nucleic Acid Test Positive in Fecal Specimens

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Abstract

Background: The outbreak of the novel coronavirus in China (COVID-19) represents a significant and urgent threat to global health. We report here five cases of COVID-19 infection patients in our clinical practices who are medically stable and presumed to successfully “cleared” the virus after antiviral treatments.

Case presentation: The clinical evaluation depends on the viral nucleic acid test in respiratory specimens by real-time PCR reverse transcription (RT-PCR) assays according to the authorized guidance. We found that the stool samples of these cured patients remain positive in RT-PCR assay while the virus is undetectable in respiratory specimens. RT-PCR molecular diagnostic assay was designed to specifically detect the presence of viral RNA. Thus, the positive result in the fecal specimens implies the existence of viable virions with the patients.

Conclusions: This highlights the importance to look closely at the assessment standard of medical treatment, as well as the need for reevaluation of the criteria for the initial screening, prevention, and care of patients with this emerging infection.

Background

The Coronavirus disease (COVID-19) outbreak, which originated from the city of Wuhan, Hubei Province, China since December 2019, has swept into at least 160 countries in the world. As of March 19, 2020, the number of confirmed cases worldwide has exceeded 200,000. It took over three months to reach the first 100,000 confirmed cases, and only 12 days to reach the next 100,000 [1]. The disease had killed more than 8000 people globally, and the World Health Organization (WHO) officially declared the outbreak as a pandemic on March 11, 2020 [2]. Chongqing municipality, a neighbor of Hubei Province, has a confirmed case number of 576 on March 19, 2020.

Up to now, there is still no vaccine or specific antiviral treatment available for this emerging infectious disease, and the effective controls are still mainly dependent on early diagnosis, quarantine of the patients and monitoring of the close contacts [3]. Thus, reliable and accurate diagnosis methods play a critical role in disease control and prevention world widely. The detections of the virus based on

real-time PCR with reverse transcription (RT-PCR) assays are currently widely used for laboratory diagnosis of virus-borne diseases. As a molecular nucleic acid amplification test (NAAT), RT-PCR has been approved by the FDA for diagnostic use, such as severe acute respiratory syndrome-related coronavirus (SARS-CoV), with higher sensitivities and specificities compared to other biochemical and immunological based assays.

According to the COVID-19 technical guidance authorized by the Chinese National Health Committee, both open reading frame *1ab* (ORF1AB) and nucleocapsid protein (N) encoding genes were selected as targets in the RT-PCR assay [4] [5]. A positive result was reported if either of these genes was detected with a Ct value of less than 37.0. Accordingly, the negative results from nasal/pharyngeal swabs tested for COVID-19 is an important criterion for the assessment of medical care and the discharge of hospitalized patients.

However, in our clinical practices, we found multiple cases of patients with controversial viral nucleic acid test results in respiratory and fecal specimens (Table 1) from multiple hospitals in the city of Chongqing. All these patients are COVID-19 confirmed and are medically stable after hospitalization for various periods of treatment. They are clinically virion-cleared according to the diagnostic guidelines, as two consecutive viral nucleic acid tests are negative with respiratory specimens.

However, fecal specimens of the patients remain positive, clearly demonstrating the presence of viral RNA, which is an indicator of the viable virus.

Case Presentation

Case 1 of our study is a 48-year old male patient who was infected via his wife, a pneumonia patient diagnosed as “novel coronavirus” infection earlier. The patient has a positive viral nucleic acid test in respiratory (nasal/pharyngeal swabs) specimens and was admitted to the hospital on January 22, 2020. The viral nucleic acid test of the respiratory secretions (nasal/pharyngeal swabs) is negative after 12 days of medical treatment, while the stool sample that collected simultaneously remains positive. We observed this phenomenon in the following three tests for both the fecal and respiratory specimens (Table 1).

Case 2 is a 9-year old boy who was diagnosed with COVID-19 and admitted to the hospital on January

22, 2020. He demonstrated mild pneumonia symptoms with a positive test for the presence of “novel coronavirus” nucleic acid in respiratory secretions. The viral nucleic acid tests with respiratory secretions were negative in three consecutive RT-PCR assays with 24 hours intervals after been hospitalized for medical treatment of 10 days. The positive RT-PCR tests, on the contrary, were reported for his stool specimens, which were collected at the same time. The viral nucleic acids in the patient’s stool samples were still detectable on day 14 of hospitalization, although at a lower viral titer (higher Ct values) (Table 1).

Case 3 is a 48-year old male patient, who was diagnosed to be COVID-19 infected with mild pneumonia syndromes. On admission, the patient has a positive viral nucleic acid test in respiratory samples. On days 10 and 11 after medical treatment, patient samples were collected for the viral nucleic acid test. The results are positive in fecal samples but negative in the respiratory specimens (Table 1).

Case 4 is a 67-year female patient who was admitted to the hospital with a positive test of the viral nucleic acid in the patient’s respiratory secretions on January 30, 2020. After 7 days of treatment, viral nucleic acids were still detectable in the fecal specimens, although marginally, while the respiratory samples collected at the same time turn negative. (Table 1).

Case 5 is a 47-year female patient, who was confirmed to be infected and admitted to the hospital on February 3, 2020. The presence of viral nucleic acids persists for two weeks with both respiratory swabs and fecal samples. A weak positive result with her fecal sample was reported after 16 days of medical treatments.

Discussion And Conclusions

In all the above cases, clinical specimens for COVID-19 diagnostic testing were obtained per the guidelines of the CDC [4, 6]. A description of the assay and sequence information for the RT-PCR primers and probes are available on the CDC Laboratory Information website for COVID-19. The procedures were conducted by experienced clinical providers, and it is unlikely that the negative results were originated from improper or poor clinical specimen collection or poor handling of a specimen after collection and before testing. Laboratory confirmation of COVID-19 was completed

and verified in two independent institutions. All patients received antiviral treatment of lopinavir and ritonavir.

In the above cases, all patients are medically stable after more than 10 days of hospitalization for medical antiviral treatment. The patients recovered well as the vital signs back to normal ranges, categorized as cured patients according to the current clinic guidelines. However, the viral nucleic acid test is still positive in the fecal specimens, despite repeated negative results observed in the specimens of respiratory secretions. We noticed this discrepancy in multiple cases with diverse clinical backgrounds, independent of genders, age ranges, and clinical backgrounds. To be pointed out, no patient in our study demonstrated gastrointestinal symptoms. It is worth to be noted that for the patient of case 2, three consecutive negative results in nasopharyngeal swabs were observed with 24-hour intervals, which satisfied the discharge standards stipulated by the Novel Coronavirus Pneumonia Prevention and Control guideline issued by the Chinese National Health Committee [4]. However, the strong positive result (lower Ct values) of viral RNA in the fecal specimens suggested the existence of viable virions with the patients, implying highly infectious and transmissible capabilities.

During the preparation of our manuscripts, two independent clinic reports demonstrated the detection of viral RNA by RT-PCR with rectal swabs after nasopharyngeal testing turn negative [7, 8]. It was then proposed that the gastrointestinal tract may shed virus and fecal-oral transmission may be possible, although *in vivo* infection evidence was not provided to show that the virus in the fecal samples is transmissible. In our study, the prolonged duration of viral RNA in the stool specimens of the patients can last for more than 20 days after hospitalization for medical treatments, and more than 8 days after free of the virus in the respiratory specimens (Table 1, case 1). Generally, it is unexpected that the viral RNA could exist for such a long time without the protection of active virions in the environment.

Our findings recommended that clinicians should pay more attention to the negative result of the viral nucleic acid tests when evaluating the treatment effect and discharge of the patients. Parallel tests should be conducted with different types of specimens to make the evaluation or assessments

accurate, such as saliva, sputum, alveolar lavage fluid, feces, etc. Also, we proposed that viral nucleic acid tests of fecal specimens should be included in the screening of suspected patients and more precaution procedures were necessary for the medical care providers.

Molecular assays such as RT-PCR can detect viral RNA for a longer duration than other biochemical tests (e.g., antigen detection diagnostic tests). However, our clinical findings reinforced the concern that although molecular assays have high sensitivities, negative molecular assay results may not always exclude a diagnosis of infection, such as the negative respiratory specimens we reported here. The reasons underlying this issue is complicated, but it is plausible to propose that new criteria, in this scenario, need to be integrated for a comprehensive evaluation of the discharge of the patient and the cure of the disease. Moreover, strict hygiene or sanitation precautions are required during the hospitalization or quarantine, considering the extra-pulmonary viral shedding in COVID-19 patients. There are many unknown features for the novel coronavirus outbreak in 2019. Investigations are underway to better understand transmission dynamics, epidemiological and clinical characteristics of the illness [9]. We will continue to track viral nucleic acid tests of fecal and other specimens in more patients, as well as in the hospital environment, and try to reveal the associations between the presence of virions with clinical features of this emerging disease. The cell culture on positive stool PCR samples is also proposed to be conducted to prove that the virus is viable and transmissible.

Declarations

Ethics approval and consent to participate

The proposal was approved by the Ethics Committees for clinical related research of the participant affiliations, including the Public Health Medical Centre of Chongqing Municipality, Yongchuan district Centre for Disease Control and Prevention, and Yongchuan Hospital of Chongqing Medical University. Informed consent was signed from each of the participants or guardians prior to the sample collection. Each participant knows that participation in this study was voluntary and was free to decline his participation or withdraw himself from the study at any time. We protected the privacy of the participants, and no identifying information was collected during the study.

Consent for publication

Each participant or guardians signed written consent for their personal or clinical details along with

any identifying images/videos/texts to be reported in a medical publication or websites in this study.

Patients knew that they have the right to refuse to sign this consent form and refusal to sign this form will not affect their care in any way.

Availability of data and materials

All data generated or analyzed during this study are included in this published article. Patients data reported here have not been reported in any other submissions/publications by us or anyone else.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Design of the work was done by MH, JZ and JJZ; acquisition data and analysis was done by JZ, WT, XW, YZ, JQ, XL, JZ, TS, YO, and ZZ; interpretation of data was done by MH and YC; conception was established by PP; MH and JJZ have drafted the work and substantively revised it. All authors read and approved the final manuscript.

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References

1. *Coronavirus disease (COVID-2019) situation reports*

[<https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/>]

2. *Statement on the second meeting of the International Health Regulations (2005) Emergency*

Committee regarding the outbreak of novel coronavirus (2019-nCoV) [[https://www.who.int/news-](https://www.who.int/news-room/detail/30-01-2020-statement-on-the-second-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-(2019-ncov))

[room/detail/30-01-2020-statement-on-the-second-meeting-of-the-international-health-regulations-\(2005\)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-\(2019-ncov\)](https://www.who.int/news-room/detail/30-01-2020-statement-on-the-second-meeting-of-the-international-health-regulations-(2005)-emergency-committee-regarding-the-outbreak-of-novel-coronavirus-(2019-ncov))]

3. *Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under*

Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19) [<https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html>]

4. *the Novel Coronavirus Pneumonia Prevention and Control guideline (Fifth Edition)*

[<http://www.nhc.gov.cn/zycqj/s7653p/202002/3b09b894ac9b4204a79db5b8912d4440.shtml>]

5. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N et al: *Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. The Lancet.* 2020. doi: 10.1016/S0140-6736(20)30251-8

6. Holshue ML, DeBolt C, Lindquist S, Lofy KH, Wiesman J, Bruce H, Spitters C, Ericson K, Wilkerson S, Tural A et al: *First Case of 2019 Novel Coronavirus in the United States. N Engl J Med* 2020.

7. Xiao F, Tang M, Zheng X, Liu Y, Li X, Shan H: *Evidence for gastrointestinal infection of SARS-CoV-2. Gastroenterology* 2020.

8. Xu Y, Li X, Zhu B, Liang H, Fang C, Gong Y, Guo Q, Sun X, Zhao D, Shen J et al: *Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding. Nature Medicine* 2020.

9. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X et al: *Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet* 2020. doi: 10.1016/S0140-6736(20)30183-5

Table

Table 1 Cases information and clinic features of the patients

Case No.	Gender	Age	Date of illness	Admission data	Hospital days when sample collected	**Sample type	RT-PCR results		Clinical Diarrhea
							(Ct value a; b)*		
1	M	48	1/21/20	1/22/20	12	R	0; 0		
					13	F	33.0434.46	No	
					13	R	0; 0		
					13	F	31.5937.14		
					15	R	0; 0		
					15	F	34.4133.62		
					20	R	0; 0		
					20	F	34.5332.76		
2	M	9	1/24/20	1/24/20	10	R	0; 0		
					11	R	0; 0	No	
					12	R	0; 0		
					12	F	32.32; 31.7		
					14	R	0; 0		
					14	F	33.56; 32.02		
3	M	48	1/18/20	1/30/20	7	R	36.4635.05	No	
					7	F	39.1336.88		
					10	R	0; 0		
					10	F	28.07; 27.07		
					11	R	0; 0		
					11	F	36.90; 0		
4	F	67	2/2/20	2/4/20	2	R	30.8128.95		
					2	F	37.2436.58	No	
					7	R	0; 0		
					7	F	39.5038.76		
5	F	47	1/24/20	2/3/20	10	R	30.43;29.96		
					10	F	35.07;34.41		
					11	R	0; 0		
					12	R	39.35;0		
					12	F	33.94;34.70	No	
					14	R	0;38.82		
					14	F	0;41.59		
					16	R	0; 0		
16	F	0;40.45							

* a, b represents the value from *ORF1ab* and *N* genes respectively.

** Sample type: F: Fecal; R: Respiratory.