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How to Prevent Rehospitalization in Patients with COVID-19

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Abstract

Aim: False-negativity of reverse-transcriptase–polymerase-chain-reaction (RT-PCR) for patients with 2019 Novel Coronavirus (COVID-19) via throat swab was discussed.

Method: A total of 260 patients were diagnosed with COVID-19 RT-PCR for samples obtained via throat swab.

Result: Positive rates of chest Computed tomography (CT) imaging were 96.99% (252/260). Positive rates of RT-PCR were 87.70% for the first time, 92.30% for two times, and 100% for three times. Multiple RT-PCR assay tests resulted in a high positive rate of RT-PCR. Combined chest CT scans and RT-PCR lead to a positive rate of 100% even for the first testing, which is conducive to COVID-19 diagnosis. Two patients with COVID-19 were discharged from hospital with false-negative results of RT-PCR using the oropharyngeal swab. There was a significant progression on CT images and an increase in infective markers during their second admission to hospital, which indicated the progressing inflammation. Patients with complete recovery were selected. Symptoms were relieved; dissipation almost completely disappeared on CT image; and infective markers significantly decreased to normal levels, which indicated that the inflammation was not progressing. percent of lymphocyte percent (LYM) increased to normal level.

Conclusion: Serial follow-up chest CT scans are quite important for confirming the patients with COVID-19 who resulted negative for RT-PCR of 2019-nCoV nucleic acid. A combination of the RT-PCR test for 2019-nCoV nucleic acid and other detective methods, such as CT imaging are conducive to diagnosis. The roadmap of how to avoid being rehospitalized for patients with COVID-19 was provided. Patients were not allowed to be discharged from hospital even with negative result of RT-PCR of 2019-nCoV nucleic acid.

Keywords: False-negativity, RT-PCR; 2019-nCoV, Samples obtained via throat swab, Serial follow-up chest CT scans, Monitoring of infective markers

Abbreviations: 2019-nCoV: 2019 Novel Coronavirus; RT-PCR: Reverse-Transcriptase–Polymerase-Chain-Reaction; RNA: Ribonucleic Acid; COVID-19: Corona Virus Disease 2019; NCP: Novel Coronavirus Pneumonia; NCP: Novel Coronavirus Pneumonia; CFDA: the China Food and Drug Administration; CDC: the Centers for Disease Control; ARDS: Acute Respiratory Distress Syndrome; MOF: Multiple Organ Function Failure; ACE-2: Angiotensin-Converting Enzyme-2; BALF: Bronchoalveolar Lavage Fluid; WBC: White Blood Cell; NE: Neutrophile Granulocyte; CRP: C-Reactive Protein; PCT: Procalcitonin; CT: Computed Tomography

Introduction

Since December 2019, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) caused by 2019 Novel Coronavirus (2019-nCoV) has resulted in 89,000 cases of Corona Virus Disease 2019 (COVID-19), formerly known as Novel Coronavirus Pneumonia (NCP) in China, including 2,450 deaths [1]. In the meantime, the epidemic has spread to 25 other countries. On January 30, 2020, WHO declared that the outbreak of COVID-19 constitutes a Public Health Emergency of International Concern. Respiratory droplets and contact are considered as the

main routes of transmission.

According to the diagnostic criteria [2], the diagnosis of COVID-19 is confirmed by the positive result of a 2019-nCoV nucleic acid test of a swab sample, as the key indicator for isolation or hospitalizations. Reverse transcription-polymerase chain reaction (RT-PCR) tests are the most widely used method for nucleic acid assay. However, it is possible for samples obtained via throat swab to give false negative results of RT-PCR. Thus, suspected or affected patients should be isolated until they have received two consecutive 2019-nCoV nucleic acid negative results.

Chest CT is a typical, routine tool used for diagnosing COVID-19, which can be immediately performed. Dynamic changes on the serial follow-up chest CT scans have not been reported for the patients with positive RT-PCR tests that turned negative, especially for the patients with false negative results of RT-PCR. Serial follow-up chest CT scans with time-interval of 3 days or more are quite essential to confirm the patients with negative results of RT-PCR for 2019-nCoV nucleic acid, which is conducive to the diagnosis of COVID-19.

In the present study, we used RT-PCR as a reference standard against which we tested the performance of follow-up chest CT scan in diagnosing COVID-19. The dynamic conversion of RT-PCR results was studied and compared with the serial follow-up chest CT scans.

Materials and Methods

This is a retrospective study of patients who were confirmed for COVID-19 by the positive result of epidemiological history, symptoms, and signs (fever, cough, fatigue and/or shortness of breath), Serial follow-up chest CT images and/or RT-PCR or gene sequencing of 2019-nCoV nucleic acid. in 2020.

Patients

A total of 260 patients who were diagnosed with COVID-19, and were subsequently admitted to and discharged from our hospital, underwent both chest CT and RT-PCR tests for several times between January 21 and March 6, 2020. The repeated RT-PCR tests were conducted up to and including one day after the initial test was performed.

According to the latest guidelines of Diagnosis and Treatment of COVID-19 (trial seventh version) published by the China government, the diagnosing the infection of 2019-nCoV must be confirmed by the positive result of epidemiological history, symptoms and signs, chest CT images and/or RT-PCR or gene sequencing of 2019-nCoV nucleic acid. In addition, patients with COVID-19 could be discharged from hospital following the improvement of their clinical character and lesions on CT scan, and two consecutive negative results of 2019-nCoV nucleic acid.

RT-PCR assay

The RT-PCR assays were performed by using TaqMan One-Step RT-PCR Kits, which were approved by the China Food and Drug Administration (CFDA). Using viral nucleic acid extracted from oropharyngeal swab, the RT-PCR assay was carried out. To accurately determine the detection limits, we serially tested diluted positive control plasmids with a dynamic range of at least seven orders of magnitude (2 × 10–4-2000 TCID50/reaction) in these assays. In our preliminary trial, reactions with ≥ 10 copies of this positive control plasmids were consistently positive. The amplification efficiencies of RT-PCR assay were proven.

CT scan

All chest CT images were obtained on the CT system (Optima 660, GE, America or Somatom Definition AS+, Siemens Healthineers, Germany) with patients in the supine position and adopting the following parameters: tube voltage of 120 kVp, automatic tube current modulation between 30 and 70 mAs, a pitch between 0.99 and 1.22 mm, matrix with 512 × 512, slice thickness with 10 mm, and a field of view with 350 mm × 350 mm. Images were reconstructed with a slice thickness between 0.625 and 1.250 mm with the same increment.

Statistical analysis

All statistical analyses were performed using SPSS 21.0. Quantitative data were presented as the mean ± standard deviation (minimum-maximum), and the counting data were presented as the percentage of the total.

Result

The patients in our study were confirmed for COVID-19 by the positive result of epidemiological history, symptoms, and signs (fever, cough, fatigue and/or shortness of breath), chest CT images and/or RT-PCR or gene sequencing of 2019-nCoV nucleic acid. The positive rates of chest CT imaging in our cohort were 96.99% (252/260) for patients with positive and negative RT-PCR results. The typical characteristics found on chest CT in patients with COVID-19 include ground-glass 4 opacities, multifocal patchy consolidation, and/or interstitial changes with a peripheral distribution. If patients were initially positive or negative for RT-PCR, typical imaging features were observed on the chest CT scan before or within six days of the initial positive RT-PCR results, in all cases. Serial follow-up chest CT scans with a time-interval of three days or more, are crucial for confirming the patients with negative results of RT-PCR for 2019-nCoV nucleic acid, which is conducive to the diagnosis of COVID-19. In our study, follow-up chest CT scans showed improvement in 87.7% (228/260) cases; in 4.61% (12/260) cases, especially in 2 patients who were discharged from hospital with

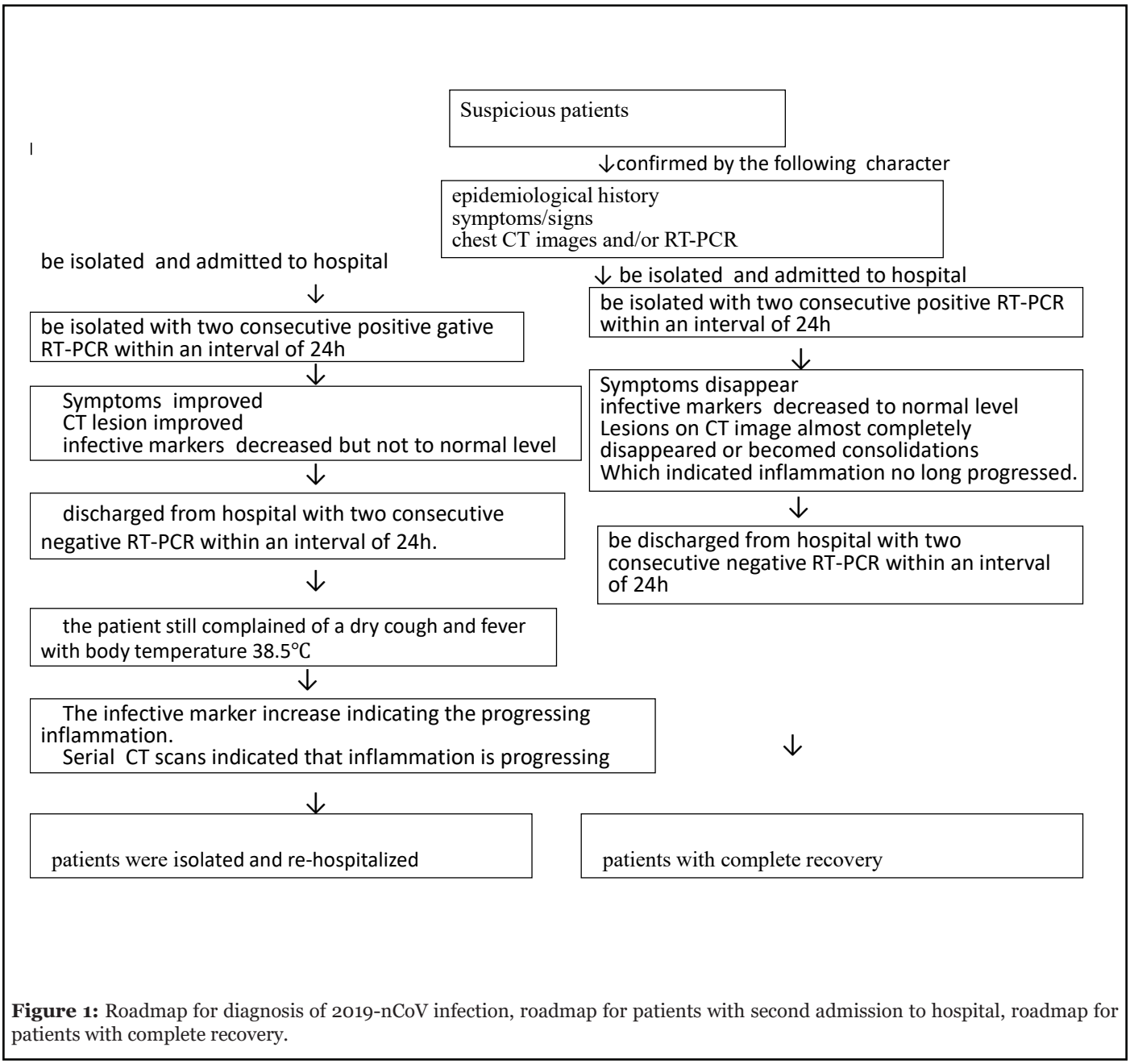

Figure 1: Roadmap for diagnosis of 2019-nCoV infection, roadmap for patients with second admission to hospital, roadmap for patients with complete recovery.

false-negative of RT-PCR, accentuations were observed; while in 7.69% (20/260) cases, there was no significant improvement. About 96.99% (252/260) cases had initial positive CT consistent with or parallel to the diagnosis of COVID-19 (Figure 1).

The RT-PCR test for 2019-nCoV nucleic acid with samples obtained via throat swab has become the standard method for diagnosis of COVID-19, which have many limitations such as high false negative rate. Among 260 patients in the present study, 87.70% (228/260) showed positive RT-PCR results for the first time, 92.30% (240/260) had positive RT-PCR results for two times, and 100% (260/260) had positive RT-PCR results for three times. Multiple RT-PCR assay tests may result in a high positive rate of RT-PCR. Combination of chest CT scans and RT-PCR lead to positive rate of 100% even for the first testing, which is conducive to COVID-19 diagnosis (Figure 2).

By analyzing serial RT-PCR assays and CT scans, the mean interval time between initial positive RT-PCR test and subsequent negative RT-PCR result was 10.6 ± 1.7 days with a range of 9–15 days (n =65) for 260 patients discharged from our hospital. For the two patients who were discharged from hospital with false-negative RT-PCR results, the mean interval time between the initial positive result and the negative result was 10 and 5 days and 4 and 9 days, respectively, during the second admission to our hospital (Figure 2).

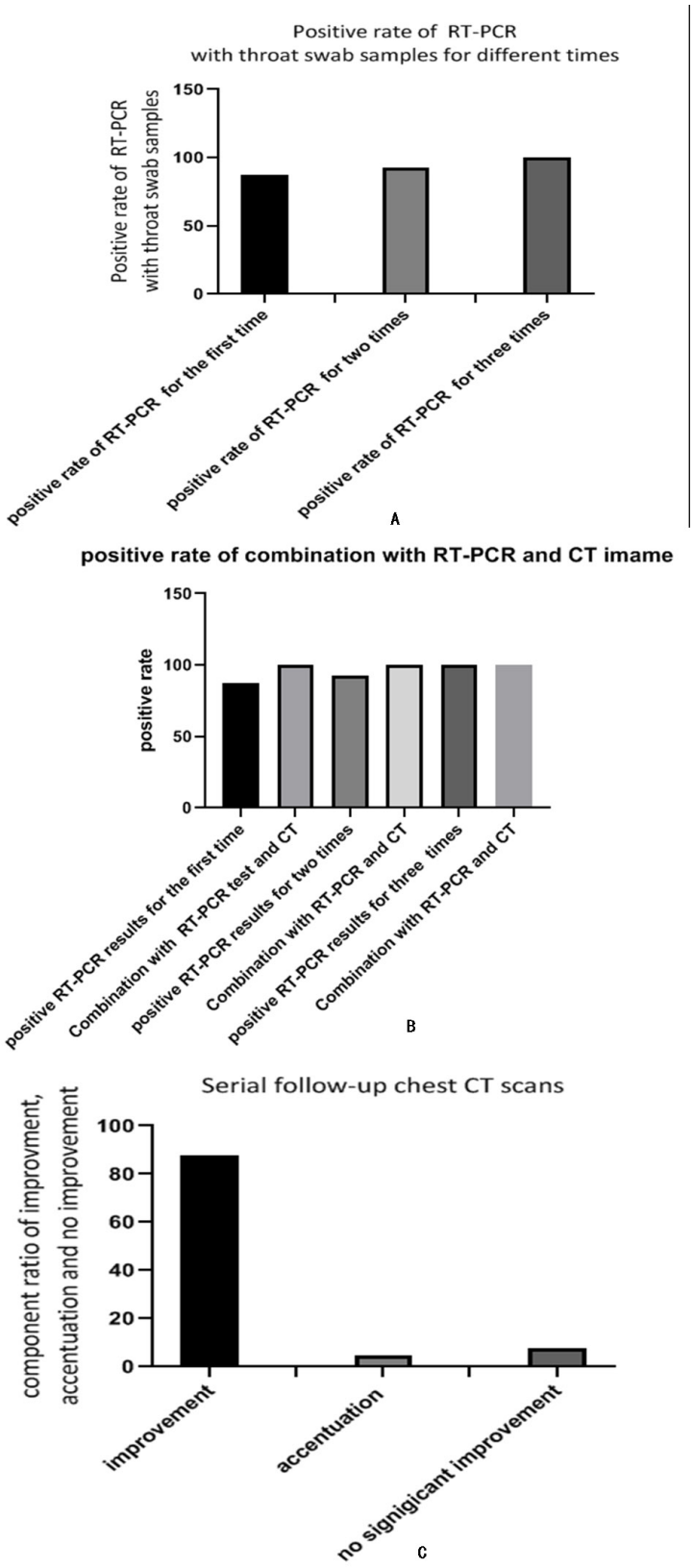

Figure 2: Combination of chest CT scans and RT-PCR led to positive rate of 100%. A. Positive rate of single/multiple RT-PCR assays with samples obtained via throat swab. 87.70% for the first testing, 92.30% for two times and 100% for three times. **B.** Positive rate of p chest CT. 96.99% (252/260) cases had initial positive CT. **C.** Combination of chest CT scans and RT-PCR led to a positive rate of 100%.

Case Presentation

Case 1

The patient suffered from slight nausea and discomfort for 5 days without vomiting, abdominal pain, diarrhea, cold, fever, and obvious cough on 17 January, 2020.

The epidemiological history: The patient`s cousin came back from Wuhan on 17 January, 2020. His aunt suffered from fever and was diagnosed with COVID-19 following positive test for RT-PCR of 2019-nCoV nucleic acid using the oropharyngeal swab test.

Past medical history: The patient was diagnosed with colorectal cancer, for which he was receiving chemo radiotherapy treatment.

Clinical examination and treatment: On admission, physical examination of the patient revealed normal vital signs. Lung auscultation revealed no rhonchi. The patient was negative for influenza A and B, parainfluenza, respiratory syncytial virus, adenovirus, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Rickettsia burnetii*, and *Legionella pneumophil*a. Chest CT showed double pulmonary ground-glass 4 opacities and multifocal patchy consolidation, which suggested viral pneumonia. He was positive for the RT-PCR assay of 2019-nCoV nucleic acid that was tested using the oropharyngeal swab by the Centers for Disease Control (CDC).

The patient was diagnosed with COVID-19 on February 6th, 2020 based on the epidemiological history, clinical characteristics of the respiratory tract, chest CT image, RT-PCR assay of 2019-nCoV nucleic acid tested using the oropharyngeal swab.

Medications such as oseltamivir, arbidol, lopinavir/ritonavir, and moxifloxacin were given as part of the treatment. Two weeks after being admitted to hospital, the symptoms and CT lesion improved and the body temperature was back to normal. RT-PCR of 2019-nCoV nucleic acid with the oropharyngeal swab tests was repeatedly performed for surveillance, which resulted in negative for two times during 24 hours interval. According to the criteria on discharge from the hospitals in China [2], the patient was discharged from hospital on January 29th.

After returning home, the patient still complained of a dry cough and fever with a high body temperature of 38.5°C, for which he was admitted to our hospital on February 23rd, 2020. The RT-PCR of 2019-nCoV nucleic acid with the oropharyngeal swab test resulted in positive for 2 consecutive times (more than 1 day apart). The patient was admitted to our department for confirmation of COVID-19. The patient was re-hospitalized for low fever and occasional cough with two consecutive positive RT-PCR tests within 24 hours interval (Figure 2).

One day later, his body temperature decreased to 37.3°C. One week after being admitted to hospital, the symptoms and CT lesions improved.

Serial CT scans (Figure 3 Left Panel): During the second admission to our hospital, follow-up chest CT scans showed more consolidation, ground-glass shadow, and nodules with absorbing and reducing area, which indicated that the inflammation was still progressing. The fifth scan (2020-02-27) showed consolidations with air-bronchogram inside with absorbing and reducing area. By this time, the inflammation was not progressing.

The infective marker such as procalcitonin (PCT), white blood cell number (WBC), and percent of neutrophils (NE %) increase during re-hospitalization were compared with those during the first admission to our hospital (Figure 5): WBC number and NE percent increased from 5.61 10E9/L to 8.19 10E9/L with the highest body temperature at 38.5°C. PCT (ng/ml) and CRP (mg/L) increased from 0.076 ng/ml and 19.63 mg/L to 0.304 ng/ml and 119.27 mg/L, respectively, which further confirmed that the inflammation was progressing.

After being admitted to hospital, the symptoms and CT lesion improved and the body temperature was back to normal. RT-PCR of 2019-nCoV nucleic acid using the oropharyngeal swab test was repeatedly performed for surveillance, resulting in negative for two consecutive times (more than one day apart). According to the discharge criteria of the hospitals in China, the patient was again discharged on March 1st.

The patients, in general, were not allowed to be discharged from hospital with the false-negative of RT-PCR of 2019-nCoV nucleic acid and the progressing inflammation for the first time of admission to hospital.

Case 2

The second patient suffered from fever for 1 day that reached the highest peak of 38.8°C on 23 January, 2020. He also experienced headache, chest tightness, chills, cough, stuffy and runny nose, muscle aches without nausea, vomiting, and slight pharyngitis. He came to the fever clinic at our hospital. He showed no obvious abnormalities during the physical examination with reference to liver function, renal function, myocardial enzymes, electrolyte, and serum procalcitonin. He was negative for influenza A and B, parainfluenza, respiratory syncytial virus, adenovirus, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Rickettsia burnetii*, and *Legionella pneumophila*. CT showed double pulmonary exudative lesions in multiple ground- glass samples, suggesting viral pneumonia.

The epidemiological history: The patient came from Wuhan on January 19. He was positive for the RT-PCR of

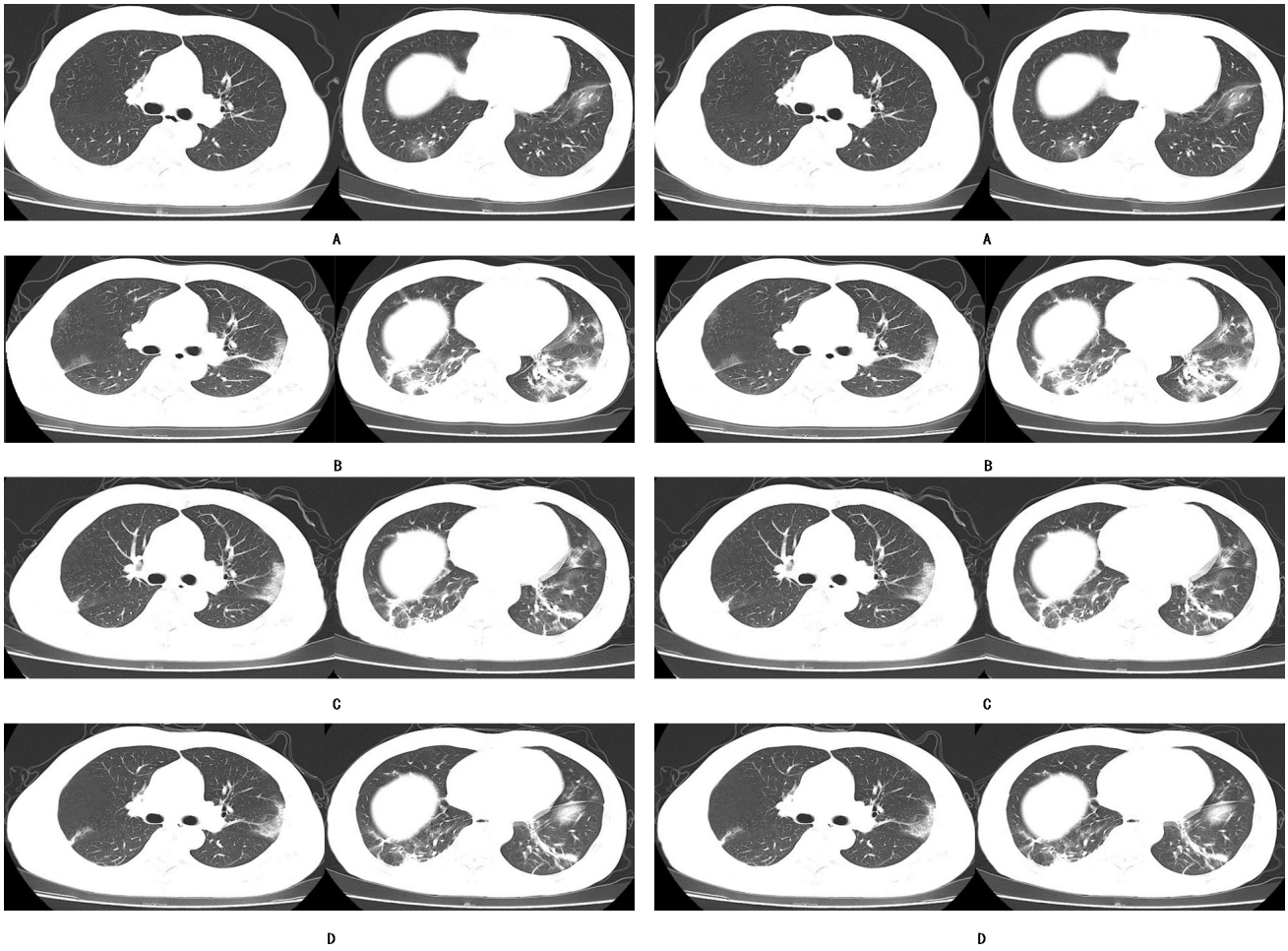

Figure 3: Serial follow-up chest CT scans of the re-hospitalized patients.

Left Panel: Serial follow-up chest CT scans of the first patient. Photo A. (2020-02-07 during the first admission to our hospital): there were scattered consolidation, ground-glass shadow and nodules in the upper and lower lobes of both the lungs(rapid progression stage). **Photo B**. (2020-02-13, during the first admission to our hospital): compared with those of 2020-02-07, there were more consolidation, ground-glass shadow and nodules in both lungs (rapid progression stage). **Photo C.** (2020-02-17, during the first admission admitted to our hospital): compared with those of 2020-02-13, there were more consolidation, ground-glass shadow and nodules with increasing density and area (consolidation stage). **Photo D.** (2020-02-24, during the second admission to our hospital): compared with those of 2020-02-17, there were consolidation, ground-glass shadow and nodules with absorbing and reducing area (consolidation stage). **Photo E.** (2020-02-27, during the second admission to our hospital): compared with those of 2020-02-24, there were consolidation, ground-glass shadow and nodules with absorbing and reducing density (dissipation stage). Photos A to D are early stage, progression stage, and consolidation stage, respectively, which indicated that the inflammation is still progressing. Photo E is dissipation stage. By this time, the inflammation is not progressing.

Right Panel: Serial follow-up chest CT scans of the second patient. Photo A. (2020-01-19, during the first admission to our hospital): there were multiple scattered, patchy, light consolidations and agglomerated ground-glass opacities (rapid progression stage). **Photo B.** (2020-02-04, during the second admission to our hospital): compared with those of 2020-01-19, there were multiple patchy consolidations in both lungs and grid-like thickness of interlobular septa (rapid progression stage). **Photo C.** (2020-02-08, during the second admission to our hospital): compared with those of 2020-02-04, there was a lot of consolidation with air-bronchogram inside with absorbing and reducing area (consolidation stage). **Photo D.** (2020-02-12, during the second admission to our hospital): compared with those of 2020-02-08, there was less consolidation, ground-glass shadow and nodules in both lungs with absorbing and reducing area (dissipation stage). Photos A to C are early stage, progression stage, and consolidation stage respectively, which indicated that the inflammation is still progressing. Photo D is dissipation stage. By this time, the inflammation was not progressing.

2019-nCoV nucleic acid, using the oropharyngeal swab test by the CDC.

Clinical examination and treatment: The patient was diagnosed with COVID-19 on February 6th, 2020 based on the epidemiological history, the clinical character of the respiratory tract, chest CT image, and RT-PCR assay of 2019-nCoV nucleic acid that was tested using the oropharyngeal swab.

Medications such as oseltamivir, arbidol, Lopinavir/ritonavir, and moxifloxacin were given as part of the treatment. One day later, the patient's body temperature decreased to 37.0°C. One week after being admitted to hospital, the symptoms and CT lesions improved. RT-PCR of 2019-nCoV nucleic acid using the oropharyngeal swab tests were repeatedly performed for surveillance, resulting negative for two consecutive times within 24 hours interval. According to the discharge criteria of the hospitals in China, the patient was discharged from hospital on January 29th.

After returning home, the patient still complained of low fever with an occasional cough. He was re-admitted to our hospital on January 31st with a high body temperature of 38.0°C. The RT-PCR of 2019-nCoV nucleic acid obtained using the oropharyngeal swab test was positive for 2 times during 24 hours interval. The patient was admitted to our department for confirmation of COVID-19.

The patient was re-hospitalized for low fever and occasional cough with two consecutive positive RT-PCR tests within 24 hours interval (Figures 1 and 2).

Serial CT scans (Figure 3 Right Panel): During the second admission to our hospital, follow-up chest CT scans showed multiple patchy consolidations in both lungs and grid-like thickness of interlobular septa (rapid progression stage), which indicated that the inflammation was still progressing. The fifth scan (2020-02-27) showed consolidations with air-bronchogram inside with absorbing and reducing area. By this time, the inflammation was not progressing.

Clinical examination

The infective markers such as PCT, WBC number, and NE percent were high during the rehospitalization (Figure 5), which indicated the progressing inflammation. The isolation was not recommended, and the patient was not allowed to be discharged from hospital with the false-negative of RT-PCR of 2019-nCoV nucleic acid.

After being admitted to hospital, the symptoms and CT lesion improved and the body temperature was back to normal. RT-PCR of 2019-nCoV nucleic acid using the oropharyngeal swab test was repeatedly performed for surveillance, resulting in negative for two consecutive times (more than one day apart).

The patients, in general, were not allowed to be discharged from hospital for the first time of admission to hospital with the false-negative of RT-PCR of 2019-nCoV nucleic acid and the progressing inflammation of chest CT scans and infective marker.

Patients with complete recovery on serial CT scans

To further study the relationship between the dynamics of the infective marker and serial CT scans, patients with complete recovery on serial CT scans were selected, who were matched for age, gender, and disease severity and were compared with the above patients.

First patient

The first patient was diagnosed with COVID-19 on January 22, 2020 based on the epidemiological history, the clinical character of the respiratory tract, chest CT image, and RT-PCR assay of 2019-nCoV nucleic acid obtained via the oropharyngeal swab. The patient came to our hospital with fever and discomfort for 5 days with a high temperature of 38.1°C, chills, cough, stuffy nose, slight pharyngitis, and muscle aches. There was a significant progression of symptoms and on CT image, and an increase of infective markers (WBC number, NE percent, temperature, and PCT), which indicated that the inflammation was progressing. The patient was negative for influenza A and B, parainfluenza, respiratory syncytial virus, adenovirus, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Rickettsia burnetii*, and *Legionella pneumophila*. The CT scan showed scattered consolidation, ground-glass shadow and nodules in the upper and lower lobes of the left lung, which suggested viral pneumonia.

The patient came from Wuhan on January 15, 2020. He tested positive for the RT-PCR of 2019-nCoV nucleic acid performed with the oropharyngeal swab test by the CDC for two consecutive times and was confirmed of COVID-19.

Medications such as oseltamivir, arbidol, lopinavir/ritonavir, and moxifloxacin were given as part of the treatment. One day later, the body temperature decreased to 37.5°C. After admission to hospital, the symptoms improved. Accentuations of CT lesion were observed, which indicated pathological changes in the progression stage. Twelve days after being admitted to hospital, the CT lesions improved. RT-PCR of 2019-nCoV nucleic acid using the oropharyngeal swab tests was repeatedly performed for surveillance, that resulted in negative for two times within 24 hours interval. According to the discharge criteria of the hospitals in China, the patient was discharged on February 2nd, 2021.

Clinical examination

On admission to our hospital, increase of infective markers

(WBC number, NE percent, LYM percent, CRP and PCT) were observed, which indicated that the inflammation was progressing (Figure 5).

After being discharged from hospital, the levels of infective markers returned to normal and never increased, which indicated that the inflammation was no longer progressing.

Serial CT scans (Figure 4 Left Panel)

Photo E (2020-03-07): There was less consolidation with increasing density (dissipation stage). By this time the inflammation was not progressing. **Photo F (2020-04-03):** Lesions on CT image almost completely disappeared.

The patient was allowed to be discharged from hospital with the negative result of RT-PCR of 2019-nCoV nucleic acid and no progressing inflammation.

Second patient

The second patient was diagnosed with COVID-19 on January 18, 2020 based on the clinical character of the respiratory tract, chest CT image, RT-PCR assay of 2019-nCoV nucleic acid obtained via the oropharyngeal swab and epidemiological history. There was a significant progression of symptoms, progression on CT image, and increase of infective markers such as WBC number, NE percent, LYM percent, temperature and PCT, which indicated that the inflammation was progressing. The patient was negative for influenza A and B, parainfluenza, respiratory syncytial virus, adenovirus, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *Rickettsia burnetii*, and *Legionella pneumophila*. CT showed scattered consolidation, ground-glass shadow, and nodules in the upper and lower lobes of the left lung, which suggested viral pneumonia.

The patient came from Wuhan on January 14, 2020. He tested positive for the RT-PCR of 2019-nCoV nucleic acid performed with the oropharyngeal swab test by the CDC for two consecutive times. The patient was confirmed to have COVID-19.

Medications such as oseltamivir, arbidol, lopinavir/ritonavir, and moxifloxacin were given as part of the treatment. After admission to hospital, the symptoms improved. Accentuations of CT lesion were observed during the 2 weeks, which indicated pathological changes in the progression stage. Soon after, the CT lesions improved. RT-PCR of 2019-nCoV nucleic acid using the oropharyngeal swab tests was repeatedly performed for surveillance, resulting negative for two times within 24 hours interval. According to the discharge criteria of the hospitals in China, the patient was discharged on February 2nd, 2021.

Clinical examination

On admission to our hospital, increase of infective markers (WBC number, NE percent, LYM percent, CRP and PCT) were observed, which indicated that the inflammation was progressing (Figure 5).

After being discharged from hospital, the levels of infective markers returned to normal and never increased, which indicated that the inflammation was no longer progressing.

Serial CT scans (Figure 4 Right Panel)

Photo D (2020-02-02): There was less consolidation with increasing density (Dissipation stage) and by that time the inflammation was not progressing. **Photo E (2020-02-06):** Lesions on CT image almost completely disappeared.

The patient was allowed to be discharged from hospital with the negative result of RT-PCR of 2019-nCoV nucleic acid and no progressing inflammation.

Discussion

COVID-19 disease caused by 2019-nCoV constitutes a Public Health Emergency [3]. It is transmitted by respiratory droplets and contact. COVID-19 might rapidly progress to acute respiratory distress syndrome (ARDS) and/or multiple organ function failure (MOF) with highly contagious transmission and mortality rates that reach as high as 5–10%. Patients infected with 2019-nCoV, asymptomatic carriers, and patients in the incubation period positive for 2019-nCoV nucleic acid by RT-PCR are the primary sources of infection [4]. According to the guidelines [2], patients with COVID-19 should be isolated until they have obtained two consecutive negative 2019-nCoV nucleic acid tests within an interval of at least 24-h. Yet, it is possible to obtain false-negative results of RT-PCR for 2019-nCoV nucleic acid using samples obtained via throat swab during first, second, and even third testing. In our study, the positive rate was 87.70% (228/260 during the first testing time, 92.30% (240/260) during the second time testing and 100% (260/260) during the third testing), which implies that many patients with COVID-19 have not been timely identified or isolated, which constitutes a risk for infecting a larger population given the highly contagious nature of the virus.

Regardless of whether patients initially had positive or negative results of RT-PCR, typical imaging features were observed in almost all cases; 96.99% (252/260) on the chest CT scan before or within 6 days of the positive RT-PCR results. Serial follow-up chest CT scans are quite important for confirming the patients with COVID-19 who resulted negative for RT-PCR of 2019-nCoV nucleic acid. A combination of the RT-PCR test for 2019-nCoV nucleic

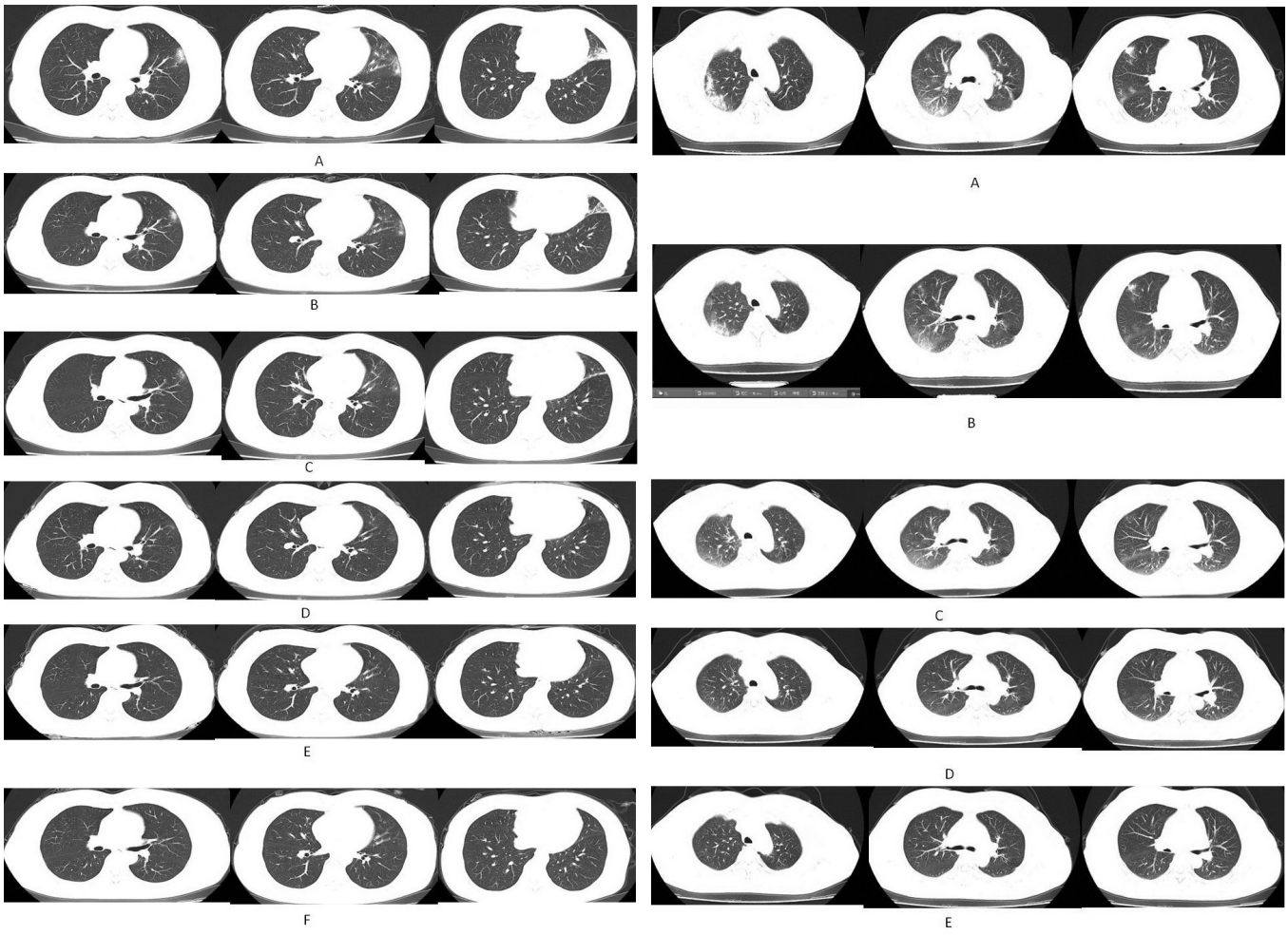

Figure 4: Serial follow-up chest CT scans of the patients with complete recovery.

Left Panel: chest CT scans of first patient in control group with complete recovery. Photo A. (2020-01-31, for the first patient in the control group with complete recovery): there were scattered consolidation in left lung (rapid progression stage). **Photo B.** (2020-02-03, for the first patient in the control group with complete recovery): compared with those of 2020-01-31, there were more consolidation in right lung (consolidation stage). **Photo C.** (2020-02-06 for the first patient in the control group with complete recovery): compared with those of 2020-02-03, there were more consolidation in left lung (consolidation stage). **Photo D.** (2020-02-17, for the first patient in the control group with complete recovery): compared with those of 2020-02-06, there were less consolidation with increasing density (dissipation stage). **Photo E.** (2020-03-07, for the first patient in the control group with complete recovery): Compared with those of 2020-02-17, there were less consolidation with increasing density (dissipation stage). Photos D and E show dissipation stage. By this time the inflammation was not progressing. **Photo F.** (2020-04-03, for the first patient in the control group with complete recovery): lesions on CT image almost completely disappeared.

Right Panel: Chest CT scans of second patient in control group with complete recovery. Photo A. (2020-01-22, for the second patient in the control group with complete recovery): there were scattered consolidation in both lungs (rapid progression stage). **Photo B.** (2020-01-27, for the second patient in the control group with complete recovery): compared with those of 2020-01-22, there were more consolidation in both lungs (consolidation stage). **Photo C**. (2020-01-30, for the second patient in the control group with complete recovery): compared with those of 2020-01-27, there were consolidation with increasing density and less area (dissipation stage). **Photo D.** (2020-02-02, for the second patient in the control group with complete recovery): compared with those of 2020-01-30 there were less consolidation with increasing density (dissipation stage). Photos C and D show dissipation stage. By this time, the inflammation was not progressing. **Photo E.** (2020-02-06, for the second patient in the control group with complete recovery): Compared with those of 2020-02-02, lesions on CT image almost completely disappeared.

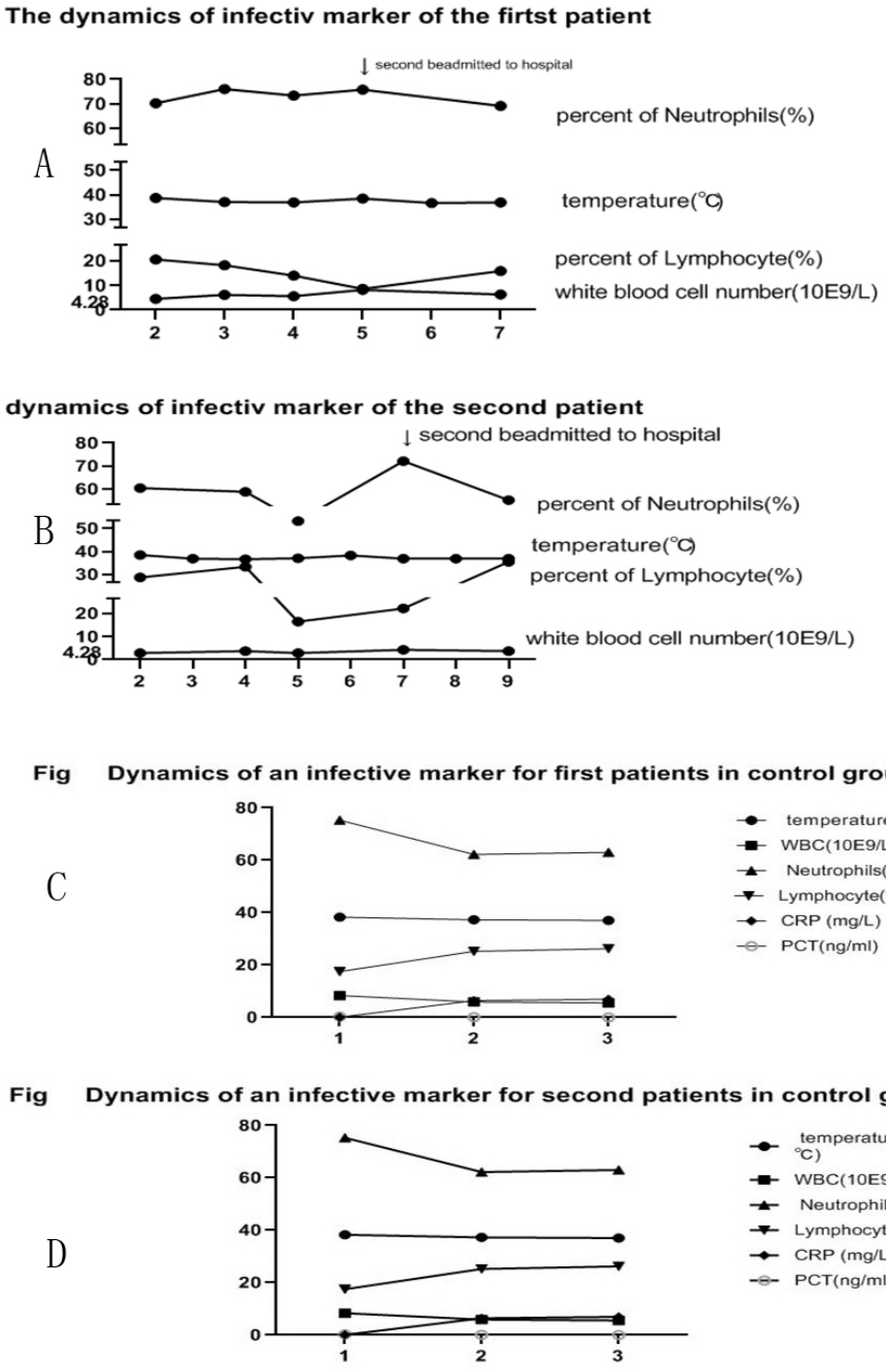

Figure 5: Dynamics of an infective marker. A. The first patient, who had a second admission to hospital. Increase of infective markers (WBC (white blood cell number (10E9/L), NE (percent of Neutrophils (%), LYM (percent of lymphocyte (%) and PCT (procalcitonin (ng/ml)) during the second admission to our hospital, which indicated that the inflammation was progressing low. **B.** The second patient, who had a second admission to hospital.

Dynamics of an infective marker for patients in control group with complete recovery. On admission to our hospital, increase of infective markers (WBC number, NE percent, LYM percent, CRP and PCT) were observed, which indicated that the inflammation was progressing. After being discharged from hospital, the levels of infective markers returned to normal and never increased, which indicated that the inflammation was no longer progressing. **C.** The first patient with complete recovery. **D.** The second patient with complete recovery.

acid and other detective methods such as CT imaging are conducive to diagnosis [5].

In our study, two patients with COVID-19 were discharged from hospital after receiving two consecutive negative results on RT-PCR testing of nCoV nucleic acid using the oropharyngeal swab within an interval of at least 24- h. Their symptoms and CT lesions improved, and their body temperature maintained normal level. According to the guidelines [2], patients were discharged from hospital. After returning home, the patients still complained of low fever with an occasional cough. Their RT-PCR test of 2019-nCoV nucleic acid obtained using the oropharyngeal swab resulted positive for two consecutive times, which indicated that patients could still transmit 2019-nCoV to other people. The period between the first discharge from hospital and the second admission to hospital was less than the incubation latent period of COVID-19, which indicated recurrence rather than reinfection of 2019-nCoV.

Re-admission and isolation of those patients were requested. There was a significant progression observed on CT images and an increase in infective markers during their second admission to hospital, which indicated the progressing inflammation. After being discharged from hospital for second time, the lesions on CT image improved significantly and levels of infective markers (WBC count, NE percent, temperature, PCT and CRP) returned to normal level and never increased, which indicated that the inflammation was not progressing. LYM percent increased to normal level, which indicated that CD4 and CD8 lymphocyte level increased to normal level. Immune recovery occurred, which was impaired by the infection of 2019-nCoV.

The period between the first discharge from hospital and the second admission to hospital was shorter than the latent incubation period of COVID-19, which indicated recurrence rather than reinfection of 2019-nCoV. The isolation was not an option, and patients could not be discharged from hospital for the false-negative of RT-PCR of 2019-nCoV nucleic acid obtained using the oropharyngeal swab.

To further study the relationship between the dynamics of the infective marker and serial CT scans, patients with complete recovery were selected, who were matched for age, gender and disease severity compared with the above patients. There was a significant progression of symptoms, progression on CT image, increase of infective markers (WBC number, NE percent, LYM percent, temperature, CRP and PCT) during admission, which indicated the progressing inflammation. After a period of therapy, symptoms were relieved; dissipation almost completely disappeared on CT image; infective markers significantly decreased to normal levels, which indicated that the inflammation was not progressing. LYM percent increased to normal level, which indicated that CD4 and CD8 lymphocyte level increased to normal level. Immune recovery occurred, which was impaired by the infection of 2019-nCoV. After being tested negative for RT-PCR of 2019-nCoV nucleic acid for two consecutive times (more than one day apart), the patients were subsequently discharged.

We assume that false-negative results of RT-PCR with the oropharyngeal swab were obtained because of the following reasons: the limitations of sample collection, transportation, and kit performance. The total positive rate of RT-PCR with samples obtained via throat swab was reported to be about 30% to 60% at the initial presentation, while the contagious period of COVID-19 has not yet been specified. 2019-nCoV nucleic acid from respiratory tract specimens may be persistent or recurrently positive during the course of COVID-19, even during recovery. Patients, asymptomatic carriers, and patients in convalescence positive for 2019-nCoV nucleic acid are all infectious and should remain in isolation until they have obtained two consecutive negative results on 2019-nCoV nucleic acid test [6,7].

In our study, two patients with COVID-19 were discharged from hospital with false-negative results of RT-PCR of 2019-nCoV nucleic acid testing performed using the oropharyngeal swab. Their CT images revealed significant progression and pulmonary fibrosis structural changes in the lungs, where there may be a lot of hidden 2019-nCoV. Angiotensin-converting enzyme-2 (ACE-2), identified as the cell entry receptor of 2019-nCoV, is highly expressed in the lungs rather than in the upper respiratory tract, such as oropharyngeal or nasopharyngeal tract. RT-PCR of 2019-nCoV nucleic acid may give false negatives when testing is performed by using the oropharyngeal swab, which can be overcome using the bronchoalveolar lavage fluid (BALF) specimen but with a higher exposure risk [7].

Regardless of patients' initial positive or negative results of RT-PCR, typical imaging features were observed in almost all cases; 96.99% (252/260) on the chest CT scan before or within 6 days of the positive RT-PCR results. Serial follow-up chest CT scans were carried out in our patients, which is conducive to the diagnosis of COVID-19. Combination with the RT-PCR test for 2019-nCoV nucleic acid and other detective methods such as CT imaging are also conducive to diagnosis [5]. Monitoring infective markers (WBC number, NE percent, LYM percent, temperature, CRP and PCT) indicate that serial follow-up chest CT scans are very important for the patients discharged from hospital with negative RT-PCR results for two consecutive times. All the discharged patients should be home-quarantined for at least 14 days and regularly tested.

Conclusion

Our results confirmed the roadmap for diagnosis of 2019-nCoV infection.

Moreover, the roadmap for patients with second admission to hospital with the false-negative of RT-PCR was confirmed, in which follow-up chest CT scans (a significant progression) and increase of infective markers showed that the inflammation was still progressing.

A combination of RT-PCR test with serial follow-up chest CT scans is conducive to COVID-19 diagnosis. Monitoring infective markers (WBC number, NE percent, LYM percent, temperature, CRP and PCT) and serial follow-up of chest CT scans are very important for the patients discharged from hospital with negative RT-PCR results for two consecutive times.

Conflict of Interest

All authors declare no conflict of interest.

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Declarations

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this paper.

Ethics Approval and Consent to Participate

The patient in the case report was available to consent. The case has been discussed with the most senior member of the staff in charge of the patient's care, who has provided consent for this, and consent was also obtained for the use of accompanying radiological images from the consultant radiologist. The study was reviewed and approved by the Hainan Provincial People's Hospital Institutional Review Board.

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Authors' Contributions

Shi Li and Chen Jie participated in the design of the study, carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript; Jiqing He contributed to revising the manuscript and performed the statistical analysis; Shi L and Chen J wrote the manuscript. All authors read and approved the final manuscript.

Data Availability Under Declaration Section

The accession numbers of any nucleic acid sequences or atomic coordinates cited in the manuscript can be found in GenBank of Medline.

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