The novel coronavirus (COVID-19) pneumonia with negative detection of viral ribonucleic acid from nasopharyngeal swabs: A case report

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Abstract

**Background**

The novel coronavirus disease 2019 (COVID-19) outbreak started in Wuhan, Hubei, China since December 2019 and cases of infection have been continuously reported in various countries. It is now clear that the COVID-19 coronavirus is transmissible from human to human. Nucleic acid detection is considered as the gold standard for the diagnosis of COVID-19. In this case report, we describe our experience in detection of COVID-19 from a confirmed patient using nucleic acid test of bronchoalveolar-lavage fluid (BALF) samples but not nasopharyngeal swabs.

**Case presentation**

We present a case of severely ill COVID-19 infected 46-year-old man with fever, coughing and chest tightness. We performed viral detection using his BALF samples and imaging method (CT) for confirmation. The patient received combination of interferon-alpha-1b and ribavirin, lopinavir and ritonavir for antiviral treatment at different stages. Other medication was also given to him in combination for anti-inflammation, intestinal microbial regulation, phlegm elimination, liver protection and pulmonary fibrosis prevention purposes. We provided oxygen supply to him using BIPAP ventilator and high-flow humidification oxygen therapy instrument to facilitate respiration. The patient was cured and discharged.

**Conclusion**

This case report described an effective supportive medication scheme to treat COVID-19 infected patient and emphasized the necessity of detection of the viral genome using BALF samples and its significance in the diagnosis and prognosis of the disease.

**Background**

The outbreak of the novel coronavirus COVID-19 started in Wuhan, Hubei, China from December 2019, and has been spread out to various parts of China, as well as multiple countries including Thailand, European countries, the United States, Japan, Korea, Iran and etc [1, 2]. As of February 24, 2020, the number of reported cases in China has reached 47672 cases (9126 severe cases) among which 2663 died.[3] The geographical distribution of initial clusters of COVID-19 infection cases is
closely related to Huanan seafood wholesale market localized in Wuhan city, Hubei Province, China. COVID-19 belongs to the genus of betacoronavirus which is usually polymorphic with a diameter of 60 to 140 nm. Its genome was found to have more than 85% homology to SARS-COV virus in bat after comparative genomic analysis. Thus, it is speculated that the natural hosts of COVID-19 are possibly wild animals such as bats and pangolins. [4, 5] However, this is still under discussion. It is clear now that this virus could spread through human-to-human transmission. [6] Further understanding on its epidemiology, transmission mechanism and spectrum of symptoms is under continuous investigation. [7–9] This case report describes the symptoms, diagnosis and treatments of one unconventional COVID-19 pneumonia case in our hospital.

Case Presentation
On 23 January 2020, a 46-year-old man was transferred to our hospital with 11-days history of fever and coughing. The patient permanently resides in Yili Development Zone, Xinjiang, China. He started low fever with dry cough, muscle ache and fatigue without known causes in Yili on 12 January. The patient disclosed that he had been in close contact with a person from Wuhan in Yili on 10 January and had a series of travel history by flights to Shanghai on 16 January 2020, from Shanghai to Ningbo on 17 January 2020, and from Ningbo to Shenzhen on January 19, 2020, without traveling or living history to Wuhan. On 18 January, there was onset of symptoms including chest tightness without chest pain and hemoptysis. Unknown medication was taken by the patient without symptomatic improvement. He was considered as a pneumonia patient and admitted to the University of Hong Kong-Shenzhen Hospital on 19 January. He received oxygen support and levofloxacin treatment in the hospital. He was then tested positive for COVID-19 from BALF specimen and transferred to the Third People’s hospital of Shenzhen for further treatment on 23 January. The patient has no history of other diseases, surgical trauma, food and drug allergy. There was no headache, dizziness, vomiting, abdominal pain, diarrhea, frequent urination, urgent urination, or urination pain claimed by the patient.

The results of physical examination on 23 January showed a body temperature of 36.1 °C, pulse of 94 times/min, respiratory rate of 26 breaths/min and blood pressure of 127/87 mmhg. Clinical laboratory
test results revealed negative results for mycoplasma, chlamydia, cytomegalovirus-IgM, influenza A/B virus and respiratory syncytial virus (rsv). Throughout the whole period of hospitalization, the patient was given 60μg of interferonalfa–1b (Beijing Tri-Prime Gene Pharmaceutical Co., Ltd., China; Shenzhen Kexing Biopharm, China) inhalation for antiviral purpose, 0.4g of Bio-Three tablets (Huizhou Jiuhui Pharmaceutical Co., Ltd, China; Toa Pharmaceutical Co., Ltd.Tatebayashi Plant, Japan) and 420mg Bifid-triple viable capsule (Inner Mongolia Shuangqi Pharmaceutical Co., Ltd, China) three times a day for regulation of intestinal microbiome, and 30mg mucosolvan (Boehringer Ingelheim Espana,S.A.) intravenous injection twice per day for phlegm elimination. A 3-day course of 0.5g ribavirin (Jiangsu Lianshui Pharmaceutical Co., Ltd., China) intravenous injection starting from 23 January was also given to the patient in combination with Interferonalfa–1b twice a day for three days for antiviral treatment of RNA virus. (Figure 2)

On 24 January, the patient was reported to have shortness of breath with respiratory rate of 30 times/min. Blood gas analysis revealed a pH value of 7.428, carbon dioxide partial pressure of 43.0mmHg, oxygen partial pressure of 64.4mmHg, actual bicarbonate level of 28.4mmol/L, and fractional concentration of inspired oxygen (FiO₂) of 41.0%. (Table 1) Supplemental oxygen was applied to the patient with non-invasive BIPAP ventilator using IPAP 14cmH₂O and EPAP 7cmH₂O with oxygen concentration of 45%. His respiratory rate was 16 times/min after receiving oxygen supplement. Shortness of breath was gradually relieved and patient’s oxygen saturation values of peripheral blood reached 99% to 100%. Routine blood test revealed a white blood cell count of 5.61×10⁹/L with 80.40% neutrophil and 14.80% lymphocyte, hemoglobin concentration of 158g/L, platelet count of 207×10⁹/L (Table 1) and erythrocyte sedimentation rate (ESR) of 71mm/h. Biochemical test results showed an elevated D-Dimer (diffuse intravascular coagulation,DIC) level (Table 1) which may induce thrombus. 0.4ml of nadroparin calcium (ASPEN Notre Dame de Bondeville, France) subcutaneous injection was given to the patient once per day for anti-coagulation until 31 January. D-DIC level decreased to normal range at 26 January. The patient was also received 40mg of esomeprazole sodium (AstraZeneca Pharmaceutical Co.,Ltd.) intravenous injection once per
day for gastro-esophageal reflux suppression until 30 January and 30mg of methylprednisolone (Pfizer Manufacturing Belgium NV) intravenous injection once every 12 hours until 28 January for anti-inflammation treatment. The increase in white blood cells (Table 1) during this period may due to the effect of methylprednisolone. However, COVID–19 ribonucleic acid test was negative using nasopharyngeal swabs done by Shenzhen Center for Disease Control (Shenzhen CDC). Treatment scheme and viral detection time points are illustrated in Figure 2.

From 25 January onwards, the patient’s syndromes had gradually relieved with only occasional dry cough. Computed tomography (CT) scan of the lungs was performed on 25th, 29th January and 12th February (Figure 1). Evidence of severe pneumonia, including multiple lesions and swollen lymph nodes, could be seen from both of the lungs on 25th January. (Figure 1A) The patient was then given 500mg lopinavir and ritonavir tablets (Abbott S. P. A., Italy) every 12 hours until 6 Feb as a combination treatment for antiviral effect. COVID–19 ribonucleic acid test was again negative using nasopharyngeal swabs done by our hospital on 26 January. To confirm the presence of COVID–19, the patient’s BALF sample was sent to Shenzhen CDC for viral nucleic acid detection. COVID–19 ribonucleic acid was tested positive for BALF sample on 27 January. Oxygen supplement by non-invasive BIPAP was replaced by high-flow humidification oxygen therapy instrument for higher oxygen flow of 45L/min and oxygen concentration of 40% on 28 January. Biochemical test results indicated an increased level of alanine aminotransferase (ALT) since 29 January. The patient was diagnosed to have toxipathic hepatitis which was possibly induced by COVID–19. The patient then received 50mg compound glycyrrhizin tablets (Akiyama Jozai Co., Ltd, Japan) three times per day from 29 January to 13 February and 50mg bicyclol tablets (Beijing Union Pharmaceutical Factory, China) three times per day from 31 January to 13 February for liver protection. (Figure 2) ALT level resumed to the normal range on 13 February. To prevent pulmonary fibrosis, the patient was given 7-day course of 0.2g acetylcysteine granules (Bio Pharmacaceutical, China) three times per day from 7 February. Obvious improvements could be seen from the subsequent CT scanning results of the lungs on 29th January (Figure 1B) and 12 February (Figure 1C). Oxygen supplement by high-flow humidification oxygen therapy instrument was discontinued on 31 January and the patient was given nasal catheter for
oxygen inhalation with a flow rate of 4L/min. His clinical conditions was stable with fractional concentration of inspired oxygen (FiO$_2$) value fluctuating within the normal range. (Table 1) On 2 February, the patient stated that there was obvious improvement of his symptoms. CT scanning of the lungs (Figure 1C) on 12 February confirmed the improvement and the patient was discharged on the following day.

Conclusion

This case report described the symptoms of a COVID-19 infected patient and treatment measures taken to the patient. The patient was in close contact with a person from Wuhan and was infected through human-to-human transmission. According to the seventh edition of Novel Coronavirus Pneumonia (NCP) treatment plan issued by National Health Commission of the People’s Republic of China, this patient in the case report is considered as a severely ill case of the COVID-19 pneumonia. The patient’s course of disease reached 11 days which is the period of acme at the time of hospital admission and showed respiratory failure symptoms. The unconventional aspect of this case is that the detection of the patient’s upper respiratory tract specimen was COVID-19 negative repeatedly while that of BALF sample was positive for COVID-19 virus. This suggests that the COVID-19 virus mainly attacks the lung. We speculate that the virus migrates from upper respiratory tract to lower respiratory tract as the infection progresses, which may explain the negative test results of nasopharyngeal swabs. As reported by Dr. Yang Yang and his group, the nucleic acid of COVID-19 could be detected from BALF samples of critically ill patients with COVID-19 pneumonia while not from the upper respiratory tract specimens of some patients. [10] In contrast, the detection of COVID-19 RNA was positive from the upper respiratory tract specimens at mild stage of illness [11]. This indicates that the distribution of this virus in the respiratory system is closely related to the severity of the disease. Thus, detection of COVID-19 RNA in BALF samples is of great importance in confirming of the infection. Although it is largely supportive, our treatment scheme was proven to be effective in helping the patient combating the virus. Our report highlights the necessity to carry out tracheoscopy and detection of COVID-19 RNA from BALF samples in addition to monitoring epidemiological changes, clinical symptoms and chest CT findings of the unconventional COVID-19 pneumonia cases.
which will be informative and clinically significant in guiding the prognosis of the disease.

Abbreviations
COVID–19: Coronavirus disease 2019; BALF: Bronchoalveolar-lavage fluid; CT: Computed tomography; BIPAP: Bilevel positive airway pressure; rsv: Respiratory syncytial virus; RNA: Ribonucleic acid; IPAP: Pressure for inhalation; EPAP: Pressure for exhalation; ESR: Erythrocyte sedimentation rate; DIC: Diffuse intravascular coagulation; CDC: Center for Disease Control; ALT: Alanine aminotransferase; FiO\textsubscript{2}: Fractional concentration of inspired oxygen.

Declarations
Ethics approval and consent to participate
This study is approved by the Ethics Committees from Shenzhen Third People’s Hospital (2020-055) and the patient is consent to participate.

Consent for publication
Written informed consent for publication of the clinical details and/or clinical images was obtained from the patient.

Authors’ contributions
PZ, WW, LP, YL, CC, LC, JL, MC, SF, XJ, FW, LY, YL contributed to the clinical patient care and management. PZ and CZ contributed to the manuscript preparation
PZ, CZ, FW and LY contributed to the data analysis. YL contributed to the manuscript verification. All authors have read and approved the manuscript.

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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Not applicable

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Table

**Table 1.** Laboratory test results. Elevated level is indicated by bold and decreased level is indicated by underline.

| Tests                        | Normal range | 24-Jan | 25-Jan | 26-Jan | 27-Jan | 28-Jan | 29-Jan | 30-Jan | 31-Jan | 1-Feb | 2-Feb | 7-Feb | 12-Feb |
|------------------------------|--------------|--------|--------|--------|--------|--------|--------|--------|--------|-------|-------|-------|-------|
| **Routine Blood Tests**      |              |        |        |        |        |        |        |        |        |       |       |       |       |
| white blood cell count (10^9/L) | 3.5-9.5     | 5.61   | 4.59   | -      | **9.89** | 9.48   | 7.41   | -      | 7.02   | -     | -     | 5.12  | 3.63  |
| Neutrophil ratio (%)         | 40-75        | **80.4** | 73.1   | -      | **87.4** | **88.3** | **80.1** | -      | **78.2** | -    | -     | **75.7** | 54.2  |
| Lymphocyte ratio (%)         | 20-50        | 14.8   | 18.7   | -      | 7.9    | 7.1    | 11.3   | 13.4   | -      | -     | 13.5  | 32.8  |
| Eosinophil ratio (%)         | 0.4-8        | 0.5    | 0.2    | -      | 0      | 0      | 0      | 0.3    | -      | -     | 1     | 2.5   |
| T cell ratio (%)             | 65-79        | -      | -      | -      | -      | -      | -      | 49.1   | -      | -     | -     | -     | 66    |
| T cell absolute count (UL)   | -            | -      | -      | -      | -      | -      | -      | 337    | -      | -     | -     | -     | 920   |
| Eosinophil ratio (%)         | -            | -      | -      | -      | -      | -      | -      | -      | 110    | -     | -     | -     | 379   |
| Ts-cell absolute count (UL)  | -            | -      | -      | -      | -      | -      | -      | -      | -      | -     | -     | -     | -     |
| Th/Ts ratio                  | 0.9-3.6      | -      | -      | -      | -      | -      | -      | 2.03   | -      | -     | -     | -     | 1.41  |
| Red blood cell (10^12/L)     | 4.3-5.8      | 5.05   | 4.94   | 4.8    | 4.53   | 4.86   | 4.86   | -      | 4.86   | -     | 4.53  | 4.14  |
| Hemoglobin (g/L)             | 130-175      | 158    | 155    | 151    | 141    | 151    | 157    | -      | 157    | -     | 142   | 129   |
| Platelet (10^9/L)            | 125-350      | 207    | 257    | 324    | 283    | 329    | 286    | -      | 286    | -     | 179   | 127   |
| Biochemical tests            |              |        |        |        |        |        |        |        |        |       |       |       |       |
| Albumin (ALB) (g/L)          | 35-50        | 39.7   | -      | 39.5   | -      | 40.3   | 38.9   | 37.9   | -      | -     | -     | 41.7  | 40.7  |
|                                | Value 1 | Value 2 | Value 3 | Value 4 | Value 5 | Value 6 | Value 7 | Value 8 |
|--------------------------------|---------|---------|---------|---------|---------|---------|---------|---------|
| Total Bilirubin (TB) (umol/L)  | 1.7-21  | 19      | 17.2    | -       | -       | 13.7    | 19.1    | 14.1    | 8.2     | 21.9    |
| Direct Bilirubin (DB) (umol/L)| 0.3-6.8 | 0       | 7.8     | -       | -       | 6.2     | 6.9     | 5.3     | 3.2     | 10.6    |
| Alanine aminotransferase (ALT) (U/L) | 21-72 | 65.2    | 51      | -       | -       | 173     | 265     | 242     | 134     | 46      |
| Aspartate aminotransferase (AST) (U/L) | 17-59 | 59      | 30      | -       | -       | 62      | 64      | 53      | 31      | 27      |
| Alkaline phosphatase (ALP) (U/L) | 45-125 | 231     | 172     | -       | -       | 123     | 95      | 97      | 96      | 77      |
| γ-glutamyl transpeptidase (GGT) (U/L) | 0-49 | 508     | 473     | -       | -       | 371     | 277     | 230     | 135     | 104     |
| Lactate dehydrogenase (LDH) (U/L) | 313-618 | 770     | -       | -       | 257     | 206     | 201     | 195     | 151     |         |
| Blood urea nitrogen (BUN) (mmol/L) | 3.2-7.1 | 4.34    | 5.38    | -       | -       | 5.17    | 4.29    | -       | 1.2     | 1.66    |
| creatinine (Cr) (umol/L)       | 57-97   | 61.8    | 55      | -       | -       | 61      | 66      | -       | 66      | 57      |
| cystatin c (Cys-C) (mg/L)      | 0-1.21  | -       | 0.7     | -       | 1.17    | 0.91    | 0.65    | 0.5     |         |
| B2 microglobulin (B2-M) (mg/L) | 1.0-3.0 | -       | 1.46    | -       | 0.73    | 1.52    | 2.27    | 2.02    |         |
| Procalcitonin (PCT) (ng/mL)    | <0.1    | 0.053   | 0.05    | 0.047   | 0.039   | 0.021   | 0.044   | 0.068   | 0.10    | 0.034   |
| C-reactive protein (CRP) (mg/L)| <10     | 177     | 48.73   | 19.37   | 9.23    | 4.94    | 3.11    | 7.43    | 11.2    | 3.93    |
| Creatine kinase (CK) (U/L)     | 55-170  | -       | 67      | -       | -       | -       | -       | -       |         |
| Creatine kinase-MB (CK-MB) (ng/mL) | 0-2.37 | <0.22   | 0.76    | -       | -       | -       | -       | -       | 0.84    |
| Myoglobin (MYO) (ng/mL)         | 0-110   | 78.3    | 103.6   | -       | -       | -       | -       | -       | 25.36   |
| Cardiac Troponin I (CTnI) (ng/mL)| <0.04 | <0.01   | <0.00   | -       | -       | -       | -       | -       | <0.006  |
| type B natriuretic peptide (BNP) (pmol/L) | 0-23.1 | 34.1    | -       | -       | -       | -       | -       | -       |         |
| Interleukin-6 (IL6) (pg/mL)     | 0-7     | 16.19   | -       | -       | -       | -       | -       | -       | <1.5    |
| Blood gas assay                |         |         |         |         |         |         |         |         |         |
| PH                             | 7.35-7.45 | 7.428 | 7.448   | 7.452   | 7.447   | 7.445   | 7.45    | 7.45    | 7.45    | 7.45    |
| PCO2 (mmHg)                    | 35-45   | 43      | 39.1    | 39.1    | 39.2    | 39.7    | 39      | 41.8    | 41.5    | 43.3    |
| PO2 (mmHg)                     | 75-110  | 64.4    | 137     | 137     | 101     | 113     | 74.2    | 71.3    | 87.1    | 75.7    |
| Actual sodium bicarbonate (mmol/L) | 21.4-27.3 | **28.4** | 27 | 27.2 | 27 | 27.3 | **27.5** | **27.9** | 27.2 | 27.5 | **27.7** | **27.9** | **27.7** |
| Whole blood alkali surplus (mmol/L) | -3-3 | **3.4** | 2.8 | 3.2 | 2.8 | 3 | **3.5** | **3.6** | **3.3** | **3.1** | 2.6 | 3 | 2.5 |
| Oxygenation index | 400-500 | 304 | 304 | 259 | 323 | 195 | 188 | 194 | 261 | 399 | 319 | 359 |
| Lactate (mmol/L) | 0.5-1.6 | 1.4 | 1.5 | 1.3 | **1.8** | 1.3 | 1.6 | 1 | 1.2 | 1.3 | 1.5 | **2.4** | 2 |
| Fractional concentration of inspired oxygen (FiO2) (%) | 15.0-100 | 41 | 45 | 45 | 39 | 35 | 38 | 38 | 45 | 29 | 21 | 29 | 29 |
| Prothrombin time activity (PTA) (%) | 70-120 | 107 - | 98 - | - - | - | - | - | - | - | - | - | - |
| Prothrombin time (PT) (Sec) | 11-15.1 | 12.7 - | 13.2 - | - - | - | - | - | - | - | - | 11.5 | - |
| Prothrombin time International normalised ration (PT INR) | 0.75-1.25 | 0.96 - | 1.01 - | - - | - | - | 0.85 | - |
| Activated partial thromboplastin time (APTT) (Sec) | 28-43.5 | 31.8 - | 30 - | - - | - | - | - | - | - | - | 28.3 | - |
| Antithrombin III (AT III) (%) | 85-135 | 100 - | 105 - | - - | - | - | - | - | - | - | 117 | - |
| Fibrinogen (FIB) (g/L) | 2-4 | **7.79** - | **5.3** - | - - | - | - | - | - | - | - | 4.62 | - |
| D-Dimer (Diffused intravascular coagulation, D-DIC) (ug/mL) | 0-0.5 | **0.95** - | 0.49 - | - - | - | - | - | - | - | - | 0.29 | - |

**Figures**
CT scanning results of the lungs. A. CT scan images on 25 January. B. CT scan images on 29 January. C. CT scan images on 12 February. Improvements in abnormalities could be seen.

Figure 2

Treatment schemes and Viral RNA detection time points.
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