A report of clinical diagnosis and treatment of nine cases of coronavirus disease 2019

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Abstract
Coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 has become an important public health issue in the world. More than 118 000 cases were confirmed around the world. The main clinical manifestations were respiratory symptoms and occasional gastrointestinal symptoms. However, there is no unified standard for the diagnosis and treatment of COVID-19. In the retrospective analysis, we report nine cases of COVID-19, describe the history of contact, clinical manifestations, the course of diagnosis and clinical treatment before, during and after treatment.

KEYWORDS
COVID-19, respiratory symptoms, SARS-CoV-2

1 | INTRODUCTION

A new virus, called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused the outbreak and global spread of pneumonia over the past 2 months.¹² Up to 13 March 2020, there are over 118 000 confirmed cases and 4000 dead in the world.³ Patients with coronavirus disease 2019 (COVID-19) had respiratory symptoms and occasional gastrointestinal symptoms including fever, cough, shortness of breath, muscle ache, confusion, headache, sore throat, rhinorrhea, chest pain, diarrhea, and so forth.⁴ It has been reported that the main ways of transmission are short-range respiratory droplets and indirect transmission, and the other routes of transmission, including mother-to-child, fecal-oral, and aerosol transmission needs further confirmation. However, epidemiological investigation shows that the new infectors had a history of close contact with confirmed SARS-CoV-2 patients.⁵ The laboratory examinations (including blood routine examination, C-reactive protein
[CRP], lymphocyte subpopulations, and SARS-CoV-2 RNA), as well as the chest computed tomography (CT) examination, play an important role in diagnosis, treatment, and assessment. Although several approved antiviral drugs including lopinavir and ritonavir, interferon, arbidol, ribavirin, and chloroquine phosphate are used for empirical antiviral therapy, the use of combination therapy of traditional Chinese and western medicine is also recommended. Currently, information regarding the clinical diagnosis and treatment of COVID-19 caused by SARS-CoV-2 is scarce. In this study, the epidemiologic history, clinical diagnosis, and treatment, as well as the changes of clinical characteristics before, during and after treatment of nine cases of COVID-19 are reported.

2 MATERIALS AND METHODS

2.1 Patient

The clinical data of nine cases infected with the SARS-CoV-2 were analyzed retrospectively. Four cases (3#, 4#, 5#, and 8#) disclosed that they had returned to their hometown from epidemic-hit Wuhan. One case (9#) stated a history of direct contact with a fellow villager from a key epidemic-hit Wuhan. There are two cases (6# and 7#) of SARS-CoV-2 infection with the familial aggregation. However, it is unknown how the two cases (1# and 2#) without the above-mentioned epidemiologic history were infected. Two cases (1# and 3#) have a history of smoking for more than 20 years, while others have no history of smoking. Written informed consent was obtained from patients for using clinical records in this study.

2.2 Laboratory tests

Nasopharyngeal swab specimens were collected according to the Centers for Disease Control and Prevention guidelines. Reverse transcription-polymerase chain reaction was employed to detect the obtained nasopharyngeal swab specimens. Three pairs of primers were designed to target three genes including RdRP, E, and N. The positive expression of the three genes, or RdRP and E genes, or RdRP and N genes indicates SARS-CoV-2 is positive. Diagnostic kit for immunoglobulin M antibodies to Mycoplasma pneumoniae, adenovirus, respiratory syncytial virus, influenza A and B viruses, parainfluenza viruses type 1, 2, and 3, and Chlamydia pneumoniae was used for detecting nine kinds of common respiratory pathogens. The subpopulations of lymphocytes including CD3+ T cells, CD3+CD4+ T cells, CD3+CD8+ T cells, CD3+CD4+CD8+ T cells, and CD4/CD8 ratio were detected by flow cytometry. Other laboratory tests were implemented as well.

3 RESULTS

3.1 Clinical symptoms before treatment

Generally, nine patients were in good health before hospitalization. Before treatment, the various clinical symptoms were reported. Most cases had fevers (range from 37.4°C to 39.3°C) except for case 7#. With the exception of cases 6# and 7#, seven cases had a slight or severe cough with or without phlegm. Only two cases (3# and 8#) of these patients had occasional chest tightness but no chest pain. Notably, cases 1# and 2# had severe or slight diarrhea, respectively but no abdominal pain, while others had no gastrointestinal symptoms.

The laboratory reports of these patients before, during and after treatment are shown (Table 1). Except for significantly decreased white-cell count (WBC) and absolute neutrophil count (ANC) in case 2#, others had basically normal or slightly decreased WBC and ANC throughout the clinical course. The absolute lymphocyte count (ALC) was low, as well as the obviously decreased percentage of lymphocyte subpopulations (including CD3+ cells, CD3+CD4+ cells, and CD3+CD8+ cells) were observed in case 1# before treatment. The absolute number and the proportion of these cells gradually returned to normal levels during and after treatment. Notably, the ALC was obviously decreased during treatment but back to normal level after treatment compared with those before treatment in cases 3# and 9#.

It was observed that CRP was obviously increased in cases 1#, 3#, and 9# before treatment, which gradually recovered to normal level during and after treatment. However, procalcitonin (PCT) had no clinically significant changes in all cases. Both of the arterial partial pressure of carbon dioxide (pCO2) and oxygen (pO2) in cases 1# and 9# were obviously decreased before treatment according to the existing records. With the exception of the significantly elevated lactic dehydrogenase in cases 1#, 8#, and 9# before treatment, the others were normal. Before treatment, slight rises of fibrinogen and D-dimer (D-D) in some patients. It was observed that the feces occult blood test was weakly positive only in case 1# with bad diarrhea before treatment. In addition, the results of SARS-CoV-2 were positive before treatment and gradually turned negative during and after treatment, while nine kinds of common respiratory pathogens were all negative in patients.

Lung lesions and outcomes of nine patients with similar findings were shown in Figure 1. Before treatment, the results were reported as showing small patchy shadows or ground-glass opacity, as well as partial pulmonary consolidation with or without bronchus sign in outer zone of single or double lung. During and after treatment, the lung lesions partially or completely resolved.

Combination therapy was performed in all cases. There were four cases (1#, 3#, 8#, and 9#) with severe infection, who were treated with interferon alfa-2b (10 million international unit [IU] daily), lopinavir and ritonavir tablets (800/200 mg daily), methylprednisolone (40 mg daily), moxifloxacin hydrochloride (0.4 g daily), and high-flow nasal oxygen therapy. Among these, additional intravenous immunoglobulin (200 mg/kg daily) and thymalfasin (1.6 mg twice a week) were employed to enhance immune of cases 1# and 8#, respectively. In addition, the traditional Chinese medicine (Qingfei Paidu Decoction) were used for preventing and treating pulmonary fibrosis in cases 1#, 8#, and 9#. The other cases (2#, 4#, 5#, 6#, and 7#) with moderate infection received interferon alfa-2b (10 million IU daily), lopinavir and ritonavir tablets (800/200 mg daily), and moxifloxacin hydrochloride (0.4 g daily). Moreover, low-flow nasal oxygen therapy was used to
| Items             | Case 1# BT/DT/AT | Case 2# BT/DT/AT | Case 3# BT/DT/AT | Case 4# BT/DT/AT | Case 5# BT/DT/AT | Case 6# BT/DT/AT | Case 7# BT/DT/AT | Case 8# BT/DT/AT | Case 9# BT/DT/AT | Reference range |
|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Age, y           | 54              | 25              | 47              | 53              | 29              | 50              | 14              | 56              | 51              | ...             |
| Sex              | Male            | Female          | Male            | Male            | Female          | Male            | Female          | Female          | Female          | ...             |
| WBC, 10^9/L      | 10.1/5.2/5.6    | 2.3/2.3/2.7     | 9.6/9.4/9.2     | 3.8/2.6/3.6     | 3.9/5.3/4.7     | 4.3/5.9/6.9     | 5.2/6.7/ND     | 9.9/8.2/5.4     | 5.4/7.1/7.1     | 4.10            |
| NEUT (%)         | 89.5/78.9/72.7  | 59.9/55.5/57.9  | 71/88.1/80.4    | 56.5/56/62.6    | 70/58.4/43.7    | 57/66.4/65.1    | 58.2/49.6/ND   | 88.8/77/51.7    | 77/78/76.2      | 50-75           |
| NEUT#, 10^9/L    | 9.0/4/1/1       | 1.4/1/1.6       | ND/8.3/7.4      | 2.2/1.4/2.3     | 2.7/31.2/2.1    | 2.4/3.9/4.5     | 3/3/ND         | 8.8/6.3/2.8     | 4.2/6.5/4.4     | 20-75           |
| LYM (%)          | 5.6/13.4/19.5   | 28.8/31.6/39.5  | 10.9/48.1/16.6  | 32.7/33/26.9    | 20.4/30/28.6    | 30.5/24/23      | 26/39/7/ND     | 8.6/19.8/40.7   | 15.9/8.3/66.3   | 20-40           |
| LYM#, 10^9/L     | 0.6/0.7/11      | 0.8/0.8/0.9     | 1.0/0.5/1.1     | 1.3/0.9/1       | 0.8/16/14       | 1.3/15.1/1.6    | 1.4/2.7/ND     | 0.9/16.2/8      | 0.9/6.1/22      | 08-40           |
| CD3+ T cells (%) | 25/66/76.7      | ND/76.3/76.2    | ND/ND/ND        | ND/ND/82.6      | ND/ND/74.7      | ND/ND/68.9      | ND/ND/76.5     | ND/74.3/81.8    | 80/ND/86.1      | 60.8-75        |
| CD3+CD4+ cells (%) | 15.5/45.6/52.5 | ND/36.3/39.1   | ND/ND/ND        | ND/ND/51.6      | ND/ND/35        | ND/ND/34.5      | ND/ND/29.7     | 27.4/52.8/51.7  | 51.3/ND/57      | 29.4-45.8      |
| CD3+CD8+ cells (%) | 8.9/15.8/22.5  | ND/31.5/32      | ND/ND/ND        | ND/ND/26.1      | ND/ND/35.2      | ND/ND/19.4      | ND/ND/38.8     | 12.5/20.7/22.2  | 26.1/ND/27      | 18-328         |
| CD4+CD8+ ratio   | 1.74/2.89/3.33  | ND/1.15/1.22    | ND/ND/ND        | ND/ND/1.98      | ND/ND/0.99      | ND/ND/1.78      | ND/ND/0.77     | 2.19/2.5/2.33   | 1.97/ND/2.2     | 105-2.5        |
| CRP, mg/L        | 111/92.4/21.1   | 1.8/2/1/0.7     | 84/5.4/ND       | 5.2/ND/21       | 3.3/2/2.53      | 1.2/4.7/ND      | 5.1/ND/ND      | 8.2/3.1/1.3     | 110/2.6/1.1     | 0-10            |
| PCT, ng/mL       | 0.09/0.03/0.08  | 0.02/0.11/0.04  | 0.24/0.1/0.12   | ND/0.12/ND      | 0.1/0.15/ND     | 0.4/0.3/ND      | ND/0.11/ND     | 0.3/0.08/0.05   | 0.14/0.07/0.08  | 0.05            |
| pCO2, mm Hg      | 25.9/ND/30.2    | 31.9/ND/ND      | 31.5/25.9/ND    | ND/ND/ND        | ND/ND/ND        | ND/ND/ND        | ND/ND/ND       | 29.7/39/40.2    | 26.6/28.7/35.9  | 35-45          |
| pO2, mm Hg       | 68.8/ND/74.4    | 127/ND/ND       | 153/88/ND       | ND/ND/ND        | ND/ND/ND        | ND/ND/ND        | ND/ND/ND       | 80/24.9/142     | 67.9/66.2/110   | 83-108          |
| sO2 (%)          | 95.4/ND/95.6    | 98.7/95.6       | 91.2/97.7/ND    | ND/ND/ND        | ND/ND/ND        | ND/ND/ND        | ND/ND/ND       | 95.6/45.4/99    | 95.8/93.5/98.6  | 93-980          |
| LDH, U/L         | 392/269/208     | 140/ND/122      | 230/204/ND      | ND/ND/ND        | ND/222/185      | 156/127/121     | 147/ND/ND      | 353/231/220     | 305/260/171     | 135-225         |
| Fib, g/L         | 4.85/ND/ND     | 2.98/3.39/ND    | 7.9/5.2/3.0     | 4.85/ND/ND      | 2.6/4.3/ND      | 3.5/ND/ND       | ND/2.8/ND      | 2.33/3.2/4/ND   | 6.04/ND/ND      | 18.4-0          |
| D-D, ug/mL       | 103/2.3/ND      | 0.33/0.28/ND    | 0.2/0.3/0.47    | 0.2/ND/ND       | 0.19/0.2/ND     | 0.6/ND/ND       | ND/0.3/ND      | 0.96/1/ND       | 0.62/ND/ND      | 0.05            |
| FOB              | WP/N/ND        | N/N/ND          | N/N/ND          | N/N/ND          | N/N/ND          | N/N/ND          | N/N/ND         | N/N/ND          | N/N/ND          | ...             |
| SARS-CoV-2       | P/N/N          | P/N/N           | P/N/N           | P/N/N           | P/N/N           | P/N/N           | P/N/N          | P/N/N           | P/N/N           | ...             |

Abbreviations: AT, after treatment (the day on which the patient was discharged); BT, before treatment; CRP, C-reactive protein; D-D, D-dimer; DT, during treatment (the day on which the virus test turned out to be negative for the first time); Fib, fibrinogen; FOB, feces occult blood; LDH, lactic dehydrogenase; LYM, lymphocyte; LYM#, absolute lymphocyte count; N, negative; ND, no data; NEUT, neutrophil; NEUT#, absolute neutrophil count; P, positive; pCO2, arterial partial pressure of carbon dioxide; PCT, procalcitonin; pO2, arterial partial pressure of oxygen; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; sO2, oxygen saturation; WBC, white-cell count; WP, weak positive.
relieve dyspnea in case 6#. The average period of treatment of nine patients who tested negative for SARS-CoV-2 for the first time was 6 days (range from 4 to 11). Based on the persistent negative results of SARS-CoV-2, the lung lesions partially or completely resolved, as well as the gradually improved clinical symptoms, nine patients were discharged. The average days of hospital stay were 14.2 (range from 9 to 20).

4 | DISCUSSION

Currently, a sharp increase in confirmed cases infected with SARS-CoV-2 have been reported in many countries around the world. Patients of COVID-19 with respiratory symptoms including fever, cough, chest tightness, and shortness of breath are viewed as the primary presenting manifestation. A small number of patients have digestive symptoms such as diarrhea and vomiting. In our nine patients, only two cases (1# with weak positive fecal occult blood and 2#) with diarrhea were treated with montmorillonite powder (3 g, three times a day) or trigeminy viable organism powder (1 g, three times a day) to arrest diarrhea or regulate intestinal flora. According to the Guideline for Diagnosis and Treatment of Novel Coronavirus-infected Pneumonia (Trial version 6), patients were clinically classified into four categories including slight, moderate, severe, and imminent types.7 In our study, there were four severely ill patients and five moderate patients. Notably, the average age of the former (52 years old, range from 47 to 56 years old) was much higher compared with that of the latter (34.2 years old, range from 14 to 53 years old). During and after treatment, although partial clinical data are not available, the improved lung lesions have a good consistency with the recovery of the laboratory characteristics including CRP, pCO2, and pO2. However, PCT as an indicator of severe bacterial, fungal, and parasitic infections did not change significantly.11 In some cases, the decreased ALC and the percentage of CD3+, CD3+CD4+, and CD3+CD8+ cells have been detected before treatment. However, these cells gradually returned to normal levels after viral clearance. Chest CT examination and the detection of SARS-CoV-2 RNA also play a vital role.10,12,13 The former is helpful for rapid clinical assessment
and taking effective segregating actions, while the latter has contributed to clinical diagnosis and specific drug therapy. Two male patients (cases 1# and 2#) have been observed with severe symptoms having had a history of smoking for more than 20 years. In addition, there were no deaths in nine patients during treatment, which was associated with the effective combination therapy of traditional Chinese and western medicine. After discharge, the patient should remain in contact isolation, and pay attention to rest more, nutrition, body temperature and the prevention of infection. The regular follow-up of blood routine examination, chest CT examination and the detection of SARS-CoV-2 is necessary.

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CONFLICT OF INTERESTS
The authors declare that there are no conflict of interests.

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