RESEARCH ARTICLE

WILEY

# Clinical features of COVID-19 convalescent patients with re-positive nucleic acid detection

Hui Zhu[1,2] (iD) | Liyun Fu[2,3] | Yinhua Jin[4] | Jiale Shao[5] | Shun Zhang[1,2] | Nanhong Zheng[6] | Lingyan Fan[6] | Zhe Yu[5] | Jun Ying[5] | Yaoren Hu[2,3] | Tongen Chen[7] | Yanglingzi Chen[1,2] | Min Chen[1,2] | Mingjue Chen[1,2] | Zi Xiong[1,2] | Junfei Kang[1,2] | Jiachang Jin[1,2] | Ting Cai[1,2] | Honghua Ye[8]

[1]Department of Experimental Medical Science, HwaMei Hospital, University of Chinese Academy of Sciences, Ningbo, China

[2]Key Laboratory of Diagnosis and Treatment of Digestive System Tumors of Zhejiang Province, Ningbo, China

[3]Department of Hepatology, HwaMei Hospital, University of Chinese Academy of Sciences, Ningbo, China

[4]Department of Radiology, HwaMei Hospital, University of Chinese Academy of Sciences, Ningbo, China

[5]Department of Respiratory, HwaMei Hospital, University of Chinese Academy of Sciences, Ningbo, China

[6]Department of Acute Infection, HwaMei Hospital University of Chinese Academy of Sciences, Ningbo, China

[7]Department of General practice, HwaMei Hospital, University of Chinese Academy of Sciences, Ningbo, China

[8]Department of Cardiology, HwaMei Hospital, University of Chinese Academy of Sciences, Ningbo, China

**Correspondence**
Honghua Ye, Department of Cardiology, HwaMei Hospital, University of Chinese Academy of Sciences, 41 Xibei Street, Ningbo 315010, Zhejiang, China.
Email: lindayenbzjch@163.com

## Abstract

**Background:** Coronavirus disease 2019 (COVID-19) is a pandemic that has rapidly spread worldwide. Increasingly, confirmed patients being discharged according to the current diagnosis and treatment protocols, follow-up of convalescent patients is important to knowing about the outcome.

**Methods:** A retrospective study was performed among 98 convalescent patients with COVID-19 in a single medical center. The clinical features of patients during their hospitalization and 2-week postdischarge quarantine were collected.

**Results:** Among the 98 COVID-19 convalescent patients, 17 (17.3%) were detected positive severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleic acid during 2-week postdischarge quarantine. The median time from discharge to SARS-CoV-2 nucleic acid re-positive was 4 days (IQR, 3-8.5).The median time from symptoms onset to final respiratory SARS-CoV-2 detection of negative result was significantly longer in re-positive group (34 days [IQR, 29.5-42.5]) than in non-re-positive group (19 days [IQR, 16-26]). On the other hand, the levels of CD3-CD56 + NK cells during hospitalization and 2-week postdischarge were higher in re-positive group than in non-re-positive group (repeated measures ANOVA, $P$ = .018). However, only one case in

re-positive group showed exudative lesion recurrence in pulmonary computed tomography (CT) with recurred symptoms.

**Conclusion:** It is still possible for convalescent patients to show positive for SARS-CoV-2 nucleic acid detection, but most of the re-positive patients showed no deterioration in pulmonary CT findings. Continuous quarantine and close follow-up for convalescent patients are necessary to prevent possible relapse and spread of the disease to some extent.

**KEYWORDS**

convalescent patients, COVID-19, SARS-CoV-2

## 1 | INTRODUCTION

A novel coronavirus, designated as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses on February 11, 2020.[1] Coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 was declared a pandemic by World Health Organization.[2] As of April 10, 2020, China had reported 83 307 cases with 3346 deaths.[3] Internationally, by the same time, 1 439 516 confirmed cases of COVID-19 had been reported in approximately 212 countries and territories with more than 85 711 deaths.[3]

Despite the large number of infected cases and the wide geographical spread of the disease, the COVID-19 has been gradually and effectively controlled by quarantine of suspected cases, analysis of epidemiology, and improvement of diagnosis and treatment in China.[4] In particular, the current use of broad-spectrum antiviral drugs such as lopinavir-ritonavir, arbidol hydrochloride, and chloroquine has achieved some desirable outcomes.[5-7] Recently, over ninety percent (77862/83307, 93.5%) of confirmed COVID-19 patients had met the criteria for hospital discharge or discontinuation of quarantine in China according to the latest diagnosis and treatment protocols from the National Health Commission of the People's Republic of China.[8]

While previous reports on COVID-19 primarily focused on epidemiological and clinical characteristics of confirmed cases,[9,10] this study was conducted to retrospectively investigate the clinical features and inflammation and immune biomarkers of COVID-19 convalescent patients with re-positive SARS-CoV-2 nucleic acid detection.

## 2 | MATERIALS AND METHODS

### 2.1 | Data sources

A retrospective study was performed on convalescent patients with COVID-19 who were treated in HwaMei Hospital, University of Chinese Academy of Sciences, Zhejiang, China. By April 2, 2020, epidemiological and clinical data were collected on 98 convalescent patients consisting of 17 cases with re-positive results to detection of respiratory SARS-CoV-2 during postdischarge quarantine and 81 cases without. Hence, we divided the convalescent patients into re-positive group and non-re-positive group.

Patients were diagnosed based on the interim guidance from the World Health Organization.[11] COVID-19 was confirmed by real-time reverse-transcriptase polymerase-chain-reaction (RT-PCR) assay for sputum or nasopharyngeal swab specimens.[12] Primers targeting open reading frame (ORF) 1a/1b and nuclear gene were used. Patient with cycle threshold (Ct) values less than or equal to 40 was considered positive.

Epidemiological characteristics, clinical symptoms and signs, laboratory findings, chest computed tomography (CT) assessments, antiviral treatment, and outcome data were extracted from electronic medical records. Epidemiological exposure was defined as a history of traveling to or residing in Wuhan city or the areas surrounding Wuhan, or a history of having contact with confirmed COVID-19 patients or patients with fever or respiratory symptoms from Wuhan city and its surrounding areas within 14 days before onset of the disease. The cluster events, such as the one when people collectively prayed in Tiantong temple on January 19, 2020, were also investigated.[13] Pulmonary CT scanning was conducted in all patients on admission. Additionally, repeat CT scans were performed every 5 days or in case of deterioration during hospitalization. The CT imaging was analyzed by two experienced radiologists. Primary parameters for CT imaging include lesion distribution, single or multiple lesions within each lobe, lesion density and interstitial pulmonary fibrosis.

Sputum and nasopharyngeal swab specimens were collected from all patients on admission, and confirmation testing for SARS-CoV-2 RNA was performed at Ningbo Municipal Centers for Disease Control (CDC) of Zhejiang Province or HwaMei Hospital, University of Chinese Academy of Sciences following the standard protocol.[12] Laboratory testing includes blood count, liver and kidney function, high-sensitivity C-reactive protein (hs-CRP), procalcitonin, creatine kinase, lactic dehydrogenase (LDH), D-dimer, electrolytes, and so on.

Activated T cells and Th1/Th2 cytokines during hospitalization and 2-week postdischarge were collected. These immunization and inflammatory indicators at baseline, the second week of hospitalization, as well as at the third day, the first week and second week after discharge from hospital were analyzed.

We analyzed the temporal changes in total serum IgM and IgG specific for SARS-CoV-2 using COVID-19 Antibody (IgM/IgG) Combined Test Kit (Medical System Biotechnology Co., Ltd) and 2019-nCoV Antibody Test Kit (Innovita Biotechnology Co., Ltd). Above two kits were used for qualitative determination of specific antibodies by the latex agglutination ethod and the colloidal gold method, respectively.

Patients have to meet the following criteria for hospital discharge: (a) temperature returned to normal for more than 3 days, (b) respiratory symptoms are relieved or resolved, (c) pulmonary computed tomography (CT) images show significant improvement in acute exudative lesions, and (d)two consecutive negative detections of respiratory SARS-CoV-2 (sample collection interval of at least 1 day).[14]

## 2.2 | Ethical approval

This study was approved by the Ethics Committee of HwaMei Hospital, University of Chinese Academy of Sciences. Written informed consents were obtained from all subjects. The procedures followed were in accordance with the ethical standards of the Helsinki Declaration.

## 2.3 | Statistical analysis

Quantitative variables were presented as means ± standard deviation (SD) or median and interquartile range (IQR), and the differences between groups were evaluated with unpaired Student's $t$ test or the Mann-Whitney $U$ test. Categorical variables were presented as absolute frequencies (n) and relative frequencies (%), and chi-square tests or Fisher's exact test was used for categorical variables. The ANOVA test with Greenhouse-Geisser correction was conducted to analyze repeated measures. All analyses were made by IBM SPSS statistics version 24.0.

# 3 | RESULT

## 3.1 | Demographic and clinical characteristics

The epidemiological and clinical data were presented in Table 1. The median age of the 98 patients was 52 years (IQR, 37.8-59), and 55 (56.1%) patients were aged 50 years or older. There were 66 female patients (67.3%), with none of them pregnant. Among the 98 patients, 86 (87.8%) patients had contact with someone with confirmed or suspected SARS-CoV-2 infection. Most of the 98 patients had no underlying disease, whereas 18 (18.4%) patients had hypertension, 8 (8.2%) diabetes, 3 (3.1%) coronary heart disease, and 3 (3.1%) chronic hepatitis B.

At onset of the illness, fever (75.5%) and cough (53.1%) were the most common symptoms. Other symptoms included chest pain and stuffiness (7.1%), nasal congestion (6.1%), fatigue (16.3%), and diarrhea (8.2%). The temperature on admission was 37.1°C (IQR,

36.8-37.7), and the maximum temperature during hospitalization was 37.9°C (IQR 37.4-38.4).

In the re-positive group, the median age was 54 years (IQR, 44-63), and 70.6% were females (Table 1). Less than 20% of re-positive group patients had underlying disease including hypertension (2 [11.8%]) and diabetes (1 [5.9%]). There was no significant difference of initial clinical symptoms between re-positive group and non-re-positive group.

## 3.2 | Chest CT features

Chest computed tomography (CT) scan on admission showed that the lesions were more apparent in the peripheral zone of lungs (73/98, 74.5%) (Table 2). Most of the patients presented blurred edge (86/98, 87.8%) and solid or mixed density (80/98, 81.6%) for lesions. Seventy (70/98, 71.4%) patients had maximum diameter of the lesion >3 cm and 16 (16/98, 16.3%) patients between 1 and 3 cm. Seventy-seven (77/98, 78.6%) patients had more than or equal to three pulmonary lesions. There was no statistically significant difference of imaging signs on admission between the two groups.

Patients who met criteria for hospital discharge or discontinuation of quarantine showed improvement in acute exudative lesions on chest CT images (Table 2). Pulmonary CT scan at discharge indicated that 54.3% (44/81) of patients in non-re-positive group, as opposed to only 29.4% (5/17) of patients in re-positive group ($P$ = .029) presented the absorption rate for acute exudative lesions greater than or equal to 50%. Non-re-positive group showed a better absorption for acute exudative lesions than positive group at discharge.

It is worth mentioning that the chest CT of one case in re-positive group presented recurrent symptoms with blurred image in the upper lobe of both lungs, more prominent on the left side during the convalescent period, but the severity of image is less than that of late period of hospitalization (Figure 1).

## 3.3 | Laboratory findings and immunological indicators

On admission, 25.5%, 34.7%, and 23.5% of cases showed leucopenia (white blood cell count <4 × $10^9$/L), lymphopenia (lymphocyte count <1.0 × $10^9$/L), and platelet suppression (blood platelet count <150 × $10^9$/L), respectively (Table 3). Re-positive group presented elevated white blood cell count compared with non-re-positive group ($P$ = .038). Meanwhile, more than half of the 98 cases showed elevated levels of hs-CRP. However, increased levels of LDH (28.6%), procalcitonin(2%), aspartate aminotransferase (AST) (10.2%), alanine aminotransferase (ALT) (15.3%), total bilirubin (TBIL) (14.3%), creatine kinase (5.1%), creatinine (9.2%), D-dimer (5.1%) were observed in a minority of the 98 convalescent patients, and no significant differences were discovered between re-positive and non-re-positive group in these blood indicators at baseline.

**TABLE 1** Demographics characteristics of convalescent patients

| Characteristics | All patients (n = 98) | Non-re-positive group (n = 81) | Re-positive group (n = 17) | P |
|---|---|---|---|---|
| Age (years) | | | | |
| Median, interquartile range | 52 (37.8-59) | 51.0 (36.5-59) | 54.0 (44.0-63.0) | .134 |
| ≥50 y | 55 (56.1) | 45 (55.6) | 10 (58.8) | 1.000 |
| <50 y | 43 (43.9) | 36 (44.4) | 7 (41.2) | |
| Gender (%) | | | | |
| Male | 32 (32.7) | 27 (33.3) | 5 (29.4) | .787 |
| Female | 66 (67.3) | 54 (66.7) | 12 (70.6) | |
| BMI (kg/m$^2$) | | | | |
| Median, interquartile range | 23.7 (21.8-26) | 23.8 (21.8-26.0) | 23.4 (21.9-26.8) | .434 |
| >28 | 14 (14.3) | 11 (13.4) | 3 (17.6) | .649 |
| 24-28 | 29 (29.6) | 25 (30.9) | 4 (23.5) | |
| 18.5-24 | 51 (52.0) | 42 (51.9) | 9 (52.9) | |
| <18.5 | 3 (3.1) | 2 (2.5) | 1 (5.9) | |
| Temperature on admission (°C) | | | | |
| Median, interquartile range | 37.1 (36.8-37.7) | 37.1 (36.8-37.8) | 36.8 (36.7-37.2) | .039 |
| Maximum temperature during hospitalization (°C) | | | | |
| Median, interquartile range | 37.9 (37.4-38.4) | 37.9 (37.4-38.4) | 37.8 (37.3-38.3) | .003 |
| Symptom | | | | |
| Fever (%) | | | | |
| Yes | 74 (75.5) | 62 (76.5) | 12 (70.6) | .757 |
| No | 24 (24.5) | 19 (23.5) | 5 (29.4) | |
| Nasal congestion (%) | | | | |
| Yes | 6 (6.1) | 6 (7.4) | 0 (0) | .586 |
| No | 92 (93.9) | 75 (92.6) | 17 (100) | |
| Cough (%) | | | | |
| Yes | 52 (53.1) | 44 (54.3) | 8 (47.1) | .605 |
| No | 46 (46.9) | 37 (45.7) | 9 (52.9) | |
| Fatigue (%) | | | | |
| Yes | 16 (16.3) | 12 (14.8) | 4 (23.5) | .469 |
| No | 82 (83.7) | 69 (85.2) | 13 (76.5) | |
| Chest pain and stuffiness (%) | | | | |
| Yes | 7 (7.1) | 5 (6.2) | 2 (11.8) | .601 |
| No | 91 (92.9) | 76 (93.8) | 15 (88.2) | |
| Diarrhea (%) | | | | |
| Yes | 8 (8.2) | 7 (8.6) | 1 (5.9) | 1.000 |
| No | 90 (91.8) | 74 (91.4) | 16 (94.1) | |
| Chronic hepatitis B (%) | | | | |
| Yes | 3 (3.1) | 3 (3.7) | 0 (0) | 1.000 |
| No | 95 (96.9) | 78 (96.3) | 17 (100) | |
| Coronary heart disease (%) | | | | |
| Yes | 3 (3.1) | 3 (3.7) | 0 (0) | 1.000 |
| No | 95 (96.9) | 78 (96.3) | 17 (100) | |
| Hypertension (%) | | | | |
| Yes | 18 (18.4) | 16 (19.8) | 2 (11.8) | .731 |
| No | 80 (81.6) | 65 (80.2) | 15 (88.2) | |

(Continues)

**TABLE 1** (Continued)

| Characteristics | All patients (n = 98) | Non-re-positive group (n = 81) | Re-positive group (n = 17) | P |
|---|---|---|---|---|
| Diabetes (%) | | | | |
| Yes | 8 (8.2) | 7 (8.6) | 1 (5.9) | 1.000 |
| No | 90 (91.8) | 74 (91.4) | 16 (94.1) | |
| Smoking (%) | | | | |
| Yes | 8 (8.2) | 8 (9.9) | 0 (0) | .344 |
| No | 90 (91.8) | 73 (90.1) | 17 (100) | |
| History of epidemiology (%) | | | | |
| Contacted with confirmed cases | 86 (87.8) | 71 (87.7) | 15 (88.2) | 1.000 |
| Undiagnosed infection | 12 (12.2) | 10 (12.3) | 2 (11.8) | |

Note: Data are presented as median and interquartile range (IQR) and n (%).

P values comparing non-re-positive cases and re-positives cases are from Mann-Whitney U test, chi-squared test, and Fisher's exact test.

Abbreviation: BMI, body mass index.

**TABLE 2** Chest CT scan of convalescent patients at discharge

| Chest CT | All patients (n = 98) [a] | Non-re-positive group (n = 81) | Re-positive group (n = 17) | P* |
|---|---|---|---|---|
| Distribution within lobes (%) | | | | |
| Peripheral | 73 (74.5) | 60 (74.1) | 13 (76.5) | 1.000 |
| Peripheral + Central | 19 (19.4) | 15 (18.5) | 4 (23.5) | |
| The maximum diameter of the lesion (%) | | | | |
| <1 cm | 6 (6.1) | 4 (4.9) | 2 (11.8) | .209 |
| 1-3 cm | 16 (16.3) | 12 (14.8) | 4 (23.5) | |
| >3 cm | 70 (71.4) | 59 (72.8) | 11 (64.7) | |
| The number of pulmonary lesions (%) | | | | |
| <3 | 15 (15.3) | 12 (14.8) | 3 (17.6) | 1.000 |
| ≥3 | 77 (78.6) | 63 (77.8) | 14 (82.4) | |
| The edge of lesion (%) | | | | |
| Clear edges | 6 (6.1) | 3 (3.7) | 3 (17.6) | .130 |
| Blurred edges | 86 (87.8) | 72 (88.9) | 14 (82.4) | |
| The density of lesion (%) | | | | |
| Ground glass opacity | 12 (12.2) | 9 (11.1) | 3 (17.6) | .822 |
| Mixed ground glass opacity and consolidation | 80 (81.6) | 66 (81.5) | 14 (82.4) | |
| Outcome (%) | | | | |
| Absorption rate <50% | 43 (43.9) | 31 (38.3) | 12 (70.6) | .029 |
| Absorption rate ≥50% | 49 (50) | 44 (54.3) | 5 (29.4) | |

Abbreviation: CT, computed tomography.

[a]Among the 98 convalescent patients, the number of patients with available image data was 92, so percentages do not total 100% owing to missing data.

*Fisher exact probability method.

The changes of T lymphocyte subsets and Th1/Th2 cytokines during hospitalization and after discharge in re-positive group and non-re-positive cases were compared (Table S1 and Table S2). The levels of CD3-CD56 + NK cells at different time during hospitalization and follow-up were overall higher in positive group than in non-positive group (repeated measures ANOVA, P = .018) (Figure 2 and Table S3). The measures of cytokines at different times found that IL-4, IL-6, IL-10, and TNF-a increased on admission and declined during recovery. However, there was no statistically significant difference between re-positive group and non-re-positive group (Table S4).
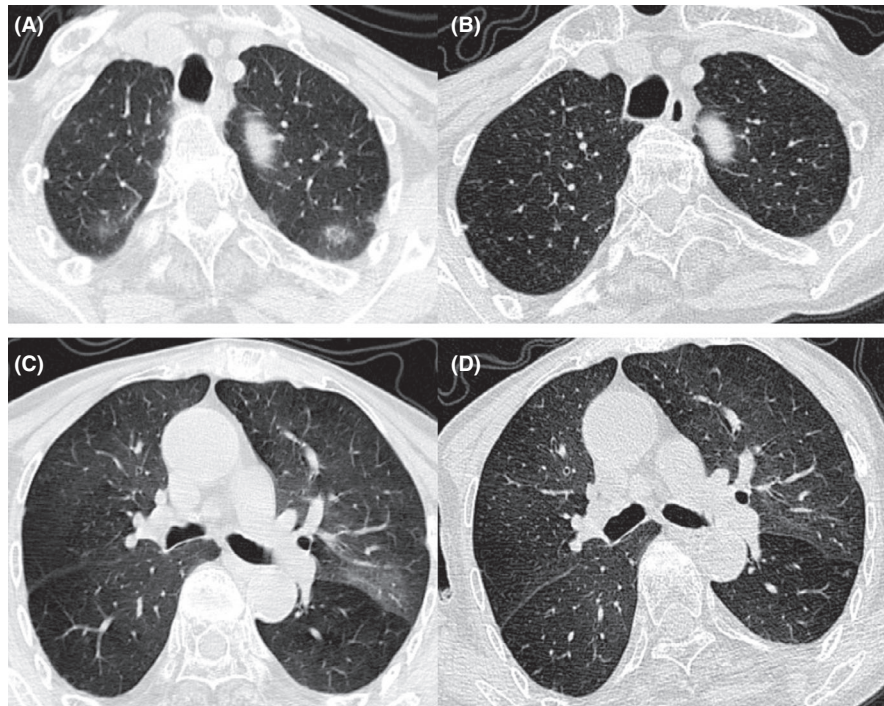
**FIGURE 1** Chest CT findings of a 77-y-old woman with COVID-19 pneumonia. The patient was hospitalized on February 2, 2020, after 3 d fever. Body temperature was normal and no other respiratory symptoms were observed during hospitalization. Positive RT-PCR result of SARS-Cov-2 for sputum and pharyngeal swab specimens was first detected on February 5, 2020. A, Chest computed tomography (CT) on admission showed bilateral scattered patchy lesions under the pleura (February 2, 2020). B, After 7 d treatment, chest CT images showed substantial absorption of acute exudative lesions (February 9, 2020). Meanwhile, consecutive RT-PCR tests of respiratory SARS-Cov-2 were negative on both February 9 and February 12, 2020. The patient was in postdischarge quarantine for further medical observation from February 12. C, Fever reappeared on February 24, 2020, and CT images demonstrated patchy lesions in upper lobe of bilateral lungs, especially in the left lung. RT-PCR tests of SARS-Cov-2 for sputum and pharyngeal swab specimens were suspected positive on February 26, 2020, and the patient was re-admitted for treatment (D) CT scan on February 28, 2020, showed lesions at upper lobe of bilateral lungs were almost absorbed. RT-PCR tests for sputum and pharyngeal swab specimens were negative on February 29, 2020

## 3.4 | Treatment and outcomes

All patients received antiviral therapy during hospitalization, and the median time from onset of symptoms to antiviral therapy was 4 days (2-8.75 days). Of the 98 patients, 71 (72.4%) patients were given chloroquine phosphate treatment, 96 (98%) lopinavir-ritonavir treatment, and 93 (94.9%) arbidol hydrochloride treatment (Table 4).

Overall, the length of hospital stay was 15 days at median (IQR, 13-19), and no significant differences were discovered between the two groups in the length of hospital stay. Among 17 cases with re-positive RT-PCR test results, the median time from discharge to nucleic acid re-positive was 4 days (IQR, 3-8.5), and notably, one of the cases had re-positive results 17 days after discharge. The time from onset of symptoms to final negative detection of respiratory SARS-CoV-2 was 21 days (IQR, 17-28), and the time was significantly longer in re-positive group (34 days [IQR, 29.5-42.5]) than in non-re-positive group (19 days [IQR, 16-26]).

Temporal changes of SARS-CoV-2–specific IgM and IgG antibodies were detected in 62 convalescent patients (Table 4). The results revealed that both antibodies remained continuously positive in 13 (13.3%) convalescent patients, while continuously negative in 3 (3.1%). Meanwhile, the specific IgG antibodies were presented in 46 (46.9%) convalescent patients and persisted for 4 weeks postdischarge, but specific IgM antibodies changed to negative during follow-up period. The time from onset of clinical symptoms to IgM antibody disappeared was 46 days at median (IQR, 40.5-50). No significant differences in dynamics of specific antibodies were observed between the re-positive group and non-re-positive group groups.

## 4 | DISCUSSION

Lan et al[14] first reported the positive results to detection of COVID-19 nucleic acid in convalescent patients after meeting the criteria for hospital discharge or discontinuation of quarantine. According to our data, 17 (17.3%) discharged cases who met the criteria for hospital discharge or discontinuation of quarantine in China presented positive results to detection of SARS-CoV-2. The median time from discharge to nucleic acid positive was 4 days (IQR, 3-8.5). Of note, one of the cases had positive RT-PCR test results 17 days after discharge.

**TABLE 3** Laboratory findings of convalescent patients

| Characteristics | Group | All patients (n = 98) | Non-re-positive patients (n = 81) | Re-positive patients (n = 17) | P |
|---|---|---|---|---|---|
| White blood cell count, ×10⁹/L | | 4.8 (3.9-6) | 4.8 (3.9-5.9) | 5.3 (4.1-7.0) | .206 |
| | <4 | 25 (25.5) | 22 (27.2) | 3 (17.6) | .038 |
| | 4-10 | 71 (72.4) | 59 (72.8) | 12 (70.6) | |
| | >10 | 2 (2.1) | 0 (0) | 2 (11.8) | |
| Lymphocyte count, ×10⁹/L | | 1.2 (0.9-1.5) | 1.1 (0.9-1.5) | 1.2 (0.9-1.7) | .362 |
| | <1 | 34 (34.7) | 30 (37.0) | 4 (23.5) | .403 |
| | >1 | 64 (65.3) | 51 (63.0) | 13 (76.5) | |
| Platelet count, ×10⁹/L | | 197.5 (151.5-246.8) | 198 (149.5-244.5) | 177 (158-278.5) | .768 |
| | <150 | 23 (23.5) | 20 (24.7) | 3 (17.6) | .755 |
| | >150 | 75 (76.5) | 61 (75.3) | 14 (82.4) | |
| Hemoglobin, g/L | | 132 (123.8-141) | 132 (121.5-141) | 133 (127-142) | .694 |
| Hypersensitive C–reactive protein, mg/L | | 9.6 (2.7-26.5) | 9.6 (2.7-28.6) | 7.1 (1.9-16.1) | .426 |
| | <6 | 37 (37.8) | 29 (35.8) | 8 (47.1) | .407 |
| | >6 | 56 (57.1) | 48 (59.3) | 8 (47.1) | |
| Procalcitonin, ng/mL | <0.5 | 94 (95.9) | 78 (96.3) | 16 (94.1) | 1.000 |
| | >0.5 | 2 (2.0) | 2 (2.5) | 0 (0) | |
| Lactose dehydrogenase, U/L | | 219 (174.8-262) | 219 (174-270) | 219 (174.5-249.5) | .715 |
| | <250 | 70 (71.4) | 57 (70.4) | 13 (76.5) | .771 |
| | >250 | 28 (28.6) | 24 (29.6) | 4 (23.5) | |
| Aspartate aminotransferase, U/L | | 23 (17.8-29) | 24 (19-31) | 18 (15-24) | .023 |
| | <40 | 88 (89.8) | 72 (88.9) | 16 (94.1) | 1.000 |
| | >40 | 10 (10.2) | 9 (11.1) | 1 (5.9) | |
| Alanine aminotransferase, U/L | | 20.5 (14.8-30.3) | 21 (15-31) | 19 (14-24.5) | .267 |
| | <40 | 83 (84.7) | 67 (82.7) | 16 (94.1) | .457 |
| | >40 | 15 (15.3) | 14 (17.3) | 1 (5.9) | |
| Total bilirubin, mmol/L | | 9.5 (6.7-13.8) | 9.3 (6.5-14) | 9.7 (7.5-12.6) | .862 |
| | <17.1 | 84 (85.7) | 68 (84.0) | 16 (94.1) | .453 |
| | >17.1 | 14 (14.3) | 13 (16.0) | 1 (5.9) | |
| Creatine kinase, U/L | | 56 (47.3-69.1) | 80 (49.5-104) | 76 (56.5-89) | .722 |
| | <200 | 93 (94.9) | 76 (93.8) | 17 (100) | .584 |
| | >200 | 5 (5.1) | 5 (6.2) | 0 (0) | |
| Creatinine, umol/L | | 78 (50.3-96.3) | 54.8 (46.3-68.8) | 59.2 (48.7-69.4) | .501 |
| | <80 | 89 (90.8) | 74 (91.4) | 15 (88.2) | .653 |
| | >80 | 9 (9.2) | 7 (8.6) | 2 (11.8) | |
| Urea, mmol/L | | 4.2 (3.2-4.9) | 4.1(3.3-4.9) | 4.4 (3-5.2) | .644 |
| Sodium, mmol/L | | 138.1 (136.6-139.8) | 138 (136.7-139.8) | 138.2 (135.6-140.1) | .747 |
| Potassium, mmol/L | | 3.9 (3.5-4.2) | 3.8 (3.5-4.2) | 4 (3.6-4.3) | .481 |
| Chloride, mmol/L | | 101 (99.3-103.2) | 101.2 (99.5-103.6) | 99.8 (97.9-102.5) | .071 |
| D-dimer, mg/L | | 100 (82.5-161.8) | 100 (82-157.9) | 100 (100-241) | .460 |
| | <500 | 87 (88.8) | 72 (88.9) | 15 (88.2) | .587 |
| | >500 | 5 (5.1) | 5 (6.2) | 0 (0) | |

*Note:* Data are presented as median and interquartile range (IQR) and n (%)

*P* values comparing non-re-positive group and re-positive group are from Mann-Whitney *U* test, chi-squared, and Fisher's exact test
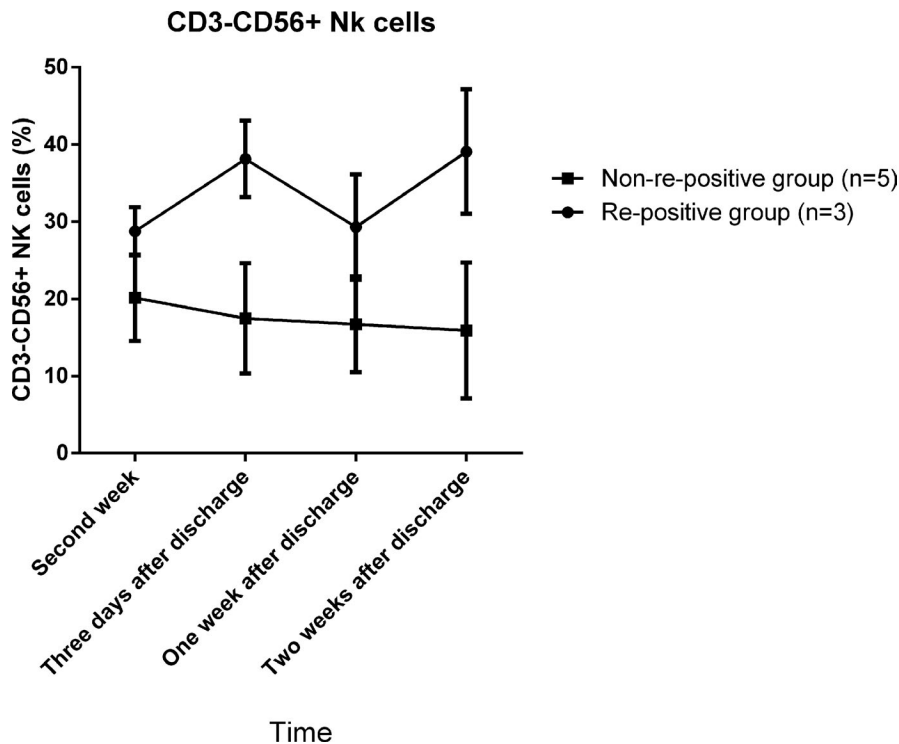
Percentages do not total 100% owing to missing data

## CD3-CD56+ Nk cells

All discharged patients in HwaMei Hospital, University of Chinese Academy of Sciences, were under medical observation for 14 days at special isolation sites and then another 14 days of home isolation with special care. A period of isolation and medical observation for convalescent patients is essential to understanding more about COVID −19 and to preventing possible spread of the disease.

No significant differences of initial clinical symptoms and chest CT scans between re-positive group and non-re-positive group on admission were observed. However, non-positive group showed a better absorption for acute exudative lesions than positive group at recovery period. Feng Panet al[13] reported four stages from onset of initial pneumonia to recovery in patients with COVID-19 pneumonia: early stage (0-4 days after onset of symptoms), progressive stage (5-8 days after onset of symptoms), peak stage (9-13 days after onset of symptoms), and absorption stage (≥14 days after onset of symptoms). Noticeably, a case report also found that progressive absorption of parenchymal lesions occurred approximately 12 days after onset of symptoms.[14] Our study revealed that slow absorption of lung lesions in patients may be associated with persistent positive RT-PCR test results. So, the result of CT scan is also one of the most valuable references for discharge, but how to quantify the CT scan requires further investigation.

In re-positive group, the chest CT scans of one nucleic acid–positive patient presented recurrent lesions accompanying with fever and throat pain, which can be defined as relapse, and which means the return of symptoms after they have apparently ceased during convalescence caused by the multiplication of pathogen. However, more cases are needed to confirm this phenomenon.

Additionally, the overall levels of CD3-CD56 + NK cells during hospitalization and rehabilitation were higher in re-positive group than in non-re-positive group. NK cells are critical for innate anti-viral immune defense due to their cytotoxic function and production of the pro-inflammatory factors IL-4 and IFN-$\gamma$.[15-17] Previous studies had shown that NK cells played a key role in the pathogenesis of acute lung injury and they could induce antifibrosis signal in the lungs to counteract the fibrogenic activity of transforming growth factor-$\beta$ (TGF-$\beta$) by producing IFN-$\gamma$.[18] However, Kim JH et al[19] disclosed that NK cells accelerate immune complex-induced acute lung injury by stimulating production of MIP-1 through both autocrine and paracrine mechanisms, and by enhancing cytokine production from alveolar macrophages and CD11c + dendritic cells (DCs). In the present study, we found that patients with re-positive (SARS-CoV-2) nucleic acid had a higher amount of NK cells. NK cells activation may be vital to the induction of adaptive immune responses that have the potential of clearing the infection.[20] The mechanism of NK cell-mediated immune response after SARS-CoV-2 infection needs to be elucidated in the future.

Our study has several limitations. First, our study was conducted in convalescent patients with mild or moderate COVID-19. Second, further follow-up study needs be conducted among these patients. Third, our study was limited to a small number of patients in Ningbo city, Zhejiang province. Multi-center research on a larger cohort is warranted in future study.

## 5 | CONCLUSIONS

In conclusion, SARS-CoV-2 nucleic acid can still be detected in a small proportion of convalescent patients based on our study.

**TABLE 4** Treatment and outcomes of convalescent patients

| Characteristics | All patients (n = 98) | Non-re-positive patients (n = 81) | Re-positive patients (n = 17) | P |
|---|---|---|---|---|
| Antiviral therapy | | | | |
| Time from onset of symptoms to antiviral therapy (days) | 4.0 (2.0-8.75) | 4 (2-9) | 4 (2-7.5) | .780 |
| Chloroquine phosphate (%) | | | | |
| Yes | 71 (72.4) | 58 (71.6) | 13 (76.5) | |
| No | 27 (27.6) | 23 (28.4) | 4 (23.5) | .774 |
| Lopinavir-ritonavir (%) | | | | |
| Yes | 96 (98.0) | 79 (97.5) | 17 (100) | |
| No | 2 (2.0) | 2 (2.5) | 0 (0) | 1.000 |
| Arbidol hydrochloride (%) | | | | |
| Yes | 93 (94.9) | 77 (95.1) | 16 (94.1) | |
| No | 5 (5.1) | 4 (4.9) | 1 (5.9) | 1.000 |
| Outcomes | | | | |
| Length of the hospital stay (days) | 15 (13-19) | 15 (13-19) | 14 (10-18.5) | .437 |
| Time from discharge to nucleic acid re-positive (days) | | | 4 (3-8.5) | |
| Time from onset of symptoms to final negative RT-PCR test results (days) | 21 (17, 28) | 19 (16-26) | 34 (29.5-42.5) | <.001 |
| IgM/IgG antibodies (%) | | | | |
| Positive IgM and IgG antibodies | 13 (13.3) | 7 (8.6) | 6 (35.3) | .164 |
| Negative IgM and IgG antibodies | 3 (3.1) | 2 (2.5) | 1 (5.9) | |
| Negative IgM and positive IgG antibodies | 46 (46.9) | 36 (44.4) | 10 (58.8) | |
| Time from onset of symptoms to IgM antibody disappeared (days) | 46 (40.5, 50) | 46 (38.5, 50) | 44.5 (40.25, 55.5) | .966 |
| Time from antiviral therapy to IgM antibody disappeared (days) | 39 (32.75, 45.25) | 39 (33, 45.25) | 39 (31.25, 46.5) | .951 |

*Note:* Data are presented as median and interquartile range (IQR) or n (%).

*P* values comparing non-re-positive cases and re-positives cases are from Mann-Whitney *U* test and Fisher's exact test.

Percentages do not total 100% owing to missing data.

Relapse might occur in COVID-19 patients. Strict quantification of pulmonary CT evaluation of inflammation absorption may improve the discharge criteria. Continuous quarantine and follow-up for convalescent patients are necessary to control possible spread to some extent.

**CONFLICT OF INTEREST**

The authors declare that they have no competing interests.

**AUTHORS' CONTRIBUTIONS**

Honghua Ye and Ting Cai designed the study. Zhu Hui and Liyun Fu analyzed the data and drafted the manuscript. Yinhua Jin, Jiale Shao, Shun Zhang, Nanhong Zheng, Lingyan Fan, Zhe Yu, Jun Ying, Jianjun Zheng, Yaoren Hu, Tongen Chen, Yanglingzi Chen, Min Chen, Mingjue Chen, Zi Xiong, Junfei Kang and Jiachang Jin contributed to the acquisition of subjects and data. Hui Zhu and Liyun Fu contributed to analysis and interpretation of data. Honghua Ye has primary responsibility for the final content.

**ORCID**

*Hui Zhu* https://orcid.org/0000-0002-3159-8549

**REFERENCES**

1. International Committee on Taxonomy of Viruses. Naming the 2019 coronavirus. https://talk.ictvonline.org/. Accessed February 25, 2020.
2. Cucinotta D, Vanelli M. WHO Declares COVID-19 a Pandemic. *Acta Biomed.* 2020;91(1):157-160.
3. World Health Organization. Coronavirus disease (COVID-19) outbreak situation. https://www.who.int/emergencies/diseases/novel-coronavirus-2019. Accessed April 10, 2020.
4. Meo SA, Alhowikan AM, Al-Khlaiwi T, et al. Novel coronavirus 2019-nCoV: prevalence, biological and clinical characteristics comparison with SARS-CoV and MERS-CoV. *Eur Rev Med Pharmacol Sci.* 2020;24(4):2012-2019.
5. Lim J, Jeon S, Shin HY, et al. Case of the index patient who caused tertiary transmission of COVID-19 infection in korea: the application of lopinavir/ritonavir for the treatment of COVID-19 infected pneumonia monitored by quantitative RT-PCR. *J Korean Med Sci.* 2020;35(6):e79.

6. Perinel S, Launay M, Botelho-Nevers E, et al. Towards optimization of hydroxychloroquine dosing in intensive care unit COVID-19 patients. *Clin Infect Dis.* 2020. https://doi.org/10.1093/cid/ciaa394

7. Wang M, Cao R, Zhang L, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res.* 2020;30(3):269-271.

8. The diagnosis and treatment protocols from the national health commission of the People's Republic of China ((trial version sixth). http://www.nhc.gov.cn/jkj/s3577/202003/4856d5b0458141fa9f376853224d41d7.shtml. Accessed March, 7 2020.

9. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA.* 2020;323(11):1061-1069.

10. Wei M, Yuan J, Liu Y, Fu T, Yu X, Zhang ZJ. Novel coronavirus infection in hospitalized infants under 1 year of age in China. *JAMA.* 2020;323(13):1313-1314.

11. WHO. Clinical management of severe acute respiratory infection when Novel coronavirus (nCoV) infection is suspected: interim guidance. Jan 28, 2020. https://www.who.int/internal-publications-detail/clinical-managementof-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected. Accessed February, 5th, 2020.

12. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet.* 2020;395(10223):497-506.

13. Qian GQ, Yang NB, Ding F, et al. Epidemiologic and clinical characteristics of 91 hospitalized patients with COVID-19 in Zhejiang, China: a retrospective, multi-centre case series. *QJM.* 2020. Epub ahead of print.https://doi.org/10.1093/qjmed/hcaa089.

14. Lan L, Xu D, Ye G, et al. Positive RT-PCR test results in patients recovered from COVID-19. *JAMA.* 2020;323(15):1502-1503.

15. Lin AW, Gonzalez SA, Cunningham-Rundles S, et al. CD56(+dim) and CD56(+bright) cell activation and apoptosis in hepatitis C virus infection. *Clin Exp Immunol.* 2004;137(2):408-416.

16. Doherty DG, Norris S, Madrigal-Estebas L, et al. The human liver contains multiple populations of NK cells, T cells, and CD3+CD56+ natural T cells with distinct cytotoxic activities and Th1, Th2, and Th0 cytokine secretion patterns. *J Immunol.* 1999;163(4):2314-2321.

17. Giroux M, Denis F. CD1d-unrestricted human NKT cells release chemokines upon Fas engagement. *Blood.* 2005;105(2):703-710.

18. Liu M, Zeng X, Wang J, et al. Immunomodulation by mesenchymal stem cells in treating human autoimmune disease-associated lung fibrosis. *Stem Cell Res Ther.* 2016;7(1):63.

19. Kim JH, Chung DH. CD1d-restricted IFN-gamma-secreting NKT cells promote immune complex-induced acute lung injury by regulating macrophage-inflammatory protein-1alpha production and activation of macrophages and dendritic cells. *J Immunol.* 2011;186(3):1432-1441.

20. Ji HF, Wang J, Yu L, Niu JQ, Ayana DA, Jiang YF. High frequencies of CD158b+ NK cells are associated with persistent hepatitis C virus infections. *Ann Hepatol.* 2013;12(4):539-547.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.