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MAJOR ARTICLE

The Clinical and Radiological Manifestations in Coronavirus Disease 2019 With Negative Nucleic Acid Results

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Background. Coronavirus disease 2019 (COVID-19) was a new emerging disease with high infectiousness. Its diagnosis primarily depended on real-time polymerase chain reaction (RT-PCR) results. This study investigated epidemiological, clinical, and radiological characteristics of COVID-19 with negative RT-PCR results before confirmation.

Methods. Patients with COVID-19 were enrolled and divided into 2 groups: a negative group with negative RT-PCR results before confirmation and a positive group with positive results at the first detection. Epidemiological and clinical features were compared. Dynamic chest computerized tomography (CT) images of the negative group were evaluated.

Results. Ninety-nine laboratory-confirmed patients with COVID-19 including 8 patients (8%) with negative RT-PCR results were included. Patients from the negative group had similar epidemiological features: the average age (50.25 ± 13.27 years in the negative group and 53.70 ± 16.64 years in the positive group) and gender distribution (males made up 50% of the negative group and 62.6% of the positive group) were comparable. No significant differences were observed in clinical symptoms between the 2 groups. We found that fever was the most common symptom for both groups, followed by cough, expectoration, chest distress, fatigue, and gastroenterological symptoms. Moreover, ground-glass opacities and consolidations were the main manifestation in chest CT of patients with COVID-19 with or without confirmed RT-PCR results.

Conclusions. Regardless of initial RT-PCR results, patients with COVID-19 had similar epidemiological, clinical, and chest CT features. Our study suggests value from early chest CT scans in COVID-19 screening and dynamic significance of radiology in disease monitoring should guide clinical decisions.

Keywords. chest CT; COVID-19; early diagnosis; RT-PCR; SARS-CoV-2.

The health crisis presented by coronavirus disease 2019 (COVID-19) has led to significant loss of life, a great burden on medical care, and severe economic consequences. This emerging 2019 novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was 1 of 7 CoVs capable causing human infection, whose complete genome showed more than 85% identity to bat SARS-like CoV [1]. As a new emerging infectious diesease, our understanding of COVID-19 has increased but it is still limitted. Guidelines established by the National Health Commission of the People's Republic of China (from version 1 to 6) set up diagnosis criteria for COVID-1 and indicates that a positive real-time polymerase chain reaction (RT-PCR) result is necessary for disease confirmation [2].

Recent publications have described epidemiological characteristics and clinical manifestations of COVID-19 [1, 3, 4], revealing that fever was the most common symptom in these patients and that approximately 66% of patients had specific exposure history. Nevertheless, in previous studies, researchers found no significant difference in clinical features between Middle East respiratory syndrome and any other community-acquired pneumonia (CAP) [5]. Because of the high rate of influenza infections during this period, atypical clinical features and laboratory tests would likely confuse clinicians who screened for COVID-19 versus CAP in early clinical decisions.

Radiological manifestations of COVID-19 showed specific subpleural ground-glass opacities (GGOs) and consolidations [6]. However, the values of these image changes in helping early diagnosing COVID-19 was not clear. SARS-CoV-2 was highly transmissible and resources for care and quarantine of infected patients were limited, doctors were under tremendous pressure to exclude sputum-negative patients with highly suspected symptoms and radiological images from COVID-19 patients.

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In this study, we retrospectively reviewed 8 COVID-19 cases whose early nucleic acid detections were inconsistent with simultaneous radiological findings. We then compared these findings with other defined patients whose RT-PCR results were positive at the first viral detection, and we explored the value of dynamic chest computerized tomography (CT) evaluation as a supplementary diagnostic method for COVID-19.

METHODS

Patients

When local health authorities in Wuhan, where the COVID-19 outbreak originated, declared an epidemiological alert on January 7, 2020, our center, in Zhejiang province, was ordered to prepare to treat COVID-19 patients. On January 19, 2020, our center, in Hangzhou city, admitted the first case of COVID-19. Because we had limited understanding of this new infectious disease, we followed guidelines provided by the National Health Commission of the People's Republic of China (from version 1 to 6) [2]. Some patients had multiple negative RT-PCR results, but typical clinical features and chest CT manifestations, and a positive RT-PCR after continued testing. We retrospectively included all COVID-19 patients hospitalized at The First Affiliated Hospital of Zhejiang University from January 19, 2020 to February 15, 2020, and we divided them into 2 groups: (1) the negative group with the first negative RT-PCR result before subsequent confirmation and (2) the positive group with a positive result at the first detection. Detection of COVID-19 in swabs or sputa was performed using RT-PCR (Shanghai BioGerm Medical Biotechnology Co. Ltd). In this retrospective study, we collected basic demographic features, epidemiological characteristics, clinical manifestations, and chest CT images from electronic medical records. Two researchers reviewed the data collection independently to double check the accuracy of all data. Epidemiological and symptomatic data were confirmed through communication with patients if the data were not clear. Approval was obtained from the ethics committee.

Evaluation of Chest Computed Tomography Images

All chest CT images were reviewed independently by 2 experienced radiologists in our hospital, and discrepancies were resolved by consensus. All images were viewed on both lung (width, 1500 Hounsfield units [HU]; level, −700 HU) and mediastinal (width, 350 HU; level, 40 HU) settings. Chest CT images were evaluated for the presence of GGOs, consolidation, mixed GGOs and consolidation, centrilobular nodules, septal thickening, perilobular opacities, reticulation, architectural distortion, subpleural bands, traction bronchiectasis, and bronchial wall thickening [7]. Each lung was divided into 3 zones: upper (above the carina), middle (below the carina up to the inferior pulmonary vein), and lower (below the inferior pulmonary vein) zones [8]. A semiquantitative score (0–4) was assigned for each lung zone: 0, no involvement; 1, less than 25% involvement; 2, 25% to less than 50% involvement; 3, 50% to less than 75% involvement; and 4, 75% or greater involvement.

Statistical Analysis

Continuous variables were expressed as mean with standard deviation and analyzed by *t* test. Computerized tomography scores of the first and second examination were analyzed by paired *t* test. Categorical variables were described as numbers with percentages and were compared by χ^2 test with Fisher's exact test. A 2-sided α of less than 0.05 was considered statistically significant. Statistical analyses were performed with the SPSS software, version 22.0.

RESULTS

Demographic, Epidemiological, and Clinical Features

We included 99 laboratory-confirmed COVID-19 patients in The First Affiliated Hospital of Zhejiang University School of Medicine from January 19, 2020 to February 15, 2020. Among them, 8 patients (8%) had the first negative RT-PCR result by swab or sputum before subsequent RT-PCR positive diagnosis. Detection of respiratory viruses, including influenza A/B, adenovirus, respiratory syncytial virus, and parainfluenza virus, was negative in these patients. The characteristics of these 8 patients are summarized in Table 1. The most common symptom in these first negative patients was fever (7 of 8), and approximately half had cough, expectoration, chest distress, and fatigue. Only 1 patient had gastroenterological symptoms, such as nausea, vomiting, abdominal pain, and diarrhea. The detection times of negative RT-PCR results before a repeat RT-PCR was positive for COVID-19 ranged from 1 to 6. Patient 2 had the longest duration from admission to infection confirmation. Because he and his 5 colleagues had contact with the same confirmed COVID-19 case, and all 5 of the colleagues had been diagnosed of COVID-19 in our hospital, we presumed that patient 2 likely contracted COVID-19, and thus we prescribed lopinavir/ritonavir as potential anticoronavirus therapy after his consent. Even though we started empiric treatment for this patient at an early stage before virological diagnosis of COVID-19, his condition still deteriorated. Most of the 8 patients also received antibiotics as empiric therapy (5 of 8), and 2 patients had oseltamivir when influenza was not excluded. Patients 1 and 2 were prescribed methylprednisolone to improve the oxygen index. Patients 3 and 5 did not have any medication because their symptoms were mild. The clinical conditions of these 8 patients all worsened at different levels from admission to infection confirmation.

There were no apparent differences in demographic characteristics or clinical symptoms between the negative (N = 8) and positive groups (N = 91). The average age was comparable with 50.25 ± 13.27 years in the negative group and

Table 1. Epidemiological Characteristics and Clinical Features of 8 COVID-19 Patients With Negative RT-PCR Results

Patient	Gender	Age	Exposure and Time[a]	Clinical Manifestation[b]	Detection[c]	Early Treatment[d]
Patient 1	Male	48y	Denied exposure. Time: NA	Fever, chills, with generalized myalgia and fatigue for 8 days and chest tightness and shortness of breath after exertion for 1 day.	3	Tienam; Methylprednisolone
Patient 2	Male	29y	Contact confirmed colleague. Time: 7 days	Dizziness, chills, and fever for 3 days.	7	LPV/r; Immunoglobulin; Methylprednisolone
Patient 3	Female	48y	Contact confirmed husband. Time: NA	No symptom before admission.	2	None
Patient 4	Female	54y	Back from Wuhan 1 day ago. Time: NA	Fever with chills, headache, and fatigue for 3 days.	3	Latamoxef
Patient 5	Female	50y	Contact confirmed husband. Time: NA	Fever, cough with expectoration, generalized myalgia, and fatigue for 2 days.	5	None
Patient 6	Female	38y	Denied exposure. Time: NA	Dry cough for 3 days and fever for 1 day.	3	Moxifloxacin
Patient 7	Male	65y	Dinner with confirmed case. Time: 15 days	Fever, with nausea, vomiting, and diarrhea for 1 day.	5	Oseltamivir; Levofloxacin
Patient 8	Male	70y	Contact confirmed case. Time: 10 days	Fever, cough and expectoration, chest tightness, and shortness of breath after exertion for 7 days.	3	Oseltamivir; Ceftriaxone

Abbreviations: COVID-19, coronavirus disease 2019; LPV/r, ritonavir-boosted lopinavir; NA, not available; RT-PCR, real-time polymerase chain reaction; y, year.

[a]Time from exposure to symptom onset.

[b]Time from symptom onset to computerized tomography examination.

[c]Detection number until the first positive RT-PCR result.

[d]Early treatment before virological diagnosis.

53.70 ± 16.64 years in the positive group. There were 50% males in the negative group and 62.6% in the positive group. In positive group, 76.9% of patients had a specific exposure history, and this proportion was 75.0% in negative group. The most common symptom was fever in both groups (negative vs positive, 87.5% vs 91.2%, $P > .05$). More than half of the patients complained of cough (negative vs positive, 62.5% vs 80.2%, $P > .05$), expectoration (negative vs positive, 37.5% vs 50.5%, $P > .05$), and pharyngalgia (negative vs positive, 12.5% vs 4.4%, $P > .05$). Chest distress and fatigue were common in both group. In addition, there were also a few patients who complained of gastroenterological symptoms, but there was no significant difference between the 2 groups.

Chest Computed Tomography Manifestations

The 8 patients' chest CT scans were taken on admission and re-examined at the time of COVID-19 confirmation. Table 2 showed the specific involvement of lung lobes and corresponding scores. In chest CT (lung window) on admission, the most affected lobes were the upper and lower lobes of left lung. The second chest CT scan was performed at virological confirmation and revealed progression in patients with new occurred or/and enlarged lesions. The mean CT involvement score of the first chest CT in 8 patients was 3.88 and increased to 5.88 at the second chest CT ($P = .015$). The highest CT involvement score was 15 at the first chest CT and increased to 21 the second time.

The main findings obtained from the chest CT for patients 1 to 4 and patients 5 to 8 from admission to laboratory confirmation are showed in Figures 1 and 2, respectively. Mixed GGOs and multifocal lobular consolidations were found in patient 1 (Figure 1A2), patient 2 (Figure 1B2), and patient 7 (Figure 2C2), which deteriorated from previous unilateral or bilateral focal GGOs and focal subpleural lobular consolidation. In patient 3 (Figure 1C2), patient 4 (Figure 1D2), and patient 5 (Figure 2A2), the size of GGOs noticeably increased compared with those in the first chest CT. In patient 6, the density of GGOs in the lower lobe of the left lung (Figure 1B1) attenuated in the repeated chest CT, but new GGOs arose below the former one (Figure 2B2). The chest CT of patient 8 (Figure 2D1) showed reticular opacities and interlobular septal thickening in the right lung and involved the region enlarged in the follow-up CT scan (Figure 2D2); meanwhile, multifocal GGOs were also shown in these patients.

DISCUSSION

The current diagnosis for COVID-19 is mainly dependent on laboratory confirmation of viral nucleic acid detection. Among all 99 COVID-19 patients enrolled in this study, 8 patients had negative RT-PCR results in swab and/or sputum at the beginning of admission, and 7 of them had at least two negative results before laboratory confirmation. According to the guidelines, these patients could be excluded for COVID-19 and did not need to be isolated. Our study describes the clinical features and chest CT characteristics of these COVID-19 patients with initially negative RT-PCR results. Moreover, we suggest the significance of CT examinations

Table 2. Chest CT Manifestations of 8 COVID-19 Patients With Negative RT-PCR Result

Patient	Left Lung on Admission			Right Lung on Admission			First Score	Left Lung on Diagnosis			Right Lung on Diagnosis			Second Score[a]
	Upper Lobe	Lingual Segment	Lower Lobe	Upper Lobe	Middle Lobe	Lower Lobe		Upper Lobe	Lingual Segment	Lower Lobe	Upper Lobe	Middle Lobe	Lower Lobe	
Patient 1	+	+	+	+	+	+	15	+	+	+	+	+	+	21
Patient 2	-	-	+	-	+	-	2	-	-	+	-	+	+	4
Patient 3	+	-	-	-	-	-	1	+	+	-	-	-	-	2
Patient 4	+	+	+	+	-	+	6	+	+	+	+	+	+	8
Patient 5	+	-	-	-	-	+	2	+	+	-	-	-	+	3
Patient 6	-	-	+	-	-	-	1	-	-	+	-	-	-	1
Patient 7	-	-	-	+	-	-	1	-	-	+	+	-	-	3
Patient 8	+	+	+	-	-	-	3	+	+	+	+	-	+	5

Abbreviations: COVID-19, coronavirus disease 2019; CT, computerized tomography; RT-PCR, real time-polymerase chain reaction.

NOTE: Score, CT involvement score; +, lesions involved in this area; -, no lesion involved in this area.

[a]Paired *t* test was used to compare the CT involvement scores on admission and virological diagnosis with $P < .05$.

for clinical diagnosis according to its consistent patterns with COVID-19 pneumonia, which will be valuable in directing early intervention strategies.

In this study, we found no significant difference in age, sex, and clinical symptoms between initial negative and positive groups, indicating that clinical features cannot be used to predict RT-PCR results. Consistent with previous researches [4, 9], the most common symptom in our patients was fever followed by respiratory symptoms and systemic discomfort, whereas gastroenterological symptoms occurred in a few patients. The proportion of patients with expectoration was also low, between 37.5% and 50.5% in 2 groups, and was reported to be as low as 28% in other literature. In our center, sputum and pharyngeal swabs were the most frequently collected specimens for virological detection for convenience. However, low sensitivity of the RT-PCR test was reported to be 58% for SARS-CoV detection [10]. Peiris et al [11] noted a positive rate of 32% in nasopharyngeal aspirates from 75 SARS patients at initial presentation, which doubled to 68% after 14 days. The optimal way to collect specimens and timing of collections for COVID diagnosis remains uncertain.

The underlying target of SARS-CoV-2 might be located in lower respiratory tract rather than upper site because few cases developed significant upper respiratory symptoms [11]. Therefore, a throat swab may not have an adequate quantity of virus to reach detection limits during the incubation stage. Expectorated sputum was also difficult to obtain from patients who mainly presented with a dry cough or were in the recovery stage. These factors could partially explain the low positive detection rate in sputa and swabs. However, lower respiratory tract sampling with bronchoscopy and bronchoalveolar lavage was not widely available in resource-limited settings, and this was also associated with increased risk of transmission [12]. However, it might be inappropriate to completely exclude COVID-19 diagnosis based only on negative RT-PCR results.

Our results suggest that more emphasis should be placed on the value of serial chest CT scans in early diagnosis and evaluation of disease progression. We show that COVID-19 primarily presents nonspecific manifestations such as acute fever and respiratory illness. The laboratory results indicated nonspecific changes of viral infections including leukopenia or lymphopenia. A recent study summarized the typical features of chest CT images of COVID-19 including multiple bilateral GGO and consolidation [6], which was consistent with our findings. Furthermore, we revealed that deterioration of chest CT in COVID-19 patients could occur with consolidation of former GGOs, enlargement of lesions, and newly developed lesions in other lobes. These findings suggest that exclusion of suspected patients by false-negative RT-PCR results may lead to missed diagnosis and delayed treatment. Previous studies reported that timely initiation of antiviral therapy in

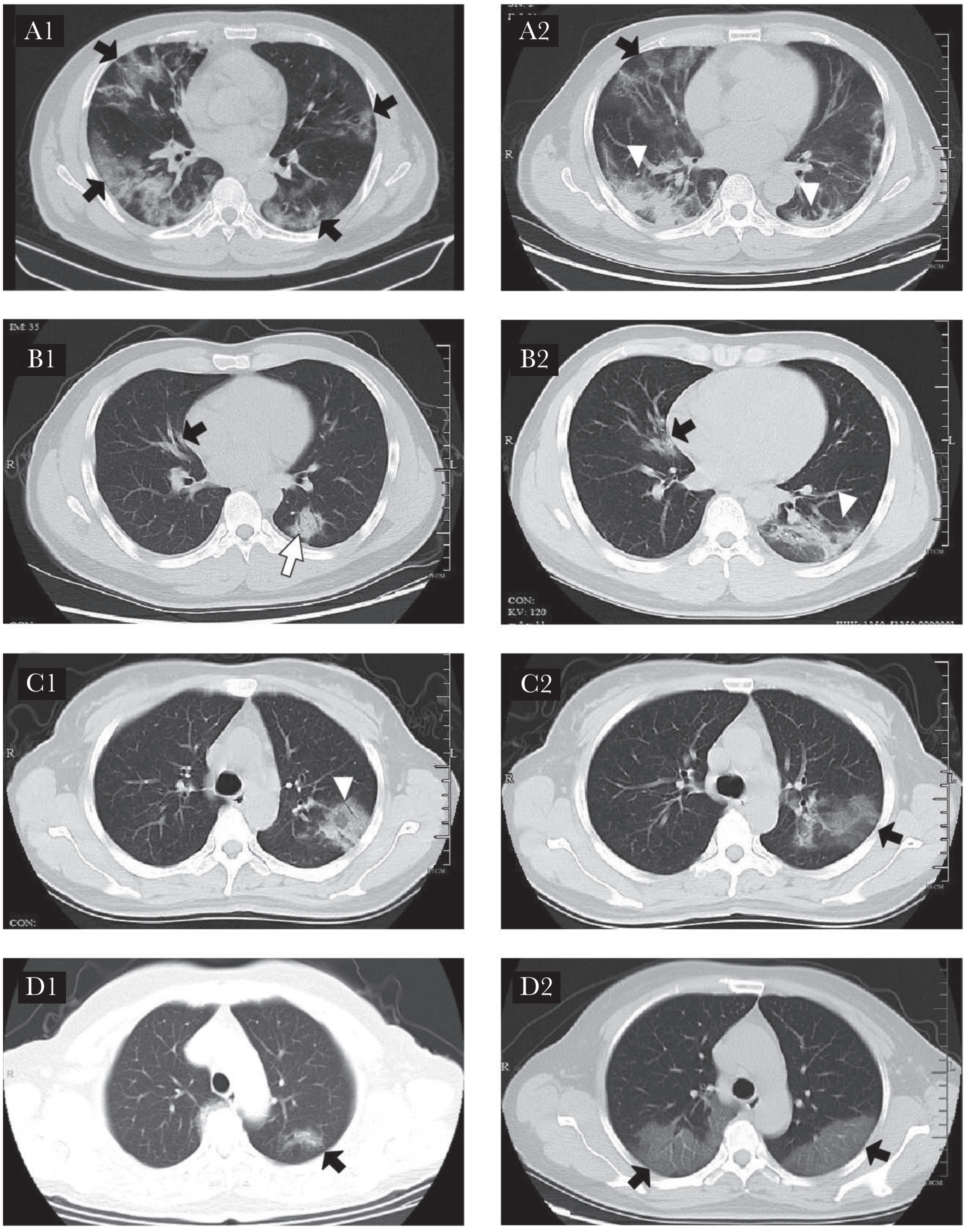

Figure 1. Progression of chest computerized tomography (CT) images in patients 1 to 4. A1, B1, C1, and D1 were axial CT images of patients 1 to 4 at the time point of admission, and the real-time polymerase chain reaction results were all negative. A2, B2, C2, and D2 were follow-up images of same patient at the time point of laboratory confirmation of coronavirus disease, respectively. Black arrow, unilateral or bilateral focal ground-glass opacities (GGOs); white arrowhead, mixed GGOs and multifocal lobular consolidations; white arrow, lobular consolidations.

the therapeutic window of SARS could inhibit viral replication, minimize subsequent immune hyperactivity, and thus improve clinical outcome [13]. In a recent study, researchers revealed that early diagnosis was of paramount importance to prevent disease progression of COVID-19 [14], whereas data about benefits from early treatment for COVID-19 were rare.

There were some limitations in this research. This was a single-center study with a small number of cases. Although we conducted repeated sampling during observation, only 1 specimen from a single location was detected. At each sampling, multiple specimens from different sites (such as oropharynx, nasopharynx, sputum, and feces) should be collected and tested together to increase positive rates. Quantitative RT-PCR for viral load was also lacking in this study. Some patients in this work were still under treatment in our hospital, and thus their outcomes and prognosis remained unknown. Future work on follow up is needed to provide more clues regarding diagnostic therapy for COVID-19 patients with negative RT-PCR results in the early stage.

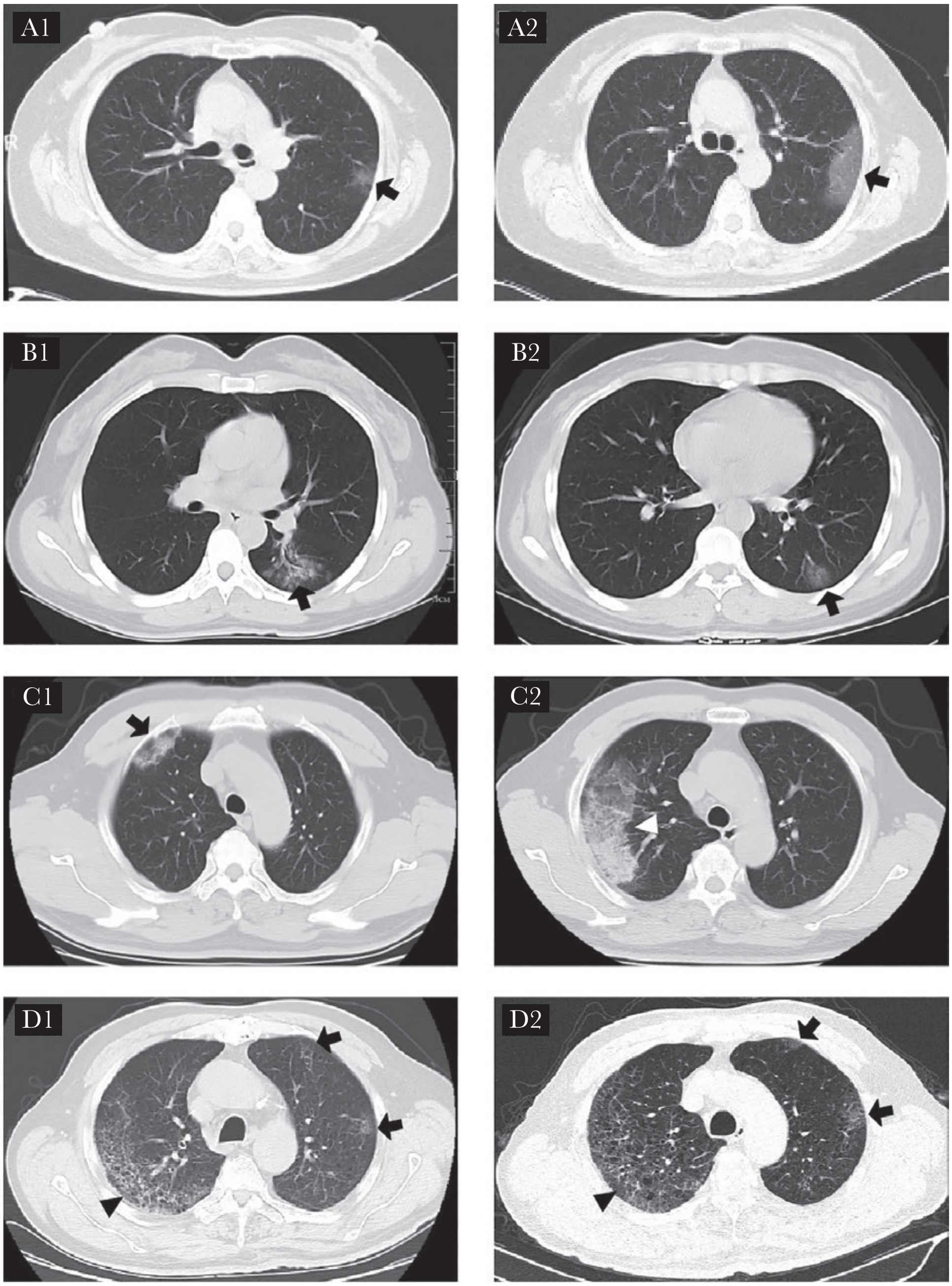

Figure 2. Progression of chest computerized tomography (CT) images in patients 5 to 8. A1, B1, C1, and D1 were axial CT images of patients 5 to 8 at the time point of admission, and the real-time polymerase chain reaction results were all negative. A2, B2, C2, and D2 were follow-up images of the same patient at the time point of laboratory confirmation of coronavirus disease, respectively. Black arrow, unilateral or bilateral focal ground-glass opacities; black arrowhead, reticular opacities and interlobular septal thickening.

CONCLUSIONS

Our work suggested early progression of disease on chest CT even when the viral nucleic acid detection was negative. The COVID-19 patients might be undiagnosed for the negative detection for viral nucleic acid, which could delayed the treatment opportunity and increased fatality. Thus, repeated sampling following standardized procedures and optimized detection methods were required. Our study suggests the value of a chest CT scan in COVID-19 screening and its roles for early medication in those negative RT-PCR patients with COVID-19.

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References

1. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med **2020**; 382:727–33.
2. Beijing: China National Health Commission. [Diagnosis and treatment of pneumonitis caused by new coronavirus (Trial version 6)]. Available at: http://www.nhc.gov.cn/yzygj/s7653p/202002/8334a8326dd94d329df351d7da8aefc2.shtml. Accessed 5 February 2020.
3. Chan JF, Kok KH, Zhu Z, et al. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerg Microbes Infect **2020**; 9:221–36.
4. Chang D, Lin M, Wei L, et al. Epidemiologic and clinical characteristics of novel coronavirus infections involving 13 patients outside Wuhan, China. JAMA **2020**; 323:1092–3.
5. Al-Tawfiq JA, Hinedi K, Ghandour J, et al. Middle East respiratory syndrome coronavirus: a case-control study of hospitalized patients. Clin Infect Dis **2014**; 59:160–5.
6. Chung M, Bernheim A, Mei X, et al. CT imaging features of 2019 novel coronavirus (2019-nCoV). Radiology **2020**; 295:202–7.
7. Ajlan AM, Ahyad RA, Jamjoom LG, Alharthy A, Madani TA. Middle East respiratory syndrome coronavirus (MERS-CoV) infection: chest CT findings. AJR Am J Roentgenol **2014**; 203:782–7.
8. Ooi GC, Khong PL, Müller NL, et al. Severe acute respiratory syndrome: temporal lung changes at thin-section CT in 30 patients. Radiology **2004**; 230:836–44.
9. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet **2020**; 395:497–506.
10. Tsui PT, Kwok ML, Yuen H, Lai ST. Severe acute respiratory syndrome: clinical outcome and prognostic correlates. Emerg Infect Dis **2003**; 9:1064–9.
11. Peiris JS, Chu CM, Cheng VC, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. Lancet **2003**; 361:1767–72.
12. Kain T, Fowler R. Preparing intensive care for the next pandemic influenza. Crit Care **2019**; 23:337.
13. Chu CM, Cheng VC, Hung IF, et al. Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. Thorax **2004**; 59:252–6.
14. Xu XW, Wu XX, Jiang XG, et al. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: retrospective case series. BMJ **2020**; 368:m606.