Letter to the Editor

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Next-generation sequencing and RT-PCR to identify a 32-day SARS-CoV-2 carrier

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To the Editor,

Since the outbreak of SARS-CoV-2 in Wuhan (Hubei, China) in December 2019 [1], it has spread rapidly and has become a major public health emergency worldwide. The World Health Organization announced the official name of the disease caused by this virus as coronavirus disease 2019 (COVID-19). According to the “Diagnosis and Treatment of COVID-19 (Trial Version 7)”, the disease must be confirmed by RT-PCR or pathogen gene sequencing from respiratory,

digestive or blood samples. RT-PCR is often used for clinical detection. Recently, negative RT-PCR results have been reported for nasopharyngeal swabs of patients exhibiting COVID-19 clinical symptoms [2]. Thus, it is necessary to determine the causes underlying these multiple RT-PCR negative results.

We report here the case of a middle-aged woman who worked in a hotel and returned home on January 23. She had no contact with people returning from affected areas, no travel history to Wuhan and has denied any contact with wild animals. After her colleagues were diagnosed with COVID-19, she was transferred to the local county people's hospital for isolation and observation on February 2. It was later learned that about eight colleagues were diagnosed with COVID-19. The patient had been in close contact with one confirmed patient and belonged to the third or fourth generation of transmission patients [3]. During intensive medical observation from February 2 to February 6, the patient showed no other clinical symptoms. The nucleic acid tests of pharyngeal swab samples were negative on February 7, 8, 11, and 13. However, CT scans (Figure 1) conducted on February 15 showed patchy ground-glass opacity in her lungs, and the patient was highly suspected of SARS-CoV-2 infection. Subsequently, the results of two RT-PCR tests were negative.

To further improve the relevant examination and treatment, the patient was transferred to The First People's Hospital of Ziyang City on February 21 for further isolation and treatment. The patient had no chronic history of hypertension, diabetes, coronary heart disease, etc. At the time of admission, the patient had been coughing for more than 20 days, but dyspnea, diarrhea, and vomiting were absent. Even during hospitalization, there were no clinical symptoms such as fever, diarrhea and vomiting except coughing. Since February 7, a total of 17 SARS-CoV-2 nucleic acid tests were performed. Only sputum samples tested positive, while the rest tested negative. Nucleic acid tests were still negative on February 22 and single-target positive on February 23. Until February 24, the ninth practical sputum nucleic acid test was positive. The specific RT-PCR results and patient's symptoms are shown in Table 1.

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Figure 1: The patient's chest CT images on February 15. There was ground-glass opacity (black arrows indicate) in the outer area of the lower lobe of both lungs.

Table 1: RT-PCR results and Patient's symptoms.

	February 7	February 8	February 11	February 13	February 15 ^a	February 17	February 22	February 23	February 24	February 29	March 1	March 2	March 4	March 7	March 8	March 10	March 11
RT-PCR results	-	-	-	-	-	-	-	-	+	-	+	+	+	-	+	-	-
N + Ct									41.88		38.89	42.56	40.72			37.65	
E + Ct									38.09		35.20	38.33	36.68			34.36	
R + Ct									41.89		38.95	42.44	40.62			37.92	
Symptoms	AS	S	S	S	S	S	S	S	S	S	S	S	AS	AS	AS	AS	AS

-, negative; +, positive; N, nucleocapsid gene; E, envelop gene; R, RdRp gene; Ct, cycle threshold; AS, asymptomatic; S, symptomatic. ^aPerformed a CT scan.

Figure 2: Chest radiographs. Image on February 28. 92 × 106 mm (300 × 300 DPI).

Arbidol tablets were administered on the day of admission for 10 consecutive days and chloroquine phosphate tablets were continuously used for 8 days. Imaging examination revealed lung lesion absorption (Figure 2). On March 3, arbidol tablets were discontinued and lopinavir/ritonavir tablets were used instead. Administration of arbidol in combination with moxifloxacin antibacterial therapy and immunotherapy alleviated the symptoms. After 20 days of hospitalization, the patient's physical examination showed promising results. Although the patient still had occasional cough and a small amount of white foam-like sputum, clinical symptoms such as cold, fever, dyspnea, abdominal pain and diarrhea were absent, indicating that her general condition was normal. After discussion by the expert group, the patient was found to be meeting the discharge standards and was discharged on March 12.

Three RT-PCR samples were selected for sequencing. Next-generation sequencing detected the partial genome of SARS-CoV-2 in all three samples (Table 2). The resultant clean

reads accounted for more than 98% of the raw reads, and most reads were successfully assigned. Notably, SARS-CoV-2 reads were found in all three samples, even in the samples that were undetermined with RT-PCR (samples 1 and 2). As SARS-CoV-2 reads were not abundant, genomes could not be assembled. The sequencing results obtained from the present study have been deposited into GenBank under Accession No. PRJNA637285.

Similar to that of the SARS coronavirus, the functional receptor targeted by SARS-CoV-2 is angiotensin-converting enzyme 2 (ACE2) [4–6]. Since RT-PCR detection requires cells containing a sufficient amount of viral load, the virus-containing cells must be sampled when collecting specimens. Recent studies have shown that the viral load in sputum samples is greater than that in throat swab samples [7]. Our patient's RT-PCR positive nucleic acid samples during the hospitalization were all deep sputum samples collected after being atomized by 3% sodium chloride injection. These results, combined with other reported cases [8], suggest that the cause of several negative nucleic acid tests in highly suspected patients may be related to the sample collection site.

In this study, next-generation sequencing detected a partial genome of SARS-CoV-2 that could not be detected by RT-PCR. Therefore, it is necessary to combine efficient SARS-CoV-2 capture sequencing to detect viral nucleic acids, and aid clinical diagnoses. In addition, because the RNA virus is susceptible to mutation, sequencing of pathogen genome using samples from asymptomatic and occult-infected patients could be a feasible strategy for studying the viral mutations and could provide a basis for subsequent virus tracing and epidemiological investigations.

In summary, we report the epidemiological history and clinical information of a patient who was diagnosed with COVID-19 despite multiple false-negative RT-PCR results, owing to the detection of some viral genes in the negative samples by next-generation sequencing. This case indicates that a negative RT-PCR result alone does not rule out the possibility of SARS-CoV-2 infection, and it is necessary to combine laboratory results, clinical symptoms and nucleic acid detection methods.

Table 2: Sequencing results of samples with RT-PCR.

Samples	Sample type	Date	E	N	R	Raw reads	Clean reads	Map_to_host reads	SARS-CoV-2 reads
1	Throat swab	February 22	ND	38	ND	126,921,364	124,777,940	108,003,919	4,035
2	Throat swab	February 23	39	ND	ND	103,839,486	102,031,982	48,156,781	1,938
3 ^a	Sputum	February 24	38	42	42	22,839,828	22,527,378	12,810,594	739

N, nucleocapsid gene; E, envelop gene; R, *RdRp* gene; ND, not detected; raw reads, the original sequencing data; clean reads, remove low quality, adapter, etc. from raw reads; Map_to_host reads, reads which mapped on human genome. ^aConfirmed as COVID-19 patient.

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Ethical approval: This study was approved by the Ethics Committee of The First People’s Hospital of Ziyang City.

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