

CASE REPORT

Distinct RT-PCR diagnosis profiles of father and son patients of COVID-19 using nasopharyngeal and alveolar lavage fluid samples

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The detection of viral nucleic acid by real time RT-PCR is the main confirmative diagnostic method for COVID-19 in clinical practice in China and worldwide. However, its sensitivity is unclear. Here we report two cases in a family in Guizhou, southwestern China. The father had a history of long stay in Wuhan. Surprisingly, although the son was diagnosed positive using the nasopharyngeal swab specimen and the rRT-PCR method, the father was diagnosed negative continuously for multiple times. Only after the alveolar lavage fluid sample was used, the father's rRT-PCR diagnosis turned positive. Their CT diagnosis and clinical symptoms did not completely align with their rRT-PCR diagnostic results. The underlying mechanisms and their implications to clinical practice are discussed.

Keywords: COVID-19, SARS-CoV-2, nasopharyngeal swab, alveolar lavage fluid, viral nucleic acid

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; CT, computerized tomography; rRT-PCR, real-time reverse transcription-polymerase chain reaction; BALF, alveolar lavage fluid

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Introduction

The recent outbreak of COVID-19 caused by SARS-CoV-2 poses a huge threat to global public health^[1-3]. As of May 22, 2020, 84,522 cases of COVID-19 have been diagnosed in China, and 4,645 have died. New cases have been reported in Japan, Korea, Thailand, the United States^[4], Germany, Italy,

Australia, and other countries outside China^[5]. A total of 5,084,920 cases have been reported in at least 214 countries. SARS-CoV-2 belongs to the same coronavirus family as SARS-CoV and MERS-CoV. The latter two have caused severe SARS and MERS epidemics in China and the Middle East, respectively, causing 10% (916/8098) and 34.4% (851/2468) deaths^[6,7]. At present, the source, transmission

Day of Hospitalization(DH)						DH 1	DH 2	DH 3	DH 4	DH 5	DH 9	DH 11	DH 12	DH 13	DH 14	DH 15	DH 16
Day of Illness(DI)		Onset				DI 7	DI 8	DI 9	DI 10	DI 11	DI 15	DI 17	DI 18	DI 19	DI 20	DI 21	DI 22
				Onset		DI 4	DI 5	DI 6	DI 7	DI 8	DI 12	DI 14	DI 15	DI 16	DI 17	DI 18	
Symptoms	Fever(°C)	38.5	37.8	36.4	38.8	38.5	38.8	38.9	38.2	36.4	37.3	36.7	37.2	37.2	37.1	36.8	
				subjective	37.8	36.4	36.5	37.2	36.7	36.4	36.7	37.1	36.8	36.2	36.4	36.5	
	Cough																
	Shortness of breath																
	Fatigue																
Lab finding	SARS-CoV-2 RNA test by rRT-PCR	Nasopharyngeal swab				Negative	Negative	NT*				NT	Negative	NT		Negative	
						Positive	Positive								Negative	Negative	
	BALF					NT	NT	Positive				Suspicious\$	Negative	Positive			
	Chest CT					Positive		Aggravated						Improved			
					Positive									Improved			
date		Jan. 23	Jan. 24-25	Jan. 26	Jan. 27-28	Jan. 29	Jan. 30	Jan. 31	Feb. 1	Feb. 2	Feb. 6	Feb. 8	Feb. 9	Feb. 10	Feb. 11	Feb. 12	Feb. 13

Figure 1. Timeline of disease course and laboratory findings of the father and son patients. The results were recorded by starting from the initial presentation of illness (the father on Jan 23 and the son on Jan 26) and their days of hospitalization. The son was discharged on February 12. The father was transferred to another hospital on February 13, and his health condition has improved since then. *NT: denotes not tested. \$ The cycle threshold (Ct) of at least one target gene (ORF1a/b or N) lies between the positive Ct and the negative Ct.

route, pathogenic mechanism, epidemiological characteristics, and disease spectrum of this novel coronavirus, SARS-CoV-2, are not clear. There is also a lack of targeted therapies and vaccines for COVID-19.

With the COVID-19 outbreak quickly spreading worldwide, it is critical to ensure the accurate and fast diagnosis. The detection of viral nucleic acid by real-time reverse transcription-polymerase chain reaction (rRT-PCR) is the main method for confirming diagnosis in clinical practice. A positive result suggests the presence of the unique SARS-CoV-2 viral sequence. The accuracy is considered high given the sequence uniqueness. However, the sensitivity of the assay is not well studied and reported.

Here we report two cases in Guiyang, Guizhou, southwestern China, belonging to one family. The father lived in Wuhan for a long time, and became ill at 1 day after returning to Guiyang. The son developed symptoms at 4 days after his father turned to Guiyang. The son patient was diagnosed COVID-19 positive quickly using the rRT-PCR on the nasopharyngeal specimen. However, the father's rRT-PCR test returned negative results twice with nasopharyngeal swabs. Once after using alveolar lavage fluid (BALF) specimen collected at Day 9 after illness onset, his rRT-PCR test showed positive. We have also collected their epidemiological and clinical characteristics. We found that the CT results did not align with the rRT-PCR results. The mother lived with the son and father but did not get infected.

Cases Description

Father patient case

A 53-year-old male patient was admitted and hospitalized to our hospital on 29 January 2020 (Figure 1). His symptoms included fever (maximum body temperature of 38.8°C), chills, coughing, fatigue, and shortness of breath. The patient disclosed that he had returned to his wife/son's house in Guiyang on 22 January 2020 from Wuhan. He had a fever on the next day after he returned Guiyang. The symptom was once reduced after medication. Previously, the patient had a long stay in Wuhan, but no exposure history to South China seafood market, and had not consumed any wild animal. He had a medical history of well controlled hypertension.

Chest computerized tomography (CT) (Figure 2A) on the day of admission revealed multiple ground-glass changes in the lung, with the lesions being subpleural. Physical examination revealed no significant findings except for the congestion of the pharynx and elevated body temperature. But his peripheral blood oxygen saturation (SPO₂) was only 93% on room air. His nasopharyngeal swabs were collected daily for two days after his admission and used for the SARS-CoV-2 nucleic acid rRT-PCR test according to the China National Health Committee guidelines^[8]. Both results were negative. Considering his clinical presentation of viral pneumonia but negative result of influenza A virus, influenza B virus antigen in a patient with the appropriate epidemiological risk, patient's alveolar lavage fluid was collected on the 3rd day after his admission, and the SARS-CoV-2 rRT-PCR test showed positive. This confirmed that the patient had COVID-19.

For the patient's treatment, he was given oxygen supple-

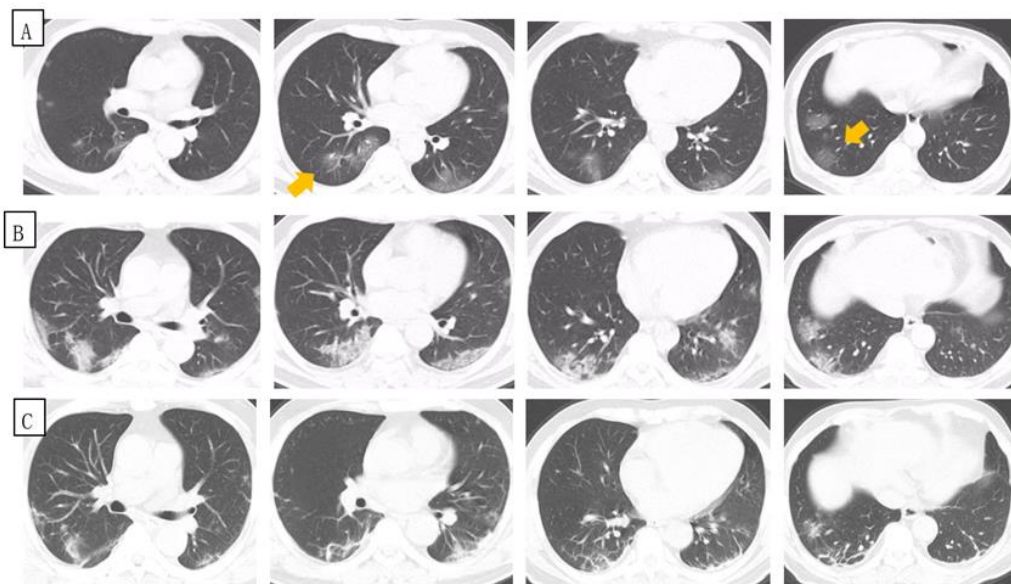


Figure 2. Chest CT images of a 53-year-old father patient of COVID-19. (A) Taken on January 29, 2020 (Illness Day 7, Hospital Day 1). Multifocal ground-glass bilateral opacities in lung, and the lesions mainly under the pleura (arrowheads). (B) January 31: increased opacities in lung. (C) February 9: absorption of bilateral ground glass and fibrotic changes in lung.

mentation through the mask. The medications he took included interferon alpha-2b (5 million units, twice daily, atomized inhalation), lopinavir plus ritonavir (500 mg, twice a day, oral) and moxifloxacin (0.4 g, once a day, intravenously).

After admission, the patient's chest tightness and shortness of breath worsened, his temperature reached 38.5-38.9°C for 72 hours. His oxygenation worsened, the oxygenation index was 268 mmHg, and respiratory failure combined. After 48 hours, chest CT (Figure 2B) showed progressive leaching and diffuse glands in the patient's lungs. Because the patient has shortness of breath and hypoxemia, methylprednisolone (40 mg, twice a day×5d, intravenously) is used to reduce inflammation of the lung.

On the 5th day of hospitalization, it was found that his lymphocyte and cellular immune function was reduced (Supplemental Table 1), and thus thymopolypeptides was given to improve the body's immunity. From the 4th day of hospitalization, the patient's temperature dropped to normal. From the 5th day, the patient's symptoms (such as cough and dyspnea) gradually reduced and then disappear, and the oxygen saturation was increased to 95% after his receiving nasal catheter oxygen therapy (FiO₂:0.29). On the 9th day, the SARS-CoV-2 RNA test of the alveolar lavage fluid showed suspiciously positive. On the 12th day, the chest was reviewed. CT showed the absorption of bilateral ground glass and fibrotic changes in lung (Figure 2C), which was better than the lung profile found in the previous examination. On the 12th day, the alveolar lavage fluid detection of SARS-CoV-2 RNA by rRT-PCR still showed a positive result. On the 14th day, the nasopharyngeal swab for SARS-CoV-2 nucleic acid was

negative. On the 16th day of hospitalization, he was transferred to another designated hospital for inpatient treatment, and his health condition has continuously improved since then.

Son patient case

The above father patient's son, a 25-year-old man with a 3-day history of mild fever (up to a maximum of 37.8°C) and cough, was admitted to our hospital on 29 January 2020, the same day when his father was hospitalized (Figure 1). He had no chest pain and shortness of breath. Physical examination revealed no significant findings. His oxygen saturation was 94%-98% on room air. Chest computed tomographic images showed ground glass opacity in right lung middle lobe and left basilar (Figure 3A). SARS-Cov-2 RNA was detected and confirmed in nasopharyngeal swabs by rRT-PCR for two continuous days after his admission. The patient underwent an "appendectomy" four years ago without other underlying diseases. Laboratory findings were in the normal range except for lymphopenia and elevated lactate dehydrogenase (Supplemental Table 2). Nasopharyngeal swabs were negative for RNA of influenza virus A and influenza virus B.

After admission, the son patient was administered abidol hydrochloride, lopinavir plus ritonavir (500 mg, twice a day, oral), interferon alpha-2b (5 million units, twice daily, atomized inhalation) as antiviral therapy, and moxifloxacin (0.4 g, once a day, intravenously) to prevent secondary infections and received nasal catheter oxygen therapy (FiO₂: 0.29). Due to his cellular immune disorders (Supplemental Table 2), the patient was given thymopolypeptides to improve the immunity. Due to nausea and bloating, pantoprazole and

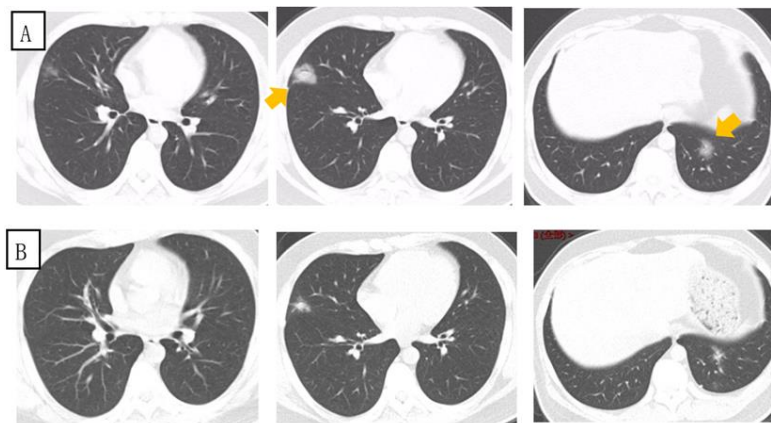


Figure 3. Chest CT images of a 22-year-old son patient of COVID-19. (A) Taken on January 29, 2020 (Illness Day 4, Hospital Day 1), showing bilateral ground glass opacities (arrowheads). (B) February 9: absorbed and reduced shadows in lung.

Clostridium butyricum were administered.

After admission, the patient had chest tightness and dry cough, but no fever. Symptoms gradually eased after treatment. On the 12th day of admission (February 9, 2020), a review of chest CT showed that compared with the film at 10 days ago, the range of opacities was absorbed and reduced (Figure 3B). According to the persistent negative results of SARS-CoV-2 (nasopharyngeal swab specimens, rRT-PCR) on day 13 and 14, as well as the lung lesions partially absorbed, the patient was discharged on day 15 (February 12) (Figure 1).

As another member of the family, the mother had been living with his son in Guiyang before his sickness, and has not had an illness so far. The mother was also tested together with her son and husband. Both SARS-CoV-2 nucleic acid tests using nasopharyngeal swabs and BALF were normal. Her CT scanning examinations were also normal. Overall, the mother appeared to be less susceptible and more resistant to the SARS-CoV-2 infection.

Discussion

In the family cases, one major unusual finding is related to diagnosis using the SARS-CoV-2 nucleic acid real time RT-PCR. Since the day of hospital admission, the son's temperature had been normal, but his two continuous rRT-PCR tests were positive with his nasopharyngeal samples. However, the father patient had fever on the day of admission and for a few days afterwards, but his rRT-PCR test with nasopharyngeal specimen was negative for all the times tested, and only the rRT-PCR test with his alveolar lavage fluid samples was positive. The false negative of nucleic acid detection with the nasopharyngeal samples is likely due to the no or low viral load in the collected samples. A recent study of 17 COVID-19 patients in Zhuhai, China reported that high viral load was detected in most nasal swabs and throat swabs

shortly after the onset of symptoms, and decreased significantly after 1 week^[9]. Another recent study using 82 infected individuals showed that the viral loads of SARS-CoV-2 in throat swab and sputum samples peaked at around 5-6 days after symptoms onset^[10]. Our findings with the nasopharyngeal swabs on the father and son patients are aligned with this report. However, these two recent studies^[9,10] did not have samples of alveolar lavage fluid. Only when the alveolar lavage fluid samples from the father patient were used, positive results showed up. Combining all these results, we can reasonably hypothesize that as the disease progresses, the virus quickly replicates and gradually transferred into the lung area, leading to the increasingly lower viral load in the upper respiratory tract and increasingly higher viral load in the lower respiratory tract. This viral transferring trend might be the reason why the father's nasopharyngeal swab test turned to be false negative at day 7 after disease onset. Given also that the same diagnosis obtained positive result with the son patient at day 4 after disease onset, we may conclude that different stages of the disease course may affect the true or false diagnosis result with the rRT-PCR method, which is an alarm for our routine diagnosis practice. A remaining question is when the lung samples start to show positive results with rRT-PCR.

In our father and son cases, the nucleic acid test results were not completely synchronized with the dynamics of CT images and clinical symptoms. While the RT-PCR diagnosis results differ significantly, the CT tests at the 1st day of hospitalization were positive for both father and son patients. On the 18th day after the disease onset, although the father's BALF nucleic acid was positive, his chest CT showed that the lung shadows had improved compared with the most severe CT, his temperature has already returned to normal, and the symptoms of cough and shortness of breath had also eased. Therefore, the remission of symptoms may precede the elimination of the

virus. After the son was hospitalized, he no longer had fever, but his viral nucleic acid test was still positive. Although there has been much discussion on the sensitivity of nucleic acid detection, the role of the etiology conformation in the diagnosis of infectious diseases is undoubted. However, a comprehensive epidemiological history, clinical symptoms, and chest imaging are needed for consideration, as illustrated in the patient case. Improving sample quality (including optimized sampling locations and sampling techniques) is an important factor in improving accuracy.

Another interesting observation is related to our drug therapy to these patients. Both patients had decreased lymphocyte counts and weakened cellular immune functions (Supplemental Table 1 and 2). Accordingly, they both received the immunomodulatory therapy using the thymosin hormone, which is able to stimulate the production of T cells. Eventually the cellular immune function of the patients improved and their lymphocytes also returned to normal. In the first report of the pathological anatomy of a COVID-19 patient, Xu et al. showed that the counts of CD4⁺ and CD8⁺ lymphocytes in the peripheral blood of the patient were greatly reduced while their cell status hyperactivated, showing severe immune injury in this patient^[11]. Our treatment of the two patients with the immunomodulatory drug might have resulted in their increased production of T cells and thus helped their immune systems fight against the infections. It is also noted that although the symptoms were relieved, the drug therapy results might not promptly change the diagnosis results within a short period of time.

Overall, this is the first case report that shows the distinct profiles of diagnosis of father and son patients with COVID-19 using nasopharyngeal and alveolar lavage fluid samples. One take-home message is that the sensitivity of the rRT-PCR method depends on the location of collected samples in different disease stages. The result may be negative with the nasopharyngeal samples at day 7 after disease onset as shown in the father patient in this report. However, the lung samples may become more sensitive at the late stage of infection. A reality is that it is not a conventional method in clinical to use lower respiratory tract samples for diagnosis due to its relative difficulty in sample collection. In our cases, BALF needs to be obtained through invasive procedures, which increases the pain of patients and increases the risk of infection transmission. However, for more reliable diagnosis, we would propose the consideration of the nucleic acid testing using the lower respiratory tract samples, especially for patients at a potentially later stage of infection and whose epidemiological and other clinical manifestation are highly suggestive (for example, typical CT images, like our patients). Mechanistically, it is critical to understand the viral load and distribution at different stages in the course of the disease, and their relationships with the severity and transmission of the disease. Solving these problems through further research will help diagnose and control the COVID-19 disease.

Ethics Approval

This case series was approved by the institutional ethics board of Guizhou Province People's Hospital (No. 202005), and consent was obtained from patients.

Patient Consent for Publication

A written informed consent was obtained from the patients for publication of the information about them that appears within this case report.

Conflicting Interests

The authors declare that they have no conflict of interests.

Acknowledgments

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Supporting Information

Table S1. Clinical laboratory test results of the father patient.

Measure	Reference Range	Hospital Day 1	Hospital Day3	Hospital Day5	Hospital Day8	Hospital Day 13	Hospital Day15
White-cell count, $\times 10^9/L$	3.5-9.5	3.57	3.00	11.22	6.75	7.56	—
Absolute neutrophil count, $\times 10^9/L$	1.8-6.3	1.91		9.92	5.44	5.18	—
Absolute lymphocyte count, $\times 10^9/L$	1.1-3.2	1.2	1.08	0.76	0.80	1.27	—
LDH, U/L	120-150	303	506	288	307	225	—
Creatine kinase, U/L	50-310	402	399	214	199	38	—
Creatine kinase-MB, U/L	0-25	22	—	—	22	—	—
PaO ₂ :FiO ₂ , mm Hg	>300	—	268	368	—	—	—
CD3+ cell count, / μ l	770-2860	—	—	388	356	—	476
CD4+ cell count, / μ l	500-1440	—	—	260	192	—	280
CD8+ cell count, / μ l	238-1250	—	—	104	140	—	168
CD4/CD8	1-2.47	—	—	2.50	1.37	—	1.67
C-reactive protein, mg/L	0-5	—	43.58	19.76	5.45	93.97	80.39
Procalcitonin, ng/mL	0-0.046	0.08	0.06	0.07	0.04	—	0.05

*The table shows that the lymphocyte counts of patient on the 3rd, 5th and 8th days of hospitalization all decreased, but returned to normal on the 13th day. The counts of CD3+, CD4+, and CD8+ lymphocytes all decreased on the 5th and 8th day of hospitalization, and then increased on the 15th day, but did not return to normal. The patient's PaO₂: FiO₂ decreased on the 3rd day of hospitalization and returned to normal on the 5th day. The patient's LDH and CRP increased slightly during the hospitalization.

Table S2. Clinical laboratory test results of the son patient.

Measure	Reference Range	Hospital Day 1	Hospital Day2	Hospital Day4	Hospital Day6	Hospital Day10	Hospital Day14
White-cell count, $\times 10^9/L$	3.5-9.5	4.13	3.25	4.26	4.26	9.88	5.6
Absolute neutrophil count, $\times 10^9/L$	1.8-6.3	2.66	1.28	1.71	2.14	8.01	2.72
Absolute lymphocyte count, $\times 10^9/L$	1.1-3.2	0.86	1.54	1.92	1.6	1.10	2.05
LDH, U/L	120-150	181	179	153	128	127	143
Creatine kinase, U/L	50-310	107	—	54	—	37	22
Creatine kinase-MB, U/L	0-25	9	—	15	—	7	9
CD3+ cell count, / μl	770-2860	—	—	—	1040	412	1072
CD4+ cell count, / μl	500-1440	—	—	—	276	144	464
CD8+ cell count, / μl	238-1250	—	—	—	696	240	536
CD4/CD8	1-2.47	—	—	—	0.40	0.60	0.87
C-reactive protein, mg/L	0-5	1.10	0.83	2.19	0.9	0.24	2.37
Procalcitonin, ng/mL	0-0.046	0.06	—	0.03	0.03	0.03	0.11

*The table shows that the patient's lymphocyte count decreased on the first day of hospitalization, and then returned to normal. The CD3+ lymphocyte count decreased on the 10th day and returned to normal on the 14th day. The CD4+ lymphocyte count decreased on both the 5th and 10th days of hospitalization, but on the 14th day, the CD4+ lymphocyte count increased to close to normal. The patient's LDH increased slightly during the first 4 days of hospitalization.