

Difficulties in False Negative Diagnosis of Coronavirus Disease 2019: A Case Report

Qinjian Hao

1 The Center of Gerontology and Geriatrics, West China Hospital, Sichuan University, Chengdu, Sichuan, China. 2 National Clinical Research Center for Geriatrics, Chengdu, Sichuan, China.

Hongmei Wu (✉ drwhm@163.com)

1 The Center of Gerontology and Geriatrics, West China Hospital, Sichuan University, Chengdu, Sichuan, China. 2 National Clinical Research Center for Geriatrics, Chengdu, Sichuan, China.

Qiang Wang

3 Mental Health Center and Psychiatric Laboratory, West China Hospital of Sichuan University, Chengdu, Sichuan, China.

Case Report

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Abstract

Background: In December 2019, a novel coronavirus emerging in china and spread rapidly globally. Early identification and effective quarantine are essential to reduce the spread of the disease. However, the presence of false-negative makes the diagnosis difficult, especially in the early stages of the disease.

Case presentation: A 34-year-old man who had an epidemiological link to Wuhan, presenting with intermittent fever and cough, with chest computed tomography showing ground-glass opacity, and repeated detection of negative 2019 novel coronavirus(2019-nCoV) nucleic acid by real-time reverse transcription-polymerase chain reaction assay, which was eventually diagnosed as coronavirus disease 2019(COVID-19).

Conclusions: This case highlights that a single negative result of the test, particularly if it is based on an upper respiratory tract specimen, in highly suspected cases, does not exclude COVID-19. Repeat and multiple-site sampling and testing in combination with dynamic imaging changes in the chest are strongly recommended in progressive disease.

Background

At the end of 2019, a cluster of pneumonia cases of unknown etiology characterized by fever, cough, shortness of breath, and inflammatory lung infiltration have been reported in Wuhan, Hubei province, China [1]. In the early stages, most of the patients were connected to the Huanan Seafood Wholesale Market in Wuhan, China, and then the disease spread rapidly, resulting in an epidemic throughout China and other countries. A novel coronavirus was quickly identified as a potential etiology [2]. Subsequently, an interim name 2019 novel coronavirus (2019-nCoV) was coined, and then officially named the disease as Coronavirus Disease 2019(COVID-19) by World Health Organization. Epidemiological investigations revealed that person-to-person transmission of COVID-19 has occurred [3, 4], and the number of people infected has surged. As of February 21, 2020, a total of 76,769 cases were confirmed in more than 20 countries around the world [5], and the cases are increasing over time. Therefore, early correct identification and confirmation of infected cases are definitely important for making prompt and effective quarantine and providing management to reduce the spread of the disease.

However, there are some problems in obtaining the correct diagnosis in the early stage of COVID-19. Limited by the lack of diagnostic reagents and the existence of false-negative diagnosis, some patients may not be correctly diagnosed in time. Here, we report a case whose throat swab specimen of 2019-nCoV nucleic acid assay tested negative repeatedly by real-time reverse transcription-polymerase chain reaction (rRT-PCR) at the initial stage, but it was eventually diagnosed as COVID-19. Furthermore, the potential causes of the false-negative test result of 2019-nCoV nucleic acid and the corresponding strategies have been explored and discussed based on the reviews of the currently available research studies. It is expected to be very helpful for clinicians in making diagnostic decisions.

Case Presentation

On the night of January 21, 2020, a 34-year-old man presented to the fever clinic of West China Hospital Sichuan University in Chengdu, Sichuan, China, with intermittent fever accompanied by chills for 1 day, and the maximum temperature reached 38.5°C without cough, shortness of breath, and muscle ache. He disclosed that he came from Wuhan to Chengdu by train to visit his relatives and had got fever before he left Wuhan. He had no history of contact with a confirmed or probable case of COVID-19 and a direct contact with or consumption of wild animals. The patient was a non-smoker without a known history of medical problems. Physical examination revealed that the body temperature was 38.2°C, blood pressure was 135/99 mmHg, heart rate was 110 beats per minute, respiratory rate was 20 breaths per minute, and blood oxygen saturation was 98% when breathing ambient air, and no positive findings were found on lungs and other physical examinations. Blood routine and rapid tests for influenza A and B antigens were negative. Furthermore, 13 pathogens of the respiratory tract were negative on the nucleic acid amplification test, including influenza A, H1N1 (2009), H3N2, influenza B, adenovirus, rhinovirus, bocavirus, parainfluenza virus, metapneumovirus, coronavirus, respiratory syncytial virus, chlamydia, and mycoplasma pneumonia nucleic acid (the report was available within 48 hours). However, emergency chest computed tomography (CT) showed mild ground-glass opacity in the apical segment of the right upper lobe (Figure 1 A1 and A2). Being a highly suspected case of COVID-19, this man was transferred to the Infectious Disease Department for quarantine on January 22, 2020. At the same time, the patient was asked to wear a surgical mask during the entire visit, examination, and waiting period. On January 23, 2020, his first oropharyngeal swab sample was negative for 2019-nCoV nucleic acid by the rRT-PCR assay. The second oropharyngeal swab sample for the 2019-nCoV nucleic acid test was reexamined to avoid a misdiagnosis in the patient. Unfortunately, the specimen tested was positive. The result was immediately reported to the Center for Disease Control and Prevention of Chengdu, and the patient was promptly transferred to a government-designated hospital in the early morning of January 25, 2020. However, two consecutive samples tested for rRT-PCR (at least at one-day interval) were negative during hospitalization. Based on these results, the patient was discharged with some cefixime capsules and antipyretics on January 28, 2020.

Due to the symptoms of recurrent fever, dry cough, and fatigue, the patient came to our hospital again on the day of discharge. A second chest CT scan was performed, and a combined nasopharyngeal and oropharyngeal swab was collected again for rRT-PCR. Chest CT images revealed

that the lesion had enlarged compared with that on January 21, 2020 (Figure 1B1 and B2). The patient was admitted to the hospital again for isolated treatment as a suspected case. The laboratory tests showed that the patient's white blood cell (WBC), hemoglobin, albumin, cholesterol, CD3, and CD8 levels were decreased and procalcitonin (PCT), C-reactive protein (CRP), and interleukin-6 (IL-6) levels were increased (Table 1). The patient was treated with moxifloxacin tablets (400 mg daily, orally) for pneumonia after admission. On day 2 of hospitalization (January 29, 2020), the specimens tested by rRT-PCR were positive for 2019-nCoV, and antiviral therapy (lopinavir and ritonavir tablets, 500 mg twice daily, orally) was prescribed for the patient. The second throat swab specimen was still positive on the next day (January 30, 2020).

Table 1: Dynamic changes of auxiliary examination results

Examination	22-Jan	29-Jan	4-Feb	8-Feb	Reference range
White-cell count($10^9/L$)	6.01	2.87↓	9.11	5.74	3.5-9.5
Absolute neutrophil count($10^9/L$)	3.37	1.02↓	7.55↑	3.02	1.8-6.3
Absolute lymphocyte count($10^9/L$)	1.62	1.32	1↓	1.98	1.1-3.2
Absolute monocyte count($10^9/L$)	1.02↑	0.5	0.55	0.69↑	0.1-0.6
Absolute eosinophil count($10^9/L$)	*	0↓	0↓	0.05↓	0.4-8
Absolute basophil count($10^9/L$)	*	0	0.1	0	0-1
Red-cell count($10^{12}/L$)	4.73	4.01↓	4.02↓	4.15↓	4.3-5.8
Hemoglobin(g/L)	148	125↓	124↓	127↓	130-175
Blood platelet count($10^9/L$)	170	124	274	229	100-300
Procalcitonin(ng/ml)	*	0.08↑	0.04	0.05	<0.046
C-reactive protein(mg/L)	*	9.34↑	2.98	1.72	<5
Interleukin-6(pg/ml)	*	13.6↑	<1.5	2	<7
Total protein(g/L)	*	61.5↓	60.1↓	56.8↓	65.0-85.0
Albumin(g/L)	*	38.5↓	37.7↓	33↓	40.0-55.0
Globulin(g/L)	*	23	22.4	23.8	20.0-40.0
Triglyceride(mmol/L)	*	0.78	1.41	3.48↑	0.29-1.83
Cholesterol(mmol/L)	*	2.35↓	3.27	3.1	2.8-5.7
Calcium(mmol/L)	*	2.08↓	2.11	1.98↓	2.11-2.52
Phosphorus(mmol/L)	*	0.79↓	0.85	0.91	0.85-1.51
Glucose(mmol/L)	*	5.23	7.45↑	5.37	3.90-5.90
Prothrombin time(Sec)	*	*	11.3↑	11.5↑	9.6-11.2
D-dimer(mg/L FEU)	*	*	2.96↑	*	<0.55
Fibrinogen(mg/L)	*	*	6.9↑	*	<5
Lactic acid(mmol/L)	*	*	2.2↑	1.9	0.7-2.1
Ferritin(ng/ml)	*	*	519↑	448↑	24-336
CD3 count(cell/ul)	*	761↓	*	1187	941-2226
CD4 count(cell/ul)	*	484	*	776	471-1220
CD8 count(cell/ul)	*	243↓	*	353	303-1003

“*”:no result; “↓”: Below the reference range; “↑”: Above the reference range.

The patient's temperature became normal on the fourth day of hospitalization, and oral antibiotics were discontinued. However, on the fifth day of hospitalization, the third chest CT images showed that the inflammatory infiltration had further expanded compared with that on January 28 (Figure 1C1 and C2), and then methylprednisolone (40 mg daily, IV drip) was used for anti-inflammation for 3 days, and atomized Recombinant Human Interferon α -2b (500 IU twice daily, nebulization) was used as an adjuvant therapy. On the eighth day of hospitalization, the fourth chest CT showed that the inflammatory infiltration had begun to reduce (Figure 1D1 and D2). In the meantime, the patient's clinical symptoms, such as fever and cough, had improved significantly. Three consecutive specimens for the rRT-PCR assay, including nasopharyngeal and oropharyngeal swabs and stool, were collected again at least at one-day interval. All of the specimens tested negative for 2019-nCoV. The patient was discharged after a total of 13 days of treatment (February 10, 2020). The fifth chest CT image (February 8, 2020) (Figure 1E1 and E2) showed that the inflammatory infiltration was almost completely absorbed, and the blood tests revealed that WBC, CD3, CD8, PCT, CRP, and IL-6 levels had become normal (Table 1).

Discussion And Conclusion

This case report highlights the possibility of false negatives in the diagnosis of COVID-19 with a nucleic acid detected by rRT-PCR, especially for patients at the early stage of COVID-19, which may be resulting in a potentially higher spread of the disease in the hospital and community

because of delayed quarantine of the missed case.

In our case, the patient was identified as a suspected case of COVID-19 by the diagnosis and treatment of pneumonia caused by novel coronavirus (trial version 4)[6] in his first visit, and it was based on (1) the clinical features of fever, normal WBC, and mild ground-glass opacity on chest CT; (2) the epidemiological history of residence in Wuhan, China within the past 14 days. In the confirmation stage, we did not find any evidence of other known respiratory viral or bacterial infection. Unfortunately, the false-negative result of the rRT-PCR assay made the diagnosis of COVID-19 uncertain. Although the final diagnosis was confirmed, the diagnosis was delayed. The phenomenon of false-negative results by the rRT-PCR assay have been reported in previous researches[7, 8], and it had also occurred in the diagnosis of Severe Acute Respiratory Syndrome (SARS) [9] and Middle East respiratory syndrome (MERS) [10]. The possible causes of false-negative result for the 2019-nCoV test may be due to the following factors: (1) the patient infected is at the early stage of disease with less viral load and below the threshold of detection; (2) because of less or no significant respiratory symptoms, such as cough and sputum, there is rare virus elimination in the patient's upper respiratory tract; (3) inappropriate sample collection, handling, shipping, and technical issues may also lead to abnormal results [11], which may be one of the causes of inconsistent results among different hospitals; (4) in addition, a variety of RT-PCR assays have been developed in a short period of time during the epidemic of COVID-19, and the reagents may be immature with an uncertain diagnostic accuracy.

How to improve the diagnostic accuracy of the suspected case of COVID-19 using rRT-PCR assays is the real challenge for clinicians. Based on the current evidence, the following strategies may be helpful: (1) to increase the positive rate of nucleic acid detection, repeated and multi-site sampling from the respiratory tract, blood, stool, and urine can be performed in the suspected cases [11]. However, extrapulmonary detection of viral RNA does not necessarily mean that the infection with the virus is present, and the clinical significance of the detection of viral RNA outside the respiratory tract is not known at this time [12]. (2) A lower respiratory tract specimen is strongly recommended in severe or progressive cases [11]. Previous studies of SARS [13] and MERS [14] have indicated that lower respiratory tract samples, such as bronchoalveolar lavage fluid and tracheal aspirates, contain higher viral loads than the upper respiratory tract samples. (3) With respect to the diagnostic accuracy of the rRT-PCR test, the assays should be conducted using strict quality control and quality assurance procedures to ensure the consistency of testing among different laboratories. (4) Furthermore, more effective detection methods should be explored and developed as soon as possible. It has been reported that serological testing may be useful to confirm the immunologic response to coronavirus infection, which could improve the positive rate of detection [15]. Second-generation sequencing can also be used to detect 2019-nCoV; however, testing is expensive, and high requirements of the test conditions limit its wide application. (5) Finally, imaging characteristics of the chest can also help the clinicians in confirming or refuting the disease diagnosis, which have been included in the diagnosis and treatment of pneumonia caused by novel coronavirus (trial version 5) [16] by the National Health Commission of People's Republic of China. Particularly, dynamic imaging changes in the chest are good indicators of disease prognosis.

In conclusion, our understanding of the clinical spectrum of COVID-19 is very limited at present. A single negative result of the rRT-PCR test, particularly if it is for an upper respiratory tract specimen from a suspected case, does not exclude the infection of 2019-nCoV. Furthermore, repeated and multiple-site sampling and testing of rRT-PCR in combination with dynamic imaging changes in the chest are strongly recommended for suspected patients in the progressive stage of the disease.

Abbreviations

2019-nCoV: 2019 novel coronavirus

COVID-19: coronavirus disease 2019

rRT-PCR: real-time reverse transcription-polymerase chain reaction

WBC: white blood cell

CT: computed tomography

PCT: procalcitonin

CRP: C-reactive protein

IL-6: interleukin-6

SARS: Severe Acute Respiratory Syndrome

MERS: Middle East Respiratory Syndrome

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

The patient provided written informed consent for publication of this case report and clinical details.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Author's Contributions

Hao and Wu conceptualized the study. Hao responsible for data collection and wrote the original draft under the guidance of Wu and Wang. Wu and Wang were responsible for resources, supervision, and revision. All authors read and approved the final manuscript.

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Figures

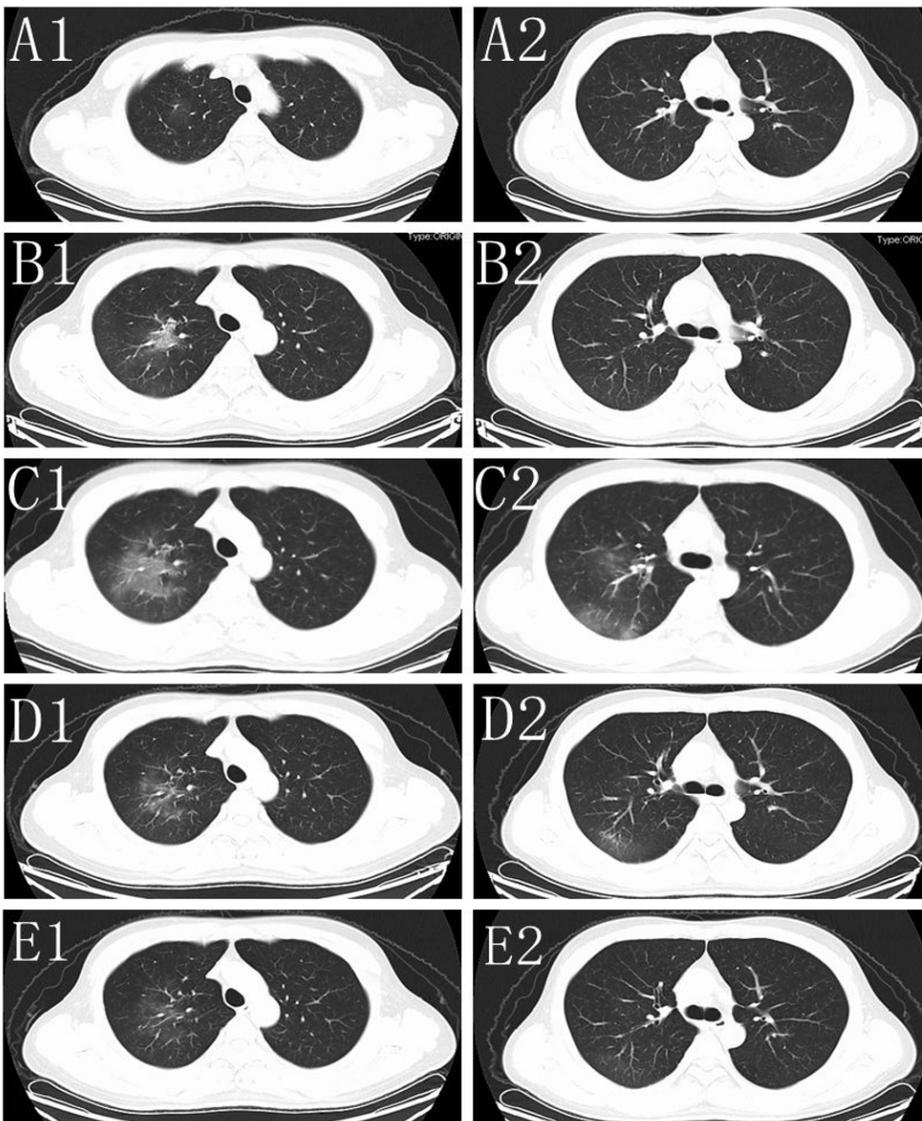


Figure 1

Figure 1: Dynamic changes in chest imaging A1&A2: Mild ground-glass opacity in the apical segment of the right upper lobe, date of January 22, 2020. B1&B2: Ground-glass opacity in the apical segment enlarged compared with A1&A2, date of January 28, 2020. C1&C2: Ground-glass opacity in the apical segment and posterior segment of the right upper lobe, enlarged compared with previous images, date of February 1, 2020.

D1&D2: Inflammatory infiltrations began to reduced, date of February 4, 2020. E1&E2: Inflammatory infiltration almost absorbed, date of February 8, 2020.