

Efficacy of Chloroquine and Lopinavir/Ritonavir in mild/general COVID-2019: a prospective, open-label, multicenter randomized controlled clinical study

CURRENT STATUS: UNDER REVIEW

 Trials  BMC

Xi Liu

The Fifth Affiliated Hospital of Sun Yat-sen University

Huili Chen

The Fifth Affiliated Hospital of Sun Yat-sen University

Yuqi Shang

The Fifth Affiliated Hospital of Sun Yat-sen University

Hongqiong Zhu

Fifth Affiliated Hospital of Sun Yat-sen University

Gongqi Chen

Fifth Affiliated Hospital of Sun Yat-sen University

Yuanli Chen

Fifth Affiliated Hospital of Sun Yat-sen University

Shaoxuan Liu

Fifth Affiliated Hospital of Sun Yat-sen University

Yaoyong Zhou

Fifth Affiliated Hospital of Sun Yat-sen University

Mingxing Huang

The Fifth Affiliated Hospital of Sun Yat-sen University

Zhongsì Hong

Fifth Affiliated Hospital of Sun Yat-sen University

Jinyu Xia

The Fifth Affiliated Hospital of Sun Yat-sen University

✉ xiajinyu@mail.sysu.edu.cn *Corresponding Author*

DOI:

10.21203/rs.3.rs-16392/v1

SUBJECT AREAS

Internal Medicine *Integrative & Complementary Medicine*

KEYWORDS

COVID-2019, Chloroquine, Lopinavir/ Ritonavir, Clinical study, Efficacy

Abstract

Background The outbreak of novel coronavirus pneumonia is very serious, and no effective antiviral treatment has been confirmed. The fresh drug research and development cycle is too long to meet clinical emergency needs, and "old drugs and brand new applications" have a huge therapeutic potential. During our previous treatment, we found that the lopinavir/ritonavir treatment recommended in the Fifth edition of the treatment plan had little effect. Earlier studies have shown that chloroquine can inhibit coronavirus replication through multiple mechanisms. Our previous use of chloroquine to treat patients with SARS-CoV-2(novel coronavirus)-infected pneumonia has a higher negative rate of nucleic acid in throat swabs within 5 days after administration than that using lopinavir/ritonavir. However, the half-life and side effects of chloroquine vary greatly among individuals.

Methods/design We plan to conduct a prospective, open-label, multicenter randomized controlled, comprehensive treatment clinical study. The study consisted of three phases: a screening period of 1-110 days, a treatment period of no more than 28 days, and a follow-up period of 1 month. Participants will be assessed at baseline and on days 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 14, 21, and 28 after the intervention begins. In this study, chloroquine and lopinavir/ritonavir tablets were used to treat patients with eligible novel coronavirus pneumonia diagnosed at various centers between February 12, 2020 and May 31, 2020. The efficacy and safety of chloroquine and lopinavir/ritonavir are to be evaluated. At the same time, explore the correlation between patient genetic polymorphisms and chloroquine steady-state concentration, therapeutic effects and adverse reactions in the body. It is an anti-virus for pneumonitis caused by novel coronavirus. The optimization and update of the antiviral treatment plan provides evidence-based evidence.

Discussion Our study is a prospective, open-label, multicenter randomized controlled,

comprehensive treatment clinical study to evaluate the efficacy and safety of chloroquine phosphate and lopinavir/ritonavir in patients with mild/general COVID-2019. The results of this study will provide valuable clinical evidence for the treatment of novel coronavirus pneumonia.

Background

In December 2019, patients with unexplained pneumonia appeared in some medical institutions in Wuhan, China, which were subsequently identified as a novel type of coronavirus. On January 30, 2020, the virus was named by the World Health Organization as “COVID-2019 (Corona Virus Disease-2019)”. At present, the COVID-2019 epidemic is spreading in every provinces and cities in China. As of February 17, 2020, 72,436 confirmed cases, 6,242 suspected cases, and 1,868 deaths have been reported.

Coronavirus is a single-stranded positive-strand RNA(Ribonucleic Acid) virus with an envelope¹. There are six HCoVs (Human Corona Viruses), except SARS-CoV-2 (Severe Acute Respiratory Syndrome Corona Virus-2), are known to cause respiratory infections, of which SARS-CoV in 2002 and MERS-CoV (Middle East Respiratory Syndrome Corona Virus) are highly pathogenic viruses that have caused outbreaks worldwide or in some regions. The other four human coronaviruses (HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1) are common pathogens that cause human upper respiratory tract infections, accounting for about 15–30% of all pathogens.

There are currently no clinically specific drugs for these 7 HCoVs. An effective treatment is urgently needed to control the COVID-2019 epidemic. The National Health Commission of China announced a new coronavirus-infected pneumonia diagnosis and treatment program (the Fifth edition), which proposed the trial of lopinavir/ritonavir(CYP3A inhibitor, also metabolized by CYP3A). However, our previous use found that the effect of

lopinavir/ritonavir on COVID-2019 was unsatisfactory (data was not shown).

Two independent research teams found that chloroquine has anti-SARS-CoV activity at the cellular level. Chloroquine phosphate can inhibit virus replication in Vero E6 cell line induced by SARS-CoV, with a 50% inhibitory concentration (IC₅₀) of $8.8 \pm 1.2 \mu\text{m}$, which is close to the chloroquine plasma concentration achieved during the treatment of acute malaria². It is significantly lower than the 50% cytostatic concentration (CC₅₀ = $261.3 \pm 14.5 \mu\text{m}$), suggesting the safety of chloroquine for this cell line. At the same time, the antiviral activity of chloroquine can be extended to 5 hours after infection without a significant decrease. A study by the United States Centers for Disease Control and Prevention further clarified that chloroquine inhibits virus replication by reducing terminal glycosylation of angiotensin-converting enzyme 2 (ACE2) receptors on Vero E6 cells and interfering with the binding of SARS-CoV and ACE2 receptors³. HCoV-229E and SARS-CoV both belong to the α group HCoVs. Chloroquine could inhibit the replication of HCoV-229E on the L132 human embryonic lung cell line by inhibiting p38 mitogen-activated protein kinase (MAPK) activation⁴. The latest study found that the S (spike) protein of SARS-CoV-2 is similar in structure with that of SARS-CoV⁵, and can also bind to the ACE2 receptor on the surface of host cell through the S protein, thereby infecting the epithelial cells of the host. At the cellular level, remdesivir (GS-5734) and Chloroquine (Sigma-C6628) can be effective inhibit the infection of SARS-CoV-2 in vitro⁶.

Based on the above evidence, we first use a prospective, open-label, multicenter randomized controlled clinical study to evaluate the efficacy of anti-viral treatment of chloroquine phosphate compared with lopinavir/ritonavir in patients diagnosed with mild/general type SARS-CoV-2 infection. Previous studies have shown that chloroquine phosphate has a good antiviral effect in the clinic (Data was not shown), and has been

highly valued by Guangdong Province and even the National Health Commission. It has been included in the National Health Commission novel coronavirus pneumonia diagnosis and treatment plan (trial version 6) on February 19, 2020. However, the effectiveness and safety of chloroquine (phosphate) require more evidence-based medical evidence.

Our objective is to evaluate the safety of the drug, and to explore the correlation between drug concentration and treatment effect and adverse reactions by measuring the blood concentration of chloroquine phosphate.

Methods/design

Background and design: This study is a prospective, open-label, multicenter randomized controlled, comprehensive treatment clinical study. Eligible participants will be randomly assigned to the experimental group (chloroquine phosphate group) and the control group (lopinavir/ritonavir), with 56 patients in each group. Participants will be recruited from 4 hospitals, including the Fifth Hospital Affiliated of Sun Yat-sen University, the Ninth People's Hospital of Dongguan, Zhongshan Second People's Hospital, and the Jiangmen Central Hospital. The study consisted of three phases: a screening period of 1-110 days, a treatment period of no more than 28 days, and a follow-up period of 1 month. Participants will be assessed at baseline and on days 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 14, 21, and 28 after the intervention begins. This study investigates the use of chloroquine and lopinavir/ritonavir tablets in patients diagnosed with novel coronavirus pneumonia (in line with the inclusion and exclusion criteria) between February 12, 2020 and May 31, 2020, to explore the safety of chloroquine and lopinavir/ritonavir tablets in the treatment of patients with COVID-2019, the appropriation of antiviral treatment, the correlation between steady-state concentration of chloroquine phosphate and treatment effects and adverse reactions, and the correlation of patient genetic polymorphisms with treatment effects and adverse reactions. The flow chart of the research process is shown in Fig. 1.

The schedule of treatment visits and data collection (also known as Clinical Research Flowchart) is shown in Table 1.

Table 1
The schedule of treatment visits and data collection.

	Baseline phase	Research Phase											End
TIMEPOINT	D0	D1	D2	D3	D4	D5	D6	D7	D...	D10	D...	D14	D28
Inclusion and grouping													
Screening cases	X												
Informed consent	X												
Inclusion group	X												
Baseline data collection	X												
Drug-assisted therapy													
Develop a Dosing Plan	X												
Assess drug-related adverse reactions		X	X	X	X	X	X	X	X	X	X	X	X
Evaluation of serious adverse events													
Clinical and laboratory data collection													
Vital signs	X	X		X		X		X		X		X	X
SOFA score	X	X		X		X		X		X		X	
Arteri	X	X		X		X		X		X		X	

al blood gas analysis													
Blood routine	X	X		X		X		X		X		X	
Coagulation index	X	X		X		X		X		X		X	
Inflammatory factors	X	X		X		X		X		X		X	
Liver and kidney function	X	X		X		X		X		X		X	
CRP	X	X		X		X		X		X		X	
PCT	X	X		X		X		X		X		X	
Muscle enzyme + myoglobin	X	X		X		X		X		X		X	
Throat swab nucleic acid	X	X	X	X	X	X	X	X	X	X		X	
ECG	X					X							
Chloroquine blood concentration	X	X		X		X		X		X		X	X

D0: Before the research; D1: The 1st day of the research; D2: The 2nd day of the research; D3: The 3rd day of the research; D4: The 4th day of the research; D5: The 5th day of the research; D6: The 6th day of the research; D7: The 7th day of the research; D10: The 10th day of the research; D14: The 14th day of the research; D28: The 28th day of the research.

Participants:

Inclusion criteria

The study inclusion criteria are as follows:(All the following criteria are met before being selected)

- (1) Age ≥18 years;
- (2) Meet all the following criteria (refer to confirmed cases in the Diagnosis and Treatment of pneumonitis caused by novel coronavirus (trial version 5)):
 - ① Epidemiological history;
 - ② Clinical manifestations (in accordance with any 2 of the following): fever; normal or decreased white blood cell counts in the early stages of disease, or decreased lymphocyte

counts; multiple small patchy shadows and interstitial changes in the early stage of chest imaging, which are evident with the extrapulmonary zones. Furthermore, it develops multiple ground glass infiltrations and infiltrates throughout both lungs. In severe cases, pulmonary consolidation may occur, and pleural effusion is rare.

③ Confirmed: The suspected case has one of the following pathogenic evidence: respiratory specimens, blood specimens, or stool specimens are detected by real-time fluorescent RT-PCR(Reverse Transcription PCR) to detect positive of novel coronavirus nucleic acid; the above-mentioned specimens are genetically sequenced and highly homologous to known novel coronavirus.

④ mild or general patients;

⑤ Those who have not used antiviral drugs.

Exclusion criteria

The exclusion criteria are as follows:

(If the subject meets any of the following conditions, they cannot enter the study)

1. Patients with a history of allergy to chloroquine phosphate, lopinavir, and ritonavir;
2. Patients with hematological diseases;
3. Patients with chronic liver and kidney disease and reaching the end stage;
4. Patients with arrhythmia and chronic heart disease;
5. Patients known to have retinal disease, hearing loss;
6. Patients known to have mental illness;
7. Patients who must use digitalis because of the original underlying disease;
8. Pancreatitis;
9. Hemophilia;
10. Broad bean disease;
11. Female patients during pregnancy.

Sample size

In this study, Logrank method was used to compare the difference in clinical recovery time between the two groups of patients. According to the existing research, the median clinical recovery time of the control group is 8 days, and the experimental group is 4 days. According to the type I error $\alpha = 0.05$, the test power is 0.85, and the ratio between the test group and the control group is 1: 1. Considering the 5% shedding rate, the number of statistical cases is no less than 56 respectively in the two groups (the experimental group and the control group), and a total of 112 subjects are included in the study.

Recruitment

Participants will be recruited from SARS-CoV-2 infected inpatients. The volunteers will be screened to determine if they meet the basic criteria. Once volunteered participants have been included or excluded from the criteria assessment, researchers will explain the research procedures in detail and require them to sign a written informed consent form (written informed consent form signed by the subject or his legal representative) . All participants can withdraw their consent at any time during the trial.

Randomization allocation and blinding

Grouping was performed using a block randomization method. Before the experiment, a statistical expert randomly assigns 1 to 112 numbers according to the statistical software to generate a random allocation table. The selected block length and random seed number are stored together with the statistical expert. According to the random allocation table in advance, the statistical expert gives random numbers (1-112) in ascending order. Each random number corresponds to an envelope. The envelope contains the corresponding random number. The envelope is sealed and given to the researchers responsible for screening. The qualified subjects are selected, and the envelopes are received in the order of enrollment. After the envelopes are opened, the random number is taken out and

given to the researcher responsible for treatment and observation. The researcher will contact the statistician. The statistician informs the group corresponding to the random number according to the random allocation table, so that the subjects will be randomly assigned to the experimental group or the control group, and the corresponding treatment and observation were performed. Each subject's random number is unique and remains the same throughout the trial.

Interventions

The study subjects were divided into experimental group and control group for corresponding treatment regimens.

1. Experimental group (chloroquine phosphate): Chloroquine phosphate tablets were administered twice a day, 0.5 g each time (equivalent to 0.3 g of chloroquine). Novel coronavirus nucleic acids in respiratory tract samples continue to be negative for 3 days before discontinuation of the drug, the total course of treatment does not exceed 10 days.

Combination prohibited: Digitaloid drugs, Amiodarone, Domperidone, Droperidol, Haloperidol, Clarithromycin, Methadone, Procainamide, Hydrochlorothiazide, Cisapride, Indapamide, Quinolones.

Avoid co-administration: Phenylbutazone, Chlorpromazine, Streptomycin, Heparin, Penicillamine, Ammonium Chloride, Monoamine Oxidase Inhibitors, Triamcinolone.

2. Control group (lopinavir/ritonavir): lopinavir/ritonavir is administered twice a day, two tablets each time (equivalent to 400mg of lopinavir / 100mg of ritonavir). Novel coronavirus nucleic acids in respiratory tract samples continue to be negative for 3 days before discontinuation of the drug, the total course of treatment does not exceed 10 days. This drug is a CYP3A inhibitor and is also metabolized by CYP3A. It cannot be combined with drugs that mainly rely on CYP3A for clearance and high blood concentrations that can cause serious or fatal adverse events.

Combination prohibited: Lovastatin, Simvastatin, Cisapride, Quetiapine, Dronedarone, Colchicine, Rifampicin, Rifapentine, Bromocriptine, Ranolazine, Midazolam (oral), Triazolam, Elbasvir, Grazoprevir, Pibrentasvir.

Avoid co-administration: Atorvastatin, Rosuvastatin, Domperidone, Amiodarone, Disopyramide, Quinidine, Voriconazole, Clarithromycin, Alprazolam, Diazepam, Clonazepam, Niratinib, Abemaciclib.

Researchers fill out the inpatient medical records at the same time when the subjects are being treated, ensuring that the data records are timely, complete, accurate and true. At the same time, the researcher fills out the case report form after the diagnosis and treatment of the subjects in time to ensure that the content of the case report form is consistent with the content on the outpatient or inpatient medical records. After each subject observes the treatment course, the researcher should fill out the relevant data on the case report form in time, and submit it to the main researcher at the center for review and signature confirmation.

Outcomes:

Primary outcome

The clinical recovery time (not more than 28 days), that is, the time (in hours) from the start of study drug intervention to normalization of body temperature, respiratory symptoms, respiratory frequency, and blood oxygen saturation. Specifically meet the following criteria at the same time:

- ①No fever, Axillary body temperature ≤ 37.2 °C;
- ②Relief of respiratory symptoms (72 consecutive hours);
- ③Respiration rate ≤ 24 / minute (resting state);
- ④Fingertip blood oxygen $> 94\%$;

Secondary outcome(not more than 28 days)

- ①Respiratory tract sample SARS-CoV-2 RT-PCR negative for two consecutive times (calculated based on the first time);
- ②Respiratory tract, blood, feces or all other samples to SARS-CoV-2 RT-PCR were negative twice in a row (both calculated at the first time);
- ③Death from all causes;
- ④Time for body temperature to return to normal (calculated from the onset of illness);
- ⑤The time of mild cough or no cough (cough is severe or moderate when enrollment);
- ⑥Time of progress to severity, according to the Diagnosis and Treatment of pneumonitis caused by novel coronavirus (trial version 5), the definition of severity is to meet any of the following: respiratory frequency greater than 30 times/minute, or fingertip blood oxygen $\leq 94\%$ or PaO_2 (Partial Pressure of Oxygen)/ FiO_2 (Fraction of inspiration O_2) $< 300\text{mmHg}$;
- ⑦The time for the improvement of chest imaging (chest CT), the improvement of imaging is determined by the professional doctor of radiology based on the reduction of the scope of the lesion and the decrease in density;
- ⑧Frequency of serious adverse events.

Safety assessment and adverse events

Test safety is monitored throughout the test. Typical laboratory safety tests include routine tests for blood, urine, liver function, eg: ALT(Alanine aminotransferase) and AST(Aspartate aminotransferase), and renal function, eg: blood urea nitrogen(BUN) and creatinine(Cr) will be performed during the treatment period. Along with treatment, safety will also be assessed by monitoring adverse events(AEs) and vital signs. An adverse event(AE) is defined as an adverse medical event that occurs during a patient or subject's treatment in a study, but is not necessarily causally related to treatment. Severe Adverse Event(SAE): Occurrence of hospitalization, prolonged hospital stay, disability, impact on

work ability, life-threatening or death during clinical research.

The investigator should carefully observe any adverse events that occur in the subject during the clinical study. When an adverse event is found, the investigator can take necessary measures according to the condition. Regardless of whether the adverse event is related to chloroquine phosphate or lopinavir/ritonavir, the investigator should record it in detail in the case report form. The record of the adverse event should include: description of the adverse event and all related events, time of occurrence, Severity, duration, measures taken, and final results and outcomes to analyze the association of adverse events with chloroquine phosphate or lopinavir/ritonavir. Sign and date when recording.

Quality control

This study will be conducted in four hospitals and the following five measures will be taken to ensure its rigor and quality.

(1) Quality control measures

Researchers should adopt standard operating procedures to ensure the quality control of clinical research and the implementation of quality assurance systems. All observations and findings in clinical research should be verified to ensure the reliability of the data and to ensure that the conclusions in the clinical research are derived from the original data. Quality control must be used at each stage of data processing to ensure that all data is reliable and processed correctly.

(2) Researchers' training

Before the formal start of clinical research, the responsibilities and attitudes of the researchers participating in the clinical research must be emphasized first. The head of the research center should train the researchers on the research plan, to help them to unify the knowledge, be familiar with the collection methods and procedures, and

understand the special requirements of the research project, to improve the internal observational consistency and inter-observer consistency of clinical research data collectors, to ensure the reliability of clinical research conclusions. At the same time, researchers are required to strictly implement standard operation procedures(SOPs) throughout the clinical research process to ensure the implementation of quality control measures for clinical research and improve the quality of clinical research and case report forms.

(3) Measures to improve subject compliance

Investigators should conscientiously implement an informed consent so that subjects fully understand the research requirements and cooperate with the research.

(4) Monitoring of clinical research

A qualified auditor is appointed, and regular on-site inspections of the research center are conducted to ensure that all the contents and requirements of the research plan are strictly observed, and the original data is checked to ensure consistency in the content on the CRF(Clinical Research Flowchart).

(5) Audit of clinical research

The clinical research management department and the project responsible unit may entrust the inspectors to conduct a systematic inspection of the clinical research to determine whether the research execution is consistent with the research plan, and whether the reported data is consistent with the records of the clinical participating units, that is, whether the data recorded in the case report form is the same as that of the medical record or other original records. The audit should be performed by personnel who are not directly involved in the clinical study.

Statistical analyses

(1) Balance analysis of baseline values

For demographic data and other baseline values such as vital signs, disease history, and basic treatment, in order to measure the balance of each group, continuous comparison test was used to compare the count data between groups. When the theoretical frequency in the fourfold table is less than 5, Fisher's exact probability method is used; Measurement data normally distributed are compared using group t-tests, otherwise, comparisons between groups are tested using Wilcoxon Rank Sum. Baseline evaluations were performed on FAS(full analysis set).

(2) Effectiveness analysis

The comparison of the main efficacy indicators' clinical recovery time was a log-rank test. If the influence of confounding factors needs to be considered, Cox proportional hazard model can be used. For the comparison of other efficacy indicators, t-test or Wilcoxon rank sum test was used for comparison of measurement data, and test or Fisher's exact probability method was used to compare the rate differences between groups.

(3) The negative rate and adverse reaction rate were the dependent variables on the 7th, 14th, and 21st days in the two groups. Its steady-state valley concentration is taken as the independent variable, Logistic regression analysis and receiver operating characteristic (ROC) curve analysis after assignment. Investigate the correlation between blood concentration and clinical efficacy and adverse reactions.

(4) The safety analysis

Use the test or Fisher's exact probability method to compare the incidence of adverse events in each group, and list the adverse events occurred in this study; the normal/abnormal changes in laboratory test results before and after the test, and the relationship with the test drug when abnormal changes occur, apply a statistical test when necessary.

Clinical trial registration

The trial was registered under the registration number ChiCTR2000029741(<http://www.chictr.org.cn/showproj.aspx?proj=49263>) on 11 February 2020. On February 10, 2020, this research was approved by the Medical Ethics Committee of the Fifth Affiliated Hospital of Sun Yat-sen University, ZDWY[2020] Lunzi No. (K15-1).

Discussion

Chloroquine is a known 4-aminoquinoline that has been used clinically since 1944. In addition to being used as an antimalarial drug, chloroquine is also used for the treatment of autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, etc. due to its immunomodulatory activity⁷. In terms of physical and chemical properties, chloroquine dissolves in water and is weakly alkaline. After entering the cell, it can accumulate in cytoplasmic acidic organelles such as lysosomes and the reverse Golgi networks through protonation, increase its pH(hydrogen ion concentration) value, destroy the structure and function of organelles. Taking typical lysosomes of acidic organelles as an example, chloroquine mediates the increase of lysosome pH in vivo, to attenuate the release of iron ions, decrease the iron ion content in cells, and then interfere with intracellular DNA(Deoxyribonucleic Acid) replication and gene expression in cells⁸. Previous studies have shown that chloroquine exerts antiviral effects through different mechanisms. Because chloroquine can change the pH value of endosomes, it has a significant inhibitory effect on viral infections that invade cells through the endosome pathway, such as Borna Disease Virus⁹, avian Leukemia Virus¹⁰, and Sika Virus¹¹. At the same time, chloroquine can affect viral replication by inhibiting viral gene expression. In vitro and in vivo experiments have shown that chloroquine can change the glycosylation pattern of HIV gp120 envelope and inhibit the replication of HIV(Human Immunodeficiency Virus) in CD4 + T cells¹². In addition, chloroquine also acts as a good autophagy inhibitor

and interferes with virus infection and replication by affecting autophagy. Animal tests have shown that the application of chloroquine can effectively inhibit autophagy in the lungs of avian influenza H5N1 mice and reduce alveolar epithelial damage¹³. Recently, it has been reported that chloroquine can block Sika Virus-induced autophagy, thereby inhibiting virus replication, and it has been shown in mouse experiments that chloroquine can cut off vertical infection of Sika Virus from the maternal-fetal pathway¹⁴.

In our research, professional researchers specialize in recording. At the same time, we appoint qualified supervisors to conduct on-site comprehensive inspection visits to the research center. In order to ensure the accuracy of numerical data, Epidata 3.1 is used for data double-checking. The data is entered and proofread. For the questions in the case report form, the data administrator will fill them in the Data Rating Questionnaire(DRQ) and send an inquiry to the researcher through the clinical monitor. The data administrator will modify the data according to the researcher's response, confirm and enter, and issue a DRQ again if necessary. The verification of data will be divided into manual verification and systematic verification. Data can be locked when the following whole conditions are met: ① all data have been entered into the database and double-checked; ② all questions have been resolved; ③ The analysis of the crowd has been defined and judged. After the data is locked, submit the database to the statistical analyst for statistical analysis according to the requirements of the statistical plan, and complete the statistical analysis report. These measures can help improve the reliability and generality of the assessment results.

The trial will be conducted in a clinical outpatient and inpatient setting by experienced clinicians, and participants will be recruited from the patients base of the other three hospitals participating in the trial. The purpose of this prospective, open-label, multicenter

randomized controlled, comprehensive clinical study is to evaluate the efficacy and safety of chloroquine phosphate and lopinavir/ritonavir in patients with mild/general COVID-2019. The results of this study will provide meaningful information and evidence for clinical practice and will help design a proven and reasonable RCT(Randomized Controlled Trial) soon.

This study will confirm the safety and effectiveness of chloroquine in the diagnosis of mild/general patients with COVID-2019, and evaluate whether chloroquine is antiviral. At the same time, investigate the correlation between drug concentration and treatment effect and adverse reactions by measuring blood concentration of chloroquine phosphate.

Limitations

Randomized controlled studies still have some design limitations. First, the sample size is relatively small and the 28-day treatment period is shorter. We will not be able to estimate the possible relapse of pneumonia after long-term treatment. Second, the pathophysiology of novel coronavirus pneumonia has not been elucidated. Only clinician assessment (including lung CT results and viral accounting load), there is no objective indicator to judge the effect of treatment on COVID-2019. Finally, the follow-up period in this study was relatively short. In light of these limitations, we will develop a more reasonable treatment cycle and follow-up period to explore the efficacy of chloroquine in patients with COVID-2019.

Trial status The trial was registered under the registration number ChiCTR2000029741(<http://www.chictr.org.cn/showproj.aspx?proj=49263>) on 11 February 2020. On February 10, 2020, this study was approved by the Medical Ethics Committee of the Fifth Affiliated Hospital of Sun Yat-sen University in Zhuhai, ZDWY[2020] Lunzi No. (K15-1). Unique Protocol ID: ZDWY.GRBK.011. Protocol version date: February 7, 2020. The first participant was randomized on February 2020, and recruitment is ongoing. It is

estimated that the recruitment will be completed on May 31, 2020. The final results will be reported next year.

Abbreviations

COVID-2019: Corona Virus Disease-2019; nCoV: novel Corona Virus; RNA: Ribonucleic Acid; HCoVs: Human Corona Viruses; SARS-CoV: Severe Acute Respiratory Syndrome Corona Virus; MERS-CoV: Middle East Respiratory Syndrome Corona Virus; IC50: 50% inhibitory concentration; CC50: 50% cytostatic concentration; ACE2: angiotensin-converting enzyme 2; MAPK: mitogen-activated protein kinase; S protein: spike protein; RT-PCR: Reverse Transcription PCR; PaO₂: Partial Pressure of Oxygen; FiO₂: Fraction of inspiration O₂; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BUN: Blood Urea Nitrogen; Cr: creatinine; AEs: adverse events; AE: adverse event; SAE: Severe Adverse Event; SOPs: standard operation procedures; CRF: Clinical Research Flowchart; FAS: full analysis set; ROC: receiver operating characteristic; DNA: Deoxyribonucleic Acid; pH: hydrogen ion concentration; HIV: Human Immunodeficiency Virus; DRQ: Data Rating Questionnaire.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

JX, Professor carried out the design of the study. MH and ZH, carried out the design of the study. XL, participated in the study design and drafted the manuscript. HC and YS, participated in the study design and drafted the manuscript. HZ and GC helped to draft the manuscript. YC, SL and YZ follow the research and help to collect data. All authors read and approved the final manuscript.

Funding

This study was not funded.

Availability of data and materials

The datasets used or analyzed in the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was reviewed and approved by the Medical Ethics Committee of the Fifth Affiliated Hospital of Sun Yat-sen University in Zhuhai on February 10, 2020, with file number ZDWY[2020] Lunzi No. (K15-1). This study is designed in accordance with the principles of the Declaration of Helsinki. All participants will provide written informed consent before enrolment.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

Department of Infectious Diseases, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, China

References

1. Fung TS, Liu DX. Human Coronavirus: Host-Pathogen Interaction. *Annu Rev Microbiol* 2019;73:529-557.
2. Keyaerts E, Vijgen L, Maes P, Neyts J, Van Ranst M. In vitro inhibition of severe acute respiratory syndrome coronavirus by chloroquine. *Biochem Biophys Res Commun* 2004;323:264-8.
3. Vincent MJ, Bergeron E, Benjannet S et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. *Virology* 2005;2:69.

4. Kono M, Tatsumi K, Imai AM, Saito K, Kuriyama T, Shirasawa H. Inhibition of human coronavirus 229E infection in human epithelial lung cells (L132) by chloroquine: involvement of p38 MAPK and ERK. *Antiviral Res* 2008;77:150-2.
5. Xu X, Chen P, Wang J et al. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Sci China Life Sci* 2020.
6. 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.
7. Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: an old drug against today's diseases? *Lancet Infect Dis* 2003;3:722-7.
8. Legssyer R, Josse C, Piette J, Ward RJ, Crichton RR. Changes in function of iron-loaded alveolar macrophages after in vivo administration of desferrioxamine and/or chloroquine. *J Inorg Biochem* 2003;94:36-42.
9. Gonzalez-Dunia D, Cubitt B, de la Torre JC. Mechanism of Borna disease virus entry into cells. *J Virol* 1998;72:783-8.
10. Diaz-Griffero F, Hoschander SA, Brojatsch J. Endocytosis is a critical step in entry of subgroup B avian leukosis viruses. *J Virol* 2002;76:12866-76.
11. Delvecchio R, Higa LM, Pezzuto P et al. Chloroquine, an Endocytosis Blocking Agent, Inhibits Zika Virus Infection in Different Cell Models. *Viruses* 2016;8.
12. Naarding MA, Baan E, Pollakis G, Paxton WA. Effect of chloroquine on reducing HIV-1 replication in vitro and the DC-SIGN mediated transfer of virus to CD4+ T-lymphocytes. *Retrovirology* 2007;4:6.
13. Yan Y, Zou Z, Sun Y et al. Anti-malaria drug chloroquine is highly effective in treating avian influenza A H5N1 virus infection in an animal model. *Cell Res* 2013;23:300-2.
14. Zhang S, Yi C, Li C et al. Chloroquine inhibits endosomal viral RNA release and

autophagy-dependent viral replication and effectively prevents maternal to fetal transmission of Zika virus. *Antiviral Res* 2019;169:104547.

Figures

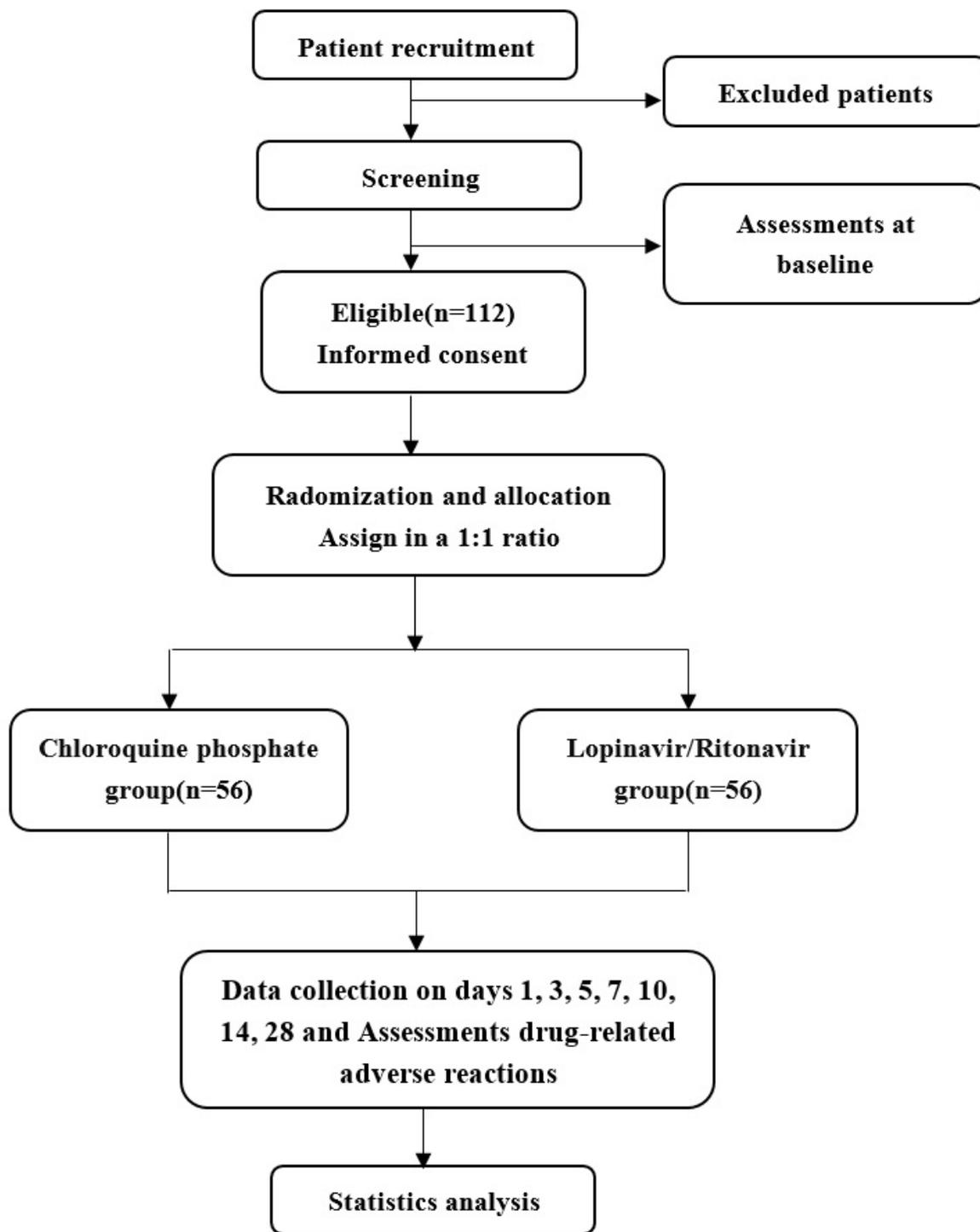


Figure 1

The flow chart of the research process

Supplementary Files

This is a list of supplementary files associated with the primary manuscript. Click to download.

Additional file. SPIRIT 2013 Checklist.docx