Study for the efficacy of Chloroquine in patients with novel coronavirus pneumonia (COVID-19): Trial Protocol

Registry name: Study for the efficacy of Chloroquine in patients with novel coronavirus pneumonia (COVID-19);

Trial ID: ChiCTR2000029542;

URL: http://www.chictr.org.cn/showprojen.aspx?proj=48968

Objectives

To evaluate the efficacy and safety of Chloroquine in hospitalized patients with 2019 novel coronavirus infections

Design

This clinical trial study was approved by the ethics committee of Fifth Affiliated Hospital of Sun Yat-sen University (Ethics approval number 2020-K09-1) and registered at Chinese Clinical Trial Registry (ChiCTR2000029542). Informed consent met the Chinese laws, regulations and clinical study requirements of our hospital. Written informed consent was obtained from all patients or their legal guardians. The study mainly aimed to evaluate the efficacy and safety of Chloroquine for treating COVID-19 infected patients. We enrolled a total of 22 patients divided into two groups treated with twice-daily oral of 500mg Chloroquine (n=10) or 400/100mg Lopinavir/Ritonavir (n=12) for 10 days. We followed the disease progression by RT-PCR for COVID-19 viral RNAs and lung pathology with CT, as well as other criteria such as fever, respiratory rate, and oxygen saturation for 14 days. The criterion of stop Chloroquine was defined as a SARS-CoV-2 RNA negative result to real-time reverse-transcriptase polymerase-chain-reaction (RT-PCR) assay for two consecutive nasal and pharyngeal swab specimens in a patient.

Participants

From January 27, 2020 through February 15, 2020, we enrolled eligible patients with COVID-19 according to the diagnosis of WHO interim guidance at Fifth Affiliated Hospital of Sun Yat-sen University in Zhuhai, China. SARS-COV-2 infection patients were eligible if they presented with mild, moderate, or severe COVID-19.

Inclusion criteria

1. Aged ≥18 years old;

2. Patients had been diagnosed with COVID-19 according to WHO interim guidance: Clinical management of severe acute respiratory infection when novel coronavirus (2019-nCoV) infection is suspected (Interim guidance, 28 January 2020).

Exclusion criteria

1. pregnant woman patients;
2. Documented allergic history to Chloroquine;
3. Documented history of hematological system diseases;
4. Documented history of chronic liver and kidney diseases;
5. Documented history of cardiac arrhythmia or chronic heart diseases;
6. Documented history of retina or hearing dysfunction;
7. Documented history of mental illnesses;
8. Use of digitalis due to the previous disease.

Data collection

Researchers conducted the regular visits from admission to discharged or SARS-CoV-2 RT-PCR negative for two consecutive detection. The epidemiological characteristics (including recent exposure history), clinical symptoms and signs, adverse reaction/events were collected with data collection forms. The clinical characteristics, laboratory findings, underlying comorbidities, chest computed tomographic (CT) scans, and treatment measures (i.e., other antiviral therapy, antimicrobial medication, corticosteroid therapy, immunoglobulin therapy, anti-hypertensive medication, respiratory support, Chinese patent medication, supplemental oxygen or non-invasive ventilation, mechanical ventilation, kidney replacement therapy) were extracted from electronic medical records. Laboratory assessments consisted of detection of SARS-CoV-2 RNA with RT-PCR, complete blood count, blood chemistry, coagulation test, liver and renal function, electrolytes, C-reactive protein, procalcitonin, lactate dehydrogenase and creatine kinase. In order to evaluate the effect of Chloroquine on immune response, T-Cell counts for patients in the Chloroquine group were recorded every 2 days. The Sequential Organ Failure Assessment (SOFA) was determined on the day of enrollment. The durations from anti-viral treatment of intervention and onset of symptom to SARS-CoV-2 RT-PCR negative was recorded.

The serum concentration of Chloroquine was measured in patients at 14 days after the Chloroquine treatment completion by the high performance liquid chromatography – tandem mass spectrometry (HPLC-MS).

All outcomes

The primary outcome was viral negative-transforming time and the negative conversion rate of SARS-CoV-2 RT-PCR at day 10, 14 of study period. Patients who were withdrawn from study treatment or who suffered from the severe adverse events before Day 10 were considered to have a

failure of treatment.

The secondary outcome was the rate of hospital discharge at Day 14, clinical recovery at day 10, CT scan improvement at Day 10 and 14, and the frequency of adverse events. The criteria of clinical recovery were: no fever, axilla temperature ≤36.6°C or oral temperature ≤37.2 °C or rectal/ tympanic temperature ≤37.8°C; respiratory rate ≤24/minute on room air; oxygen saturation >94% on room air; mild or absent of cough (the scale of cough is classified as severe, moderate, mild, absent). The criteria of hospital discharge were: the temperature returned to normal for more than 3 days; the respiratory symptoms improved significantly; the pulmonary imaging showed that the inflammation was obviously absorbed; and the detection of respiratory pathogenic nucleic acid was negative twice in a row (the sampling time is at least 1 day apart). The criteria of CT scan improvement were: exudation or consolidation of the lesion absorbed; the lesion area was gradually narrowed; and there might be residual linear fibrosis.

RT-PCR assay for COVID-19

Nasal and pharyngeal swab samples were collected for extracting SARS-CoV-2 RNA from patients suspected of having SARS-CoV-2 infection at daily basis. After collection, the total RNA of samples was extracted within 2 hours using the respiratory sample RNA isolation kit (Da'an gene Co., Ltd). The total RNA was used for RT-PCR assay of SARS-CoV-2. The realtime RT-PCR assay was performed using a SARS-CoV-2 nucleic acid detection kit according to the manufacturer's protocol (Shanghai ZJ Bio-Tech Co Ltd). Three target genes, including RNA-dependent RNA polymerase (RdRP), nucleocapsid protein (N) and envelope protein (E), were simultaneously amplified and tested during the real-time RT-PCR assay. Target 1 (RdRP): forward primer GTGARATGGTCATGTGTGGCGG; reverse primer CARATGTTAAASACACTATTAGCATA use 800 nM per reaction; and the probe 5'-FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ-3'. Target 2 (N): forward primer GGGGAACTTCTCCTGCTAGAAT; reverse primer CAGACATTTTGCTCTCAAGCTG; and the probe 5'-FAM- TTGCTGCTGCTTGACAGATT-BBQ-3'. Target 3 (E): forward primer ACAGGTACGTTAATAGTTAATAGCGT; reverse primer ATATTGCAGCAGTACGCACACA; and the probe 5'-FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ-3'. Amplifications were initially done at 45°C for 10 min and subsequently at 95°C for 3 min, followed by 45 cycles of 95°C for 15 sec and 58°C for 30 sec.

Statistical analysis

All time related data were calculated from the day of treatment initiation. Continuous variables were directly expressed as median and inter-quartile range (IQR), while categorical variables were

expressed as number (%). In order to evaluate the effect of Chloroquine on immune response, we examined the trajectory of CD3+, CD4+, CD8+ T-Cell counts from serum samples. Square root and logarithmic (base 10) transformation were applied to normalize the distribution of CD3+, CD4+, CD8+ T-Cell counts data.

Supplementary Table S1. Secondary baseline demographic and clinical characteristics of participants by treatment groups

		Treatment Group		
Characteristics	**All Patients (n=22)**	**Chloroquine (n=10)**	**Lopinavir/Ritonavir (n=12)**	***P* Value***
Comorbidities				
Hypertension, n (%)	4 (18.20)	1 (10.00)	3 (25.00)	0.59
Diabetes, n (%)	2 (9.10)	1 (10.00)	1 (8.30)	0.99
Cerebrovascular disease, n (%)	1 (4.50)	0 (0)	1 (8.30)	0.99
Laboratory parameters				
White blood cell count, 10^9/L	5.02 (4.51-5.71)	4.96 (4.41-5.50)	5.02 (4.79-7.92)	0.38
Neutrophil count, 10^9/L	2.84 (2.58-3.55)	2.67 (2.39-3.11)	3.20 (2.82-5.21)	0.06
Lymphocyte count, 10^9/L	1.56 (1.23-1.78)	1.61 (1.44-1.98)	1.44 (1.17-1.72)	0.35
Monocyte count, 10^9/L	0.57 (0.44-0.68)	0.60 (0.39-0.76)	0.57 (0.50-0.64)	0.92
Platelet count, 10^9/L	181.00 (137.25-209.25)	166.00 (145.25-184.25)	208.50 (121.5-253.25)	0.35
Prothrombin time, second	11.90 (11.30-12.50)	12.10 (11.83-12.45)	11.90 (11.20-12.55)	0.80
Activated partial thromboplastin time, second	30.20 (27.80-32.30)	32.45 (30.48-34.18)	30.10 (25.40-30.55)	0.01
D-dimer, ng/ml	99.00 (73.00-119.00)	91.50 (60.00-132.25)	109.00 (82.50-115.00)	0.99
Creatine kinase, U/L	69.00 (50.25-105.75)	70.50 (57.50-109.75)	60.00 (49.00-99.25)	0.53
Creatine kinase-MB, U/L	13.60 (11.63-14.88)	13.50 (12.15-14.43)	14.00 (10.18-15.83)	0.97
Lactate dehydrogenase, U/L	165.50 (136.25-199.00)	173.50 (141.00-188.75)	162.50 (139.00-204.00)	0.77
Alanine aminotransferase, U/L	18.90 (13.93-28.13)	18.90 (10.75-33.90)	18.55 (13.98-25.33)	0.97
Aspartate aminotransferase, U/L	19.55 (15.40-28.68)	24.25 (13.90-29.28)	19.00 (16.13-23.75)	0.77
Total bilirubin, μmol/L	7.70 (5.32-10.20)	6.89 (5.32-8.65)	7.71 (6.15-11.64)	0.46
Blood urea nitrogen, mmol/L	3.90 (2.70-4.60)	3.75 (2.60-4.45)	4.25 (3.00-5.05)	0.23
Creatinine, μmol/L	66.30 (53.80-79.83)	73.70 (56.53-82.60)	62.65 (53.15-75.93)	0.31
Procalcitonin, ng/L <0.1, N (%)	16 (72.73)	7 (70)	9 (75)	0.99
C-reactive protein, mg/L	5.51 (2.21-17.78)	4.07 (1.46-14.93)	6.95 (3.63-20.05)	0.35
Albumin, g/L	38.50 (37.60-39.38)	39.00 (37.85-42.33)	38.10 (36.88-38.65)	0.14
Signs and symptoms at admission				
Fever	14 (63.6)	7 (70)	7 (58.3)	0.67
Fatigue	4 (18.2)	2 (20)	2 (16.7)	0.99
Dry cough	8 (36.4)	4 (40)	4 (33.3)	0.99
Anorexia	1 (4.5)	0 (0)	1 (8.3)	0.99

Myalgia	3 (13.6)	1 (10)	2 (16.7)	0.99
Expectoration	2 (9.1)	1 (10)	1 (8.3)	0.99
Pharyngalgia	6 (27.3)	4 (40)	2 (16.7)	0.35
Dizziness	2 (9.1)	0 (0)	2 (16.7)	0.23
Headache	1 (4.5)	0 (0)	1 (8.3)	0.99

Data are presented as number of participants (%) or median (IQR).
Abbreviations: IQR, interquartile range.
***** *P* values indicate differences between patients in the Chloroquine group and the Lopinavir/Ritonavir group. $P < 0.05$ was considered statistically significant.

Supplementary Table S2. Incidence of virologic, clinical, and imaging outcomes

	Chloroquine (n=10)		Lopinavir/Ritonavir (n=12)		Risk Ratio (95% CI)	Rate Ratio (95% CI)
	N (%)	Person-time, day	N (%)	Person-time, day		
RT-PCR negative at						
Day 14	10 (100)	69.5	11 (91.67)	81.5	1.09 (1,1.33)	1.07 (0.44,2.56)
Day 10	9 (90)	66.5	9 (75)	75.5	1.20 (0.84,2.00)	1.14 (0.44, 2.95)
Day 7	7 (70)	59.5	7 (58.33)	64	1.20 (0.60,2.40)	1.08 (0.36, 3.20)
CT scan improvement at						
Day 14	10 (100)	101	9 (75)	134	1.33 (1.00,2.00)	1.47 (0.59, 3.75)
Day 10	2 (20)	96	1 (8.33)	120	2.4 (0.14, 12.32)	2.35 (0.19, 73.73)
Clinical outcomes						
Hospital discharge at Day 14	10 (100)	115	6 (50)	154	2 (1.33,4.00)	2.21 (0.81, 6.62)
Clinical recovery at Day 10	8 (80)	86.5	7 (58.33)	109	1.37 (0.80,2.80)	1.44 (0.51, 4.17)

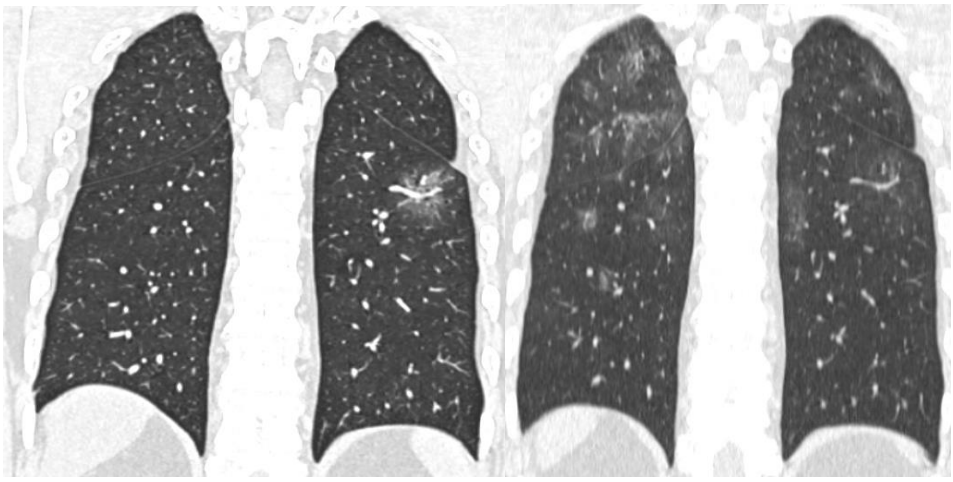
Abbreviations: CI, confidence interval; CT, computed tomographic; RT-PCR, real-time reverse-transcription-polymerase-chain-reaction.
95% CI for the risk ratio was calculated using Bootstrap method. 95% CI for the rate ratio was calculated using exact method.

Supplementary Table S3. Number of adverse events by two treatment groups

	Chloroquine (n=10) N (%)	**Lopinavir/Ritonavir (n=12) N (%)**	***P* Value***
Total No. of Adverse Events	9 (90)	10 (83.33)	0.99
Gastrointestinal			
Vomiting	5 (50)	1 (8.33)	0.06
Abdominal pain	1 (10)	2 (16.67)	0.99
Nausea	4 (40)	5 (41.67)	0.99
Diarrhea	5 (50)	8 (66.67	0.67
Neurological			
Dizziness	0 (0)	2 (16.67)	0.48
Headache	0 (0)	1 (8.33)	0.99
Psychosis	0 (0)	1 (8.33)	0.99
Rash or itchy	1 (10)	0 (0)	0.45
Respiratory			
Cough	4 (40)	6 (50)	0.69
Shortness of breath	1 (0.1)	4 (33.33)	0.32

5 patients with 9 adverse events were observed in the Chloroquine treatment period. No serious adverse events were observed during the follow-up period in the present study for all participants.
n = number of patients evaluable for adverse events, N = Number of adverse events.
***** *P* value was calculated using Fisher's exact tests.

Lung CT scan results at baseline and after Chloroquine therapy

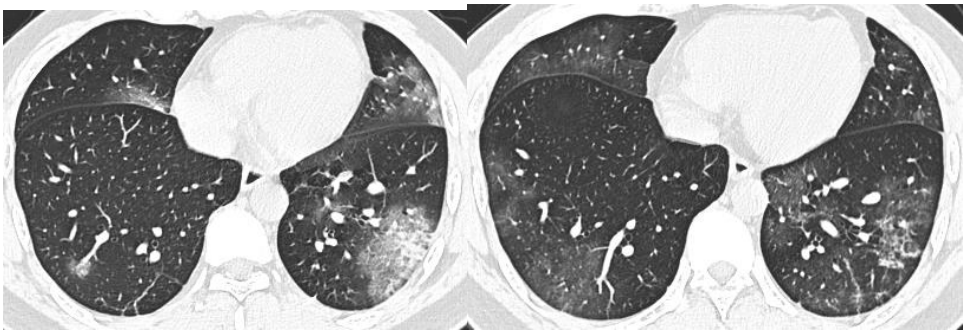


2020.01.27 2020.02.13

ID: C1, Age: 52Y, male

Baseline CT（left）：Coronal thin-section plain CT shows patchy ground-glass opacities in the dorsal segment of left lower lobe. The lesion's edge is blurred. The small vessel within the lesion is thickened.

Follow-up CT（right）：Coronal thin-section plain CT shows the size and density of lesion in the dorsal segment of left lower lobe is obviously decreasing; The density of other lesions in lung is low, and this is a manifestation of the absorption period.



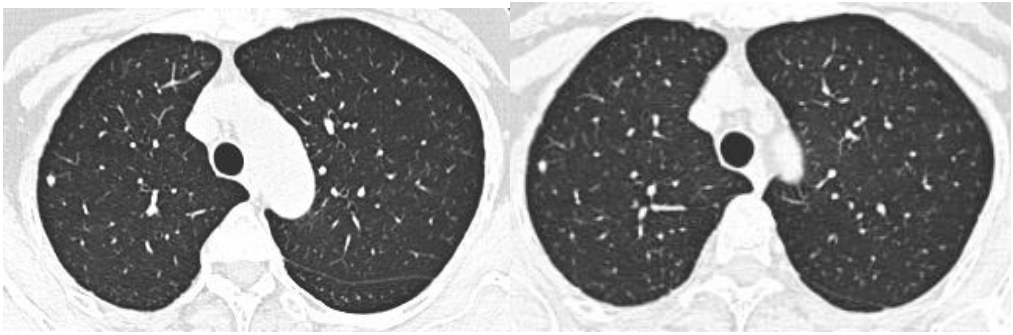
2020.01.28 2020.02.09

ID: C2, Age: 36Y, male

Baseline CT（left）：Transversal thin-section plain CT shows multiple patchy ground-glass opacities and consolidation in bilateral lung. The intralobular septal within the lesion is thickened. There is a "crazy paving" pattern in the left lower lobe.

Follow-up CT（right）：Transversal thin-section plain CT shows the area of lesion is slight

enlarge, but the density of lesion is obviously decreasing, and fibrosis is seen in the right lower lobe. This suggests that the patient is in the absorption phase.

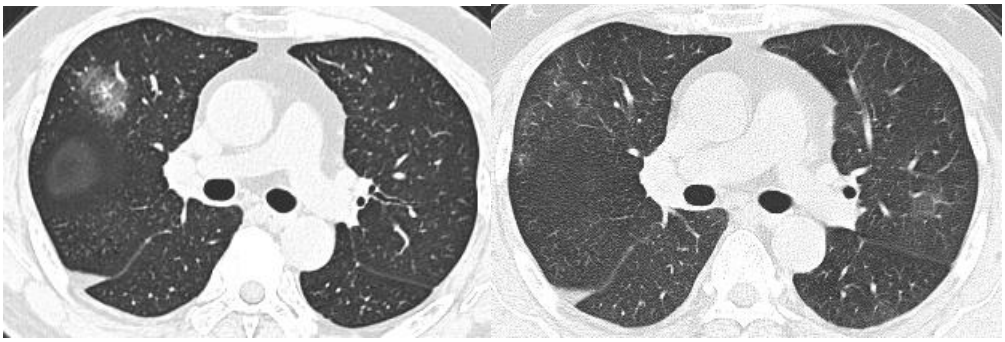


2020.01.28 2020.02.09

ID: C3, Age: 55Y, Female

Baseline CT（left）：Transversal thin-section plain CT shows a small nodule in right upper lobe.

Follow-up CT（right）：There is no change from the baseline.

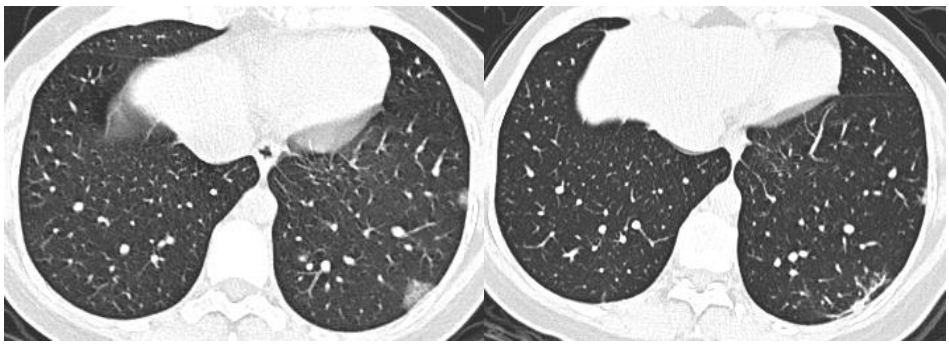


2020.01.27 2020.02.13

ID: C4, Age: 64Y, male

Baseline CT（left）：Transversal thin-section plain CT shows patchy ground-glass opacities in the right upper lobe. The lesion’s edge is blurred. The intralobular septal and small vessel within the lesion is thickened.

Follow-up CT（right）：Transversal thin-section plain CT shows the size and density of lesion in right upper lobe is obviously decreasing; The thin shadow in the left upper lobe is a manifestation of the absorption period.

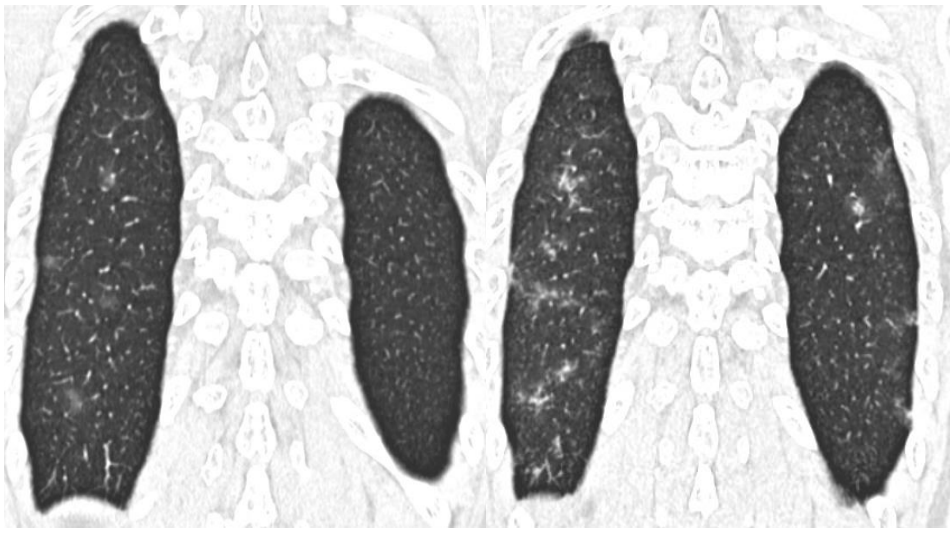


2020.01.28 2020.02.08

ID: C5, Age: 33Y, Female

Baseline CT（left）：Transversal thin-section plain CT shows patchy ground-glass opacities in subpleural zone of the left lower lobe. The intralobular septal within the lesion is thickened.

Follow-up CT（right）：Transversal thin-section plain CT shows the lesion is significantly narrowed, and fibrosis is seen.

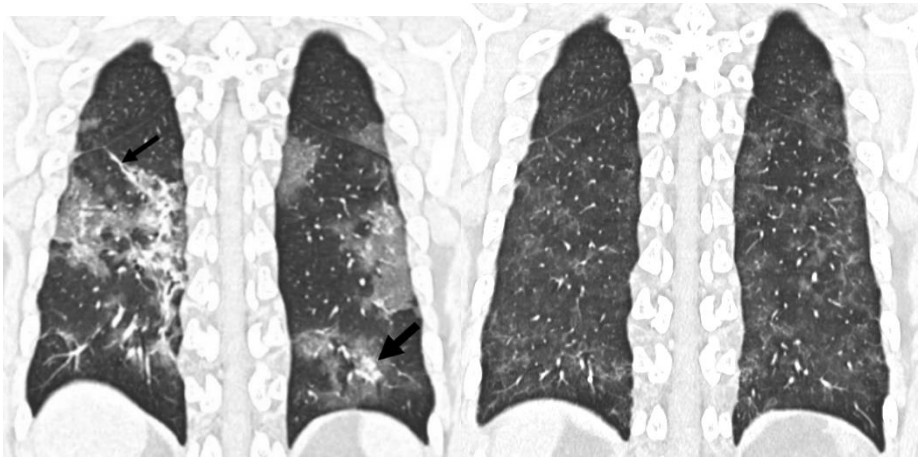


2020.01.28 2020.02.09

ID: C6, Age: 32Y, male

Baseline CT（left）：Coronal thin-section plain CT shows multiple patchy ground-glass opacities in the right lung. The lesion’s edge is blurred.

Follow-up CT（right）：Coronal thin-section plain CT shows the number of lesions is increased. However, the lesions are mainly fibrous cord lesions, rather than exudative lesions, which indicates that the patient is in the absorption phase.

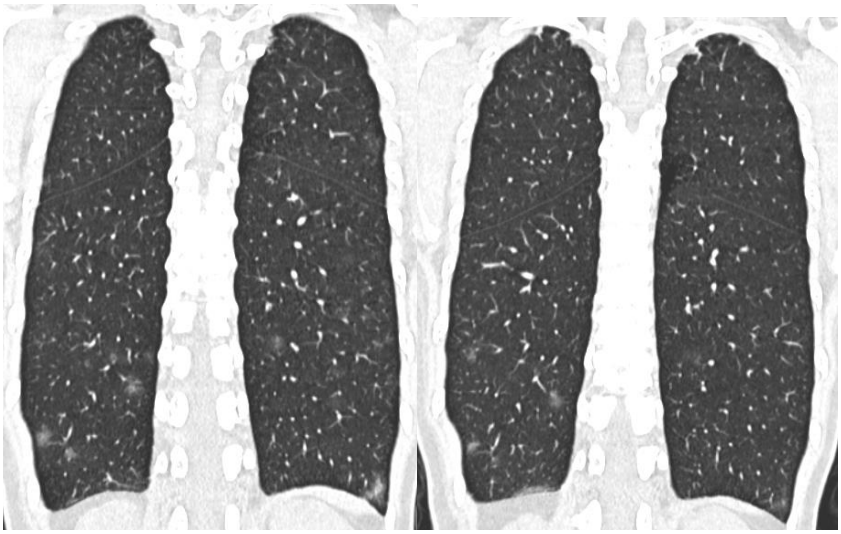


2020.01.29 2020.02.09

ID: C7, Age: 42Y, male

Baseline CT（left）：Coronal thin-section plain CT shows multiple patchy ground-glass opacities in both sides of lung. Consolidation（thick arrow） and linear opacities（thin arrow） can be found in lower lobes.

Follow-up CT（right）：The size and density of lesion in both sides of lung is reducing. The previous consolidation and linear opacities is obviously absorbed.

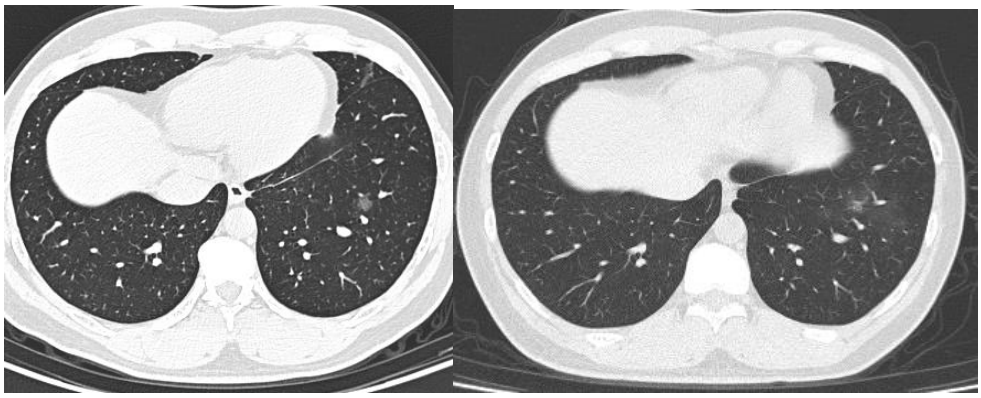


2020.01.30 2020.02.09

ID: C8, Age: 41Y, Female

Baseline CT（left）：Coronal thin-section plain CT shows multiple patchy ground-glass opacities in the left upper lobe and lower lobes of lung , with blurring edge.

Follow-up CT（right）：Coronal thin-section plain CT shows the lesion in the left upper lobe has absorbed, and the range and density of the lesion in lower lobes is decreasing.

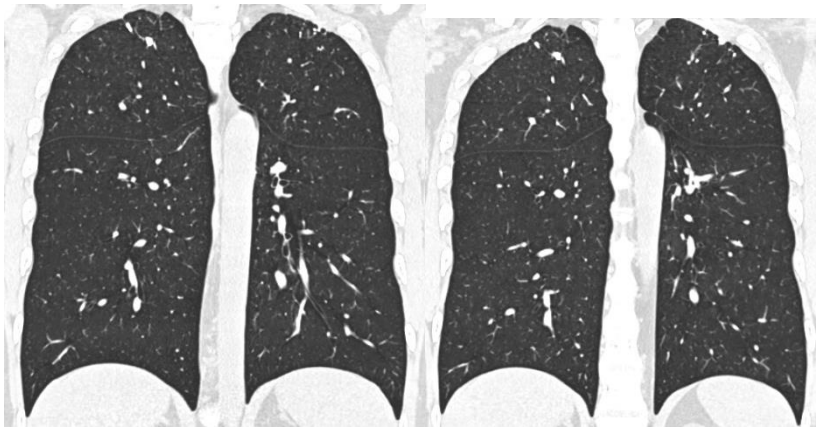


2020.01.30 2020.02.12

ID: C9, Age: 44Y, male

Baseline CT（left）：Transversal thin-section plain CT shows patchy ground-glass opacities in the left lower lobe.

Follow-up CT（right）：Transversal thin-section plain CT shows the density of the lesion in left lower lobe is decreasing. The thin shadow around the lesion is a manifestation of the absorption period.



2020.01.31 2020.02.09

ID: C10, Age: 29Y, Male

Baseline CT（left）：Coronal thin-section plain CT shows fibrosis and calcified foci at the pulmonary apex, with clear margin.

Follow-up CT（right）：There is no change from the baseline.