Preliminary evidence from a multicenter prospective observational study of the safety and efficacy of chloroquine for the treatment of COVID-19

Mingxing Huang1*, Man Li2*, Fei Xiao1,2*, Jiabi Liang3*, Pengfei Pang2,4*, Tiantian Tang5*, Shaoxuan Liu6, Binghui Chen7, Jingxian Shu3, Yingying You8, Yang Li2, Meiwen Tang9, Jianhui Zhou10, Guanmin Jiang10, Jingfen Xiang11, Wenxin Hong12, Songmei He13, Zhaofei Wang14, Jianhua Feng15, Changqing Lin16, Yinong Ye17, Zhihong Wu18, Yaocai Li19, Bei Zhong20, Ruilin Sun21, Zhongsi Hong1, Jing Liu22, Huili Chen1, Xiaohua Wang23, Zhonghe Li24, Duanqing Pei25,26†, Lin Tian3†, Jinyu Xia†, Shanping Jiang5†, Nanshan Zhong27†, Hong Shan1,2†

1. Department of Infectious Diseases, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong Province, China
2. Guangdong Provincial Key Laboratory of Biomedical Imaging and Guangdong Provincial Engineering Research Center of Molecular Imaging, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong Province, China
3. Department of Pharmacy, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong Province, China
4. Interventional Medical Center, Guangdong Provincial Key Laboratory of Biomedical Imaging, Zhuhai, Guangdong Province, China
5. Department of Respiratory and Critical Care Medicine, Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, Guangdong Province, China
6. Clinical Research Center Office, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong Province, China
7. Department of Radiology, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong Province, China

NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.
8. Department of Oral and Maxillofacial Surgery, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong Province, China

9. Department of Hematology, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong Province, China

10. Department of Clinical Laboratory, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong Province, China

11. Department of Emergency, Wuhan East West Lake Mobile Cabin Hospitals, Wuhan, Hubei Province, China

12. Department of Infectious Diseases, Guangzhou Eighth People’s Hospital, Guangzhou Province, Guangdong Province, China

13. Department of Infectious Diseases, Dongguan Ninth People’s Hospital, Dongguan, Guangdong Province, China

14. Department of Infectious Diseases, Shenzhen Third People’s Hospital, Shenzhen, Guangdong Province, China

15. Department of Infectious Diseases, Zhongshan Second People’s Hospital, Zhongshan, Guangdong Province, China

16. Department of Respiratory and Critical Care Medicine, Huizhou Central People’s Hospital, Huizhou, Guangdong Province, China

17. Department of Infectious Diseases, Foshan First people’s Hospital, Foshan, Guangdong Province, China

18. Department of Respiratory and Critical Care Medicine, Foshan Fourth People’s Hospital, Foshan, Guangdong Province, China

19. Department of Infectious Diseases, Maoming People’s Hospital, Maoming, Guangdong Province, China

20. Department of Infectious Diseases, Qingyuan People’s Hospital, Qingyuan, Guangdong Province, China

21. Department of Respiratory and Critical Care Medicine, Guangdong Second People’s Hospital, Guangzhou, Guangdong Province, China

22. Department of Respiratory and Critical Care Medicine, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong Province, China

23. Intensive Care Unit, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong Province, China
24. Department of Nephropathy, The Fifth Affiliated Hospital, Sun Yat-sen University, Zhuhai, Guangdong Province, China
25. Guangzhou Regenerative Medicine and Health Guangdong Laboratory, Guangzhou, Guangdong Province, China
26. Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, Guangdong Province, China
27. State Key Laboratory of Respiratory Diseases, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, Guangdong Province, China.

* These authors contributed equally.
† These are the co-corresponding authors.
Abstract

Background Effective therapies are urgently needed for the SARS-CoV-2 pandemic. Chloroquine has been proved to have antiviral effect against coronavirus in vitro. In this study, we aimed to assess the efficacy and safety of chloroquine with different doses in COVID-19.

Method In this multicenter prospective observational study, we enrolled patients older than 18 years old with confirmed SARS-CoV-2 infection excluding critical cases from 12 hospitals in Guangdong and Hubei Provinces. Eligible patients received chloroquine phosphate 500mg, orally, once (half dose) or twice (full dose) daily. Patients treated with non-chloroquine therapy were included as historical controls. The primary endpoint is the time to undetectable viral RNA. Secondary outcomes include the proportion of patients with undetectable viral RNA by day 10 and 14, hospitalization time, duration of fever, and adverse events.

Results A total of 197 patients completed chloroquine treatment, and 176 patients were included as historical controls. The median time to achieve an undetectable viral RNA was shorter in chloroquine than in non-chloroquine (absolute difference in medians -6.0 days; 95% CI -6.0 to -4.0). The duration of fever is shorter in chloroquine (geometric mean ratio 0.6; 95% CI 0.5 to 0.8). No serious adverse events were observed in the chloroquine group. Patients treated with half dose experienced lower rate of adverse events than with full dose.

Conclusions Although randomised trials are needed for further evaluation, this study provides evidence for safety and efficacy of chloroquine in COVID-19 and suggests that chloroquine can be a cost-effective therapy for combating
the COVID-19 pandemic.
Introduction

The coronavirus disease 2019 (COVID-19) emerged in late 2019, originating from Wuhan China\(^1,2\). The responsible virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), belongs to a distinct clade from the human severe acute respiratory syndrome CoV (SARS-CoV) and Middle East respiratory syndrome CoV (MERS-CoV)\(^3\). It has become a global pandemic, affecting over 100 countries with more than 240,000 confirmed cases and over 10,000 deaths globally as of March 20, 2020, calling for an urgent demand of effective treatment.

Chloroquine has been proved effective in vitro to inhibit the replication of SARS-CoV\(^4\), HCoV-229E\(^5\), and the newly discovered SARS-CoV-2\(^6,7\). To evaluate the efficacy and safety of chloroquine for COVID-19, we previously conducted a single-arm pilot clinical study with 10 patients (Huang et al. Journal of Molecular Cell Biology, in press). Encouragingly, all patients achieved undetectable level of viral RNA within 14 days without serious adverse events. These results led us to conduct a multicenter prospective observational study in adult patients with COVID-19 to assess the efficacy and safety of chloroquine for COVID-19.

Result

Patients

Of the 233 enrolled patients for chloroquine, 197 (84.5%) completed treatment and were included in the final analysis (Figure 1, study flowchart; Supplementary Table 1). Of the 182 patients collected as historical controls, 176 (96.7%) were included in the final analysis. Their baseline demographic and clinical features are listed in Table 1. The median age of patients were 43
years (inter-quartile range [IQR], 33 to 55 years) in the chloroquine group and
47.5 years (IQR, 35.8 to 56 years) in the non-chloroquine group. Across the
two treatment groups, the majority patients were classified as moderate cases
(93.4% in chloroquine; 89.2% in non-chloroquine). Chloroquine was added
into China’s Diagnosis and Treatment Guidelines of COVID-19 later than the
other therapies used in the non-chloroquine group. Therefore, we observed
longer interval time between symptom onset and treatment initiation in
chloroquine versus non-chloroquine (absolute difference 4 days; 95% CI 2 to
6 days; \( P < 0.0001 \)). In addition, due to the rapid rise of patients in Wuhan
and established mobile hospital in early February, the interval time between
symptom onset and treatment initiation in Wuhan (median 17 days, IQR 10.5
to 21 days) is longer than that in Guangdong Province (median 5 days, IQR 3
to 10 days; Table 1). In the subgroup of patients from the Fifth Affiliated
Hospital of Sun Yat-sen University (SYSU5), we obtained and evaluated the
viral load at baseline between chloroquine (N=21) and non-chloroquine (N=8)
group and did not observe statistically significant difference (absolute
difference in medians = 2.93, 95% CI -0.8 to 6.6, \( p = 0.09 \)).

**Outcomes**

In the analysis of the full study population, patients in the chloroquine
group have an accelerated time to undetectable viral RNA from that of
patients in the non-chloroquine group (absolute difference in medians -5.4
days; 95% CI -6 to -4; \( P < 0.0001 \); Figure 2). Secondly, by day 10 and day 14
since treatment initiation, higher proportion of patients had undetectable viral
RNA in the chloroquine group (91.4% and 95.9% respectively; Table 2)
comparing to the non-chloroquine group (57.4% and 79.6% respectively;
Table 2). In the aspect of clinical manifestations, we found that the duration of fevers is shorter in chloroquine versus non-chloroquine among patients experienced fever symptom (geometric mean ratio 0.6; 95% CI 0.5 to 0.8; \( P = 0.0029 \); Supplementary Figure S1). To note, the antipyretic effects of chloroquine may have also contributed to this result. We observed no difference in the length of hospital stay (Supplementary Figure S2). No patient died or admitted to ICU either in the chloroquine group or in the non-chloroquine group. There are 1 patient in the chloroquine group experienced aggravated symptoms from moderate to severe, while 9 patients in the non-chloroquine group have the same aggravated experience. All of the 10 patients eventually were tested negative for the viral RNA within the study period.

Due to the significant difference observed in clinical classification between chloroquine and non-chloroquine group at baseline, we further analyzed the primary and secondary outcomes in patients with moderate symptoms only. The number of patients in mild or severe subgroup were too few to compare. The benefit of chloroquine in viral suppression is consistent with the full analysis, except for non-significant difference observed for the proportion of patients with undetectable viral RNA by day 14 (Supplemental Table 2). In post hoc analysis, we examined the effect of chloroquine on the time to undetectable viral RNA stratified by different doses, types of clinical manifestation, the interaction between province and time from symptom onset to treatment initiation, and a representative center (Figure 3). Chloroquine showed beneficial effect in all stratum. However, the beneficial effect is not statistically significant in patients with severe COVID-19 symptoms, patients
from Guangdong Province treated later than 14 days after symptom onset, or patients from SYSU5.

In order to assess the effect of chloroquine in more detailed clinical improvement outcomes in post hoc analysis, we collected detailed clinical data in patients from SYSU5, including the improvement of chest CT, the monitoring of serum chloroquine concentration, and the reappearance of positive viral RNA detection after hospital discharge. In this subgroup of patients, the interval time between symptom onset and treatment initiation were comparable. The medians are 7 days in chloroquine group (N=50) and 6 days in non-chloroquine group (N=21) (absolute difference in medians 1 day; 95% CI -3 to 4 days; \( P = 0.99 \); **Supplemental Table 3**). We did not find statistically significant difference in the time to undetectable viral RNA between the two groups (absolute difference in medians -3.5 days; 95% CI -6 to 1 days). The chloroquine group have higher percentage of patients with improved chest CT by day 10 (absolute difference in proportions 9.7; 95% CI -16.0 to 35.6) and day 14 (absolute difference in proportions 6.3; 95% CI -22.2 to 32.0) than the non-chloroquine group but the difference is not statistically significant (**Supplemental Table 3**). This could be due to the small sample size or the delayed chest CT absorption\(^9\). We did not observe beneficial effect of chloroquine in the length of hospital stay and the duration of oxygen support (**Supplemental Table 3**). Unprecedentedly, we observed 3 cases of so called “re-positive” patients in the chloroquine group. They were identified with negative viral RNA test from respiratory tract samples but positive viral RNA test from fecal samples within 7 days following hospital discharge. No such
observation in the non-chloroquine group. Investigation is underway to
examine whether it is due to re-infection or other factors.

Among the 12 hospitals, one hospital explored different dosage of
chloroquine, as 500 mg once daily, which is half of the protocol dosage. We
compared the primary and secondary outcomes in patients from this subgroup
(N=29) with the non-chloroquine group in Guangdong Province. The results
mainly showed that chloroquine has benefit effect on the time to undetectable
viral RNA (absolute difference in medians -5 days; 95% CI -6.0 to -4.0 days)
and the proportion of patients with undetectable viral RNA by day 10 is higher
in chloroquine group (absolute difference in proportions 32.7; 95% CI 23.9 to
42.1). The duration of fever was also shorter than those in the non-
chloroquine group (geometric mean ratio 0.8; 95% CI 0.5 to 0.9)
(Supplemental Table 4).

Safety

A total of 53 patients (26.9%) in the chloroquine group and 57 (32.4%) in
the non-chloroquine group reported adverse events during study period
(Table 3). Gastrointestinal events including vomiting, abdominal distension,
nausea, decreased appetite, thirst were more common in chloroquine than in
the non-chloroquine group. The percentage of patients with neurological
adverse events, including dizziness and sleep order, were higher in the
chloroquine than in the non-chloroquine group. In addition, anxiety was
observed more frequently in chloroquine than in the non-chloroquine group.
We observed fewer adverse events in patients with half dose of chloroquine
than full dose (absolute difference in proportions -40; 95% CI -60 to -29).
Chloroquine phosphate has a long half-life (20-60 days)\textsuperscript{10-12} and its mean residence time is approximately 20 days\textsuperscript{10}. It may have cumulative effect\textsuperscript{13}. In order to determine whether chloroquine has a cumulative effect in the short-term treatment with COVID-19, we measured the serum concentration of chloroquine in patients from SYSU5 during and off the treatment. The results showed that the mean of serum concentration of chloroquine gradually rising, with the highest reaching 1.80(±0.49) μmol/L during medication and reduced to 0.13(±0.08) μmol/L within 28±1 days off chloroquine (Supplemental Figure 3).

DISCUSSION

In this study, we found that patients in the chloroquine group experienced significantly faster and higher rate of viral suppression comparing to the non-chloroquine group in both the full analysis and the post hoc stratified analysis. Even when the dose reduced to half, the benefit of chloroquine still remained (Figure 3). These findings indicate that chloroquine could be effective in treating patients with COVID-19. To our knowledge, this is the first and largest clinical study on chloroquine phosphate for treating COVID-19 to date.

We recognize that our study has several limitations. This study was carried out under the COVID-19 public health emergency. Due to the limited medical capacity and urgent clinical situation, we were unable to conduct a standard randomised controlled study to formally evaluate efficacy and safety of chloroquine versus placebo. As an observational study, we have to note that several factors may influence the interpretation of the result. It is reasonable to suspect that the dramatic improvement in the primary outcome
in chloroquine could be due to the later treatment initiation since symptom onset. Firstly, gaining experience in treatment management and attenuation of the virus during the course of the epidemic could contribute to the improved outcomes. Secondly, we cannot rule out the possibility that among those with longer interval time between symptom onset and treatment, some may already have been on the course of recovery. Nevertheless, post hoc analysis dividing subgroups according to the interval time did not change the conclusion that the chloroquine group had a better outcome than the non-chloroquine group. Notably, some of the strata were incomparable due to small sample size. Thirdly, although it is impossible to dissect the influence from other antiviral therapies used before chloroquine, it is a plausible assumption that chloroquine is the first antiviral therapy used in the group of patients treated within 3 days since symptom onset. In this stratum, chloroquine still benefits patients with faster viral suppression (Figure 3).

Lastly, due to the differences in personnel and technical equipment of among all hospitals, we could not fully collect clinical and laboratory data of all patients. However, detailed clinical data were obtained from the chloroquine patients enrolled from SYSU5, enabling advanced analysis of clinical outcomes and pharmacokinetics.

As of this time, there are more than 20 trials ongoing for evaluating the efficacy and safety of chloroquine or hydroxychloroquine in treating COVID-19. Magagnoli et al. recently published a retrospective study indicating that the use of hydroxychloroquine with or without azithromycin does not reduced the risk of mechanical ventilation in United States veterans hospitalized with COVID-19\textsuperscript{14}. Comparing with this study, our study population included both
genders, was much younger, has fewer patients with severe symptoms that
requires ventilation. Therefore, prospective randomised trials are needed to
see if the results can be replicated.

Till now, the mechanism of chloroquine’s effect against SARS-CoV-2
remained unelucidated. Clathrin-mediated endocytosis is required for entry
of coronavirus into host cells and meanwhile autophagy involves in viral
replication\(^\text{15}\). Chloroquine inhibits clathrin-mediated endocytosis by
suppressing acidification of endosomes, and autophagy by raising its
lysosomal PH and blocking fusion of autophagosome with lysosome and
lysosomal protein degradation\(^\text{16}\). A recent study has shown that the
development of COVID-19 disturbed metabolic patterns, which aligned with
the progress and severity of COVID-19 (Wu et al. National Science Review
2020, in press). Chloroquine has a favorable effect on glucose and lipid
metabolism\(^\text{17}\). Therefore, chloroquine may exert its antiviral effect against
SARS-CoV-2 by inhibiting endocytosis and autophagy, and stabilizing glucose
and lipid metabolism.

The adverse reactions of chloroquine drugs are of great concern to the
community. Although it is an old anti-malarial drug, its safety in treating
COVID-19 patients is still unknown. In the present study, we did not observe
serious adverse events in patients with chloroquine. All adverse events
observed during the study period are known side-effects for chloroquine
(Table 3). The main adverse events were symptoms in gastrointestinal and
neuropsychiatric systems. Chloroquine is known for its side effects in
cardiovascular system. In the chloroquine group, we did not find significantly
higher rate of adverse events in patients older than 65 or with pre-existing
conditions (Supplement Table 5). Adverse event appeared in 1 out of 29 patients (3.5%) with half dose while in 52 out 168 patients (31.0%) with full dose, indicating that the half dose group has lower adverse event rate (absolute rate difference -27.5; 95% CI -45.0 to -19.2). Although previous studies suggested that chloroquine may have cumulative effect\textsuperscript{11,18,19}, we did not observe cumulative effects among 50 patients from SYSU5 by monitoring the serum concentration of chloroquine for up to 28 days after treatment completion. Future studies are needed to determine the optimal dosing for treating COVID-19 and the cumulative effect of chloroquine in tissues and organs. Severe cases are underrepresented in the present study, and thus should be focused in the future studies to evaluate the efficacy and safety profile in this population. In addition, it will be important to study the prophylaxical use of chloroquine in areas with high rate of COVID-19 or in health professionals working with COVID-19 patients.

In conclusion, our preliminary evidence showed that chloroquine has the potential to shorten the time to SARS-CoV-2 viral suppression and duration of fever, even with reduced dose. Further randomised studies are needed to determine the optimal dose, to assess its benefit for both severe cases and to assess its benefit in settings other than secondary care. Considering that there is no better option at present, chloroquine could be a viable option to combat the coronavirus pandemic under proper management.

**METHODS**

**Study Design and participants**

This study was a multicenter prospective observational study conducted from February 7 through March 8, 2020 at 11 hospitals in
Guangdong Province and 1 mobile cabin hospital in Wuhan, Hubei Province, China. The study protocol was approved by the ethics committee of Fifth Affiliated Hospital of Sun Yat-sen University (SYSU5), located in Zhuhai, Guangdong Province, and registered at Chinese Clinical Trial Registry (ChiCTR2000029609). We did this study in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. Written informed consent was obtained from all patients or their legal guardians. During the study period, each hospital had various choices of antiviral regimen, and the sample size of Lopinavir/Ritonavir (the historical control group in the original protocol) for single-use were underpowered. Thus, we updated the inclusion criteria of the historical control group as patients receiving non-chloroquine treatment.

Eligible patients were aged 18 years or older with confirmed SARS-CoV-2 infection, tested by the local Center for Disease Control (CDC) or by a designated diagnostic laboratory, using reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assay (Shanghai ZJ Bio-Tech Co Ltd) for SARS-CoV-2 in a respiratory tract sample. Patients were ineligible if he/she met any of the following criteria: pregnant women, with known allergies to 4-aminoquinoline compounds, blood system diseases, chronic liver or kidney diseases in end-stage, arrhythmia or second/third degree heart block, with known to have retinopathy, hypoacusis or hearing loss, mental disease, glucose-6-phosphate dehydrogenase (G6PD) deficiency, had received digitalis drugs within the 7 days preceding enrollment, or is classified as critical case according to China’s Novel Coronavirus Pneumonia Diagnosis and Treatment Plan (4th Edition). Enrolled patients received 500mg
chloroquine Phosphate (equivalent of 300 mg chloroquine base, Shanghai Xinyi Pharmaceutical Co., Ltd) orally, once/twice-daily with no other antiviral therapies. The criteria of stopping chloroquine was defined as undetectable viral RNA for two consecutive respiratory tract samples. The duration of medication in chloroquine group is no more than 10 days. Patients in the historical control group were treated according to China’s Novel Coronavirus Pneumonia Diagnosis and Treatment Plan (details described in Supplemental Table 6).

Outcome and measurements

The primary outcome is the time from treatment initiation to undetectable viral RNA for two consecutive respiratory tract samples. The secondary outcomes include the proportion of patients with undetectable viral RNA by day 10 and 14, duration of fevers, time in hospital, and adverse events. The detailed definition of outcomes is described in Supplementary Methods. Respiratory tract sample was collected from patients daily to conduct RT-PCR assay for SARS-CoV-2 infection. The epidemiological characteristics, clinical symptoms and signs, adverse reactions/events were collected with data collection forms. The outcomes, clinical characteristics, laboratory findings, chest computed tomographic (CT) scans were recorded on case record forms and then double-entered into an electronic database and validated by trial staff. After hospital discharge, patients were followed up once weekly. Patients with “re-positive” viral RNA detection within one week after hospital discharge are defined as having either 2 consecutive RT-PCR positive result from either respiratory tract sample or fecal specimen. In the
subgroup of patients in SYSU5, all CT images were reviewed by two fellowship-trained cardio-thoracic radiologists by using a viewing console. Images were reviewed independently, and final decisions were reached by consensus. To fully assess the safety of chloroquine, we monitor the serum concentration of chloroquine at the day 1, 3, 5, 7, 10 during drug administration and day 1 to 7, and day 14, day 21 after treatment completion in a subgroup of samples enrolled from SYSU5 (N=50). Details about the measurement of serum concentration of chloroquine are described in Supplemental Methods.

Statistical Analysis

The original plan was to compare the efficacy between three groups, chloroquine only, Lopinavir/Ritonavir only, and chloroquine plus Lopinavir/Ritonavir. At the beginning of the outbreak, different therapies were proposed and tested for the treatment of COVID-19. Therefore, it is challenging to find sufficient patients with unified treatment across all centers. The epidemic in Guangdong had been brought under control rapidly during the study making it difficult to recruit patients as planned. The history of changes to the protocol is listed in Supplemental Table 7. Thus, a decision was made to focus on recruiting chloroquine only and compare the efficacy with historical controls. The current sample size was based on feasibility within the fixed trial recruitment window and was felt would provide sufficient precision for the estimation of plausible effects. With right-censoring in time-to-event variables, generalized Wilcoxon test was used to compare the difference in medians and the 95% confidence intervals were calculated by
bootstrapping\textsuperscript{20}. For binary outcomes, Wilson test was implemented to calculate the difference in proportions and 95\% confidence intervals. As this was an observational study, imbalance in the baseline characteristics of the two groups was expected. To adjust for this imbalance, we performed post hoc analyses within various subgroups by two dosage options, by clinical manifestation, by the interaction of province and the interval time between symptom onset and treatment initiation (\( \leq 3 \text{ days}; 3\sim7 \text{ days}; 7\sim14 \text{ days}; > 14 \text{ days} \)), and by center. For all comparative analyses, \( P < 0.05 \) was considered statistically significant. No allowance for multiplicity. All \( P \) values are two tailed. All statistical analyses were performed in R, version 3.6.1 (R Foundation for Statistical Computing)\textsuperscript{21}.

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The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data and had final responsibility for the decision to submit for publication.
Contributors
S.H, N.Z, S.J. J.X. L.T and D.P. had the idea for and designed the study and had full
access to all data in the study and take responsibility for the integrity of the data and
the accuracy of the data analysis. M.L, F.X, Y.L., M.H, J.L, P.P and T.T contributed
to writing of the report. M.L, F.X, M.H, Y.L., J.L and P.P contributed to critical
revision of the report. M.L contributed to the statistical analysis. All authors
contributed to data acquisition, data analysis, or data interpretation, and reviewed
and approved the final version.

Declaration of interest
All authors declare no competing interests.

Data sharing
The data that support the findings of this study are available from the corresponding
author on reasonable request. Participant data without names and identifiers will be
made available after approval from the corresponding author and Ministry of science
and technology and Health Committee in Guangdong province. After publication of
study findings, the data will be available for others to request. The research team will
provide an email address for communication once the data are approved to be
shared with others. The proposal with detailed description of study objectives and
statistical analysis plan will be needed for evaluation of the reasonability to request
for our data. The corresponding author and Ministry of science and technology and
Health Committee in Guangdong province will make a decision based on these
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Table 1. Baseline characteristics in chloroquine and non-chloroquine among people with COVID-19.

|                   | chloroquine (N=197) | Non-chloroquine (N=176) |
|-------------------|----------------------|-------------------------|
| Guangdong, N (%)  | 118 (60)             | 96 (54)                 |
| Hubei, N (%)      | 79 (40)              | 80 (46)                 |
| Age, mean (SD)    | 43.8 (13.1)          | 45.6 (13.5)             |
| Age ≤ 65          | 190 (96)             | 171 (97)                |
| Age > 65          | 7 (4)                | 5 (3)                   |
| Female sex, N (%) | 101 (51)             | 97 (55)                 |
| Clinical manifestation†, N (%) |         |                        |
| Mild              | 9 (5)                | 5 (3)                   |
| Moderate          | 184 (93)             | 157 (89)                |
| Severe            | 4 (2)                | 14 (8)                  |
| Comorbidities, N (%)* |                    |                        |
| Hypertension      | 13 (17)              | 11 (17)                 |
| Type 2 diabetes   | 4 (5)                | 5 (8)                   |
| Interval time from symptom onset to treatment initiation, median (IQR) | | |
| Guangdong         | 7 (3, 10.8)          | 4 (2, 7)                |
| Hubei             | 19 (17, 24.5)        | 11 (7, 16)              |
| Body temperature, median (IQR), °C | 36.7 (36.5, 37.0) | 36.6 (36.4, 37.3) |
| Pneumonia from chest CT, N (%)§ | 173 (89) | 137 (93) |

* The number of patients with valid record of comorbidities are 78 in chloroquine group and 66 in non-chloroquine group.

§ The number of patients with valid record of chest CT image are 194 in chloroquine group and 148 in non-chloroquine group.

† clinical manifestation type definitions: 1) Mild, mild clinical symptoms with no signs of pneumonia on chest radiological imaging; 2) Moderate, fever, respiratory symptoms, imaging with pneumonia changes; 3) Severe, meet any of the following criteria: shortness of breath, respiratory rate > 30 times per minute, resting stable oxygen saturation in fingertip < 93%, oxygenation index < 300, pulmonary imaging showed that the lesion progressed significantly more than 50% within 24-48 hours; 4) Critical, if any of the following occurs: respiratory failure requiring mechanical ventilation; shock, concurrent with other organ failure requires intensive care.
Table 2. Outcomes in the overall population with confirmed SARS-CoV-2 infection§.

|                                | chloroquine (N=197) | Non-chloroquine (N=176) | Difference (95% CI)† | P value |
|--------------------------------|---------------------|-------------------------|----------------------|---------|
| Time to undetectable viral RNA, median no. of days (IQR) | 3.0 (3.0, 5.0) | 9.0 (6.0, 12.0) | -6.0 (-6.0, -4.0) | < 0.0001 |
| Patients with undetectable viral RNA by, N (%) |                      |                         |                      |         |
| Day 10                          | 180.0 (91.0) | 101.0 (57.0) | 34.0 (25.6, 42.9) | < 0.0001 |
| Day 14                          | 189.0 (96.0) | 140.0 (80.0) | 16.0 (9.2, 23.3)  | < 0.0001 |
| Duration of fever*, no. of days, geometric mean (CV) | 1.2 (53.5) | 1.9 (110.0) | 0.6 (0.5, 0.8)  | 0.0029  |
| Length of hospital stay, median no. of days (IQR) | 19.0 (16.0, 23.0) | 20.0 (15.8, 24.0) | -1.0 (-3.0, 0.0) | 0.25    |

Abbreviations: CI, confidence interval; IQR, inter-quartile range; CV, coefficient of variation.

§ Definitions of outcomes are listed in Supplemental Methods.

† 95% CI for continuous variables are calculated by bootstrapping. 95% CI for binary variables are calculated with Wilson method. The difference for duration of fever is geometric mean ratio of chloroquine group to non-chloroquine group. The differences for all other variables are the absolute difference between chloroquine group and non-chloroquine group.

* The number of patients had at least one day of fever is 42 and 51 in the chloroquine and non-chloroquine group respectively.
Table 3. Summary of adverse events.

| Event                  | chloroquine (N=197) | Non-chloroquine (N=176) |
|------------------------|----------------------|-------------------------|
| Any adverse event      | 53 (26.9)            | 57 (32.4)               |
| **Gastrointestinal**   |                      |                         |
| Vomiting               | 9 (4.6)              | 2 (1.1)                 |
| Abdominal distension   | 2 (1.0)              | 1 (0.6)                 |
| Abdominal pain         | 2 (1.0)              | 2 (1.1)                 |
| Nausea                 | 18 (9.1)             | 7 (4.0)                 |
| Diarrhea               | 6 (3.0)              | 11 (6.3)                |
| Decreased appetite     | 7 (3.6)              | 0 (0)                   |
| Heart                  | 4 (2.0)              | 0 (0)                   |
| Acid reflux            | 1 (0.5)              | 0 (0)                   |
| Belching               | 1 (0.5)              | 0 (0)                   |
| **Neurological**       |                      |                         |
| Dizziness              | 20 (10.2)            | 4 (2.3)                 |
| Headache               | 3 (1.5)              | 3 (1.7)                 |
| Sleep disorder         | 10 (5.1)             | 1 (0.6)                 |
| **Psychological**      |                      |                         |
| Anxiety                | 6 (3.0)              | 0 (0)                   |
| Depression             | 1 (0.5)              | 0 (0)                   |
| Delirious              | 1 (0.5)              | 1 (0.6)                 |
| Dysphoria              | 1 (0.5)              | 0 (0)                   |
| Emotional Unstable     | 1 (0.5)              | 0 (0)                   |
| **Cardiovascular**     |                      |                         |
| Pain under xiphoid     | 1 (0.5)              | 0 (0)                   |
| Chest tightness        | 2 (1.0)              | 6 (3.4)                 |
| Ventricular premature beat | 0 (0)            | 1 (0.6)                 |
| **Other**              |                      |                         |
| Hand shaking/numbness  | 2 (1.0)              | 0 (0)                   |
| Muscle soreness        | 0 (0)                | 4 (2.3)                 |
| Blurred vision         | 3 (1.5)              | 0 (0)                   |
| Rash                   | 1 (0.5)              | 0 (0)                   |
| Weight loss            | 1 (0.5)              | 0 (0)                   |
| Fatigue / Weakness     | 2 (1.0)              | 1 (0.6)                 |
| Shortness of breath    | 1 (0.5)              | 3 (1.7)                 |
| Unsteady gait          | 1 (0.5)              | 0 (0)                   |

§ Adverse events that occurred in more than 1 patient after treatment initiation during study period are shown. Some patients had more than one adverse event.
Figure 1. Study flowchart.

COVID-19 patients from 12 hospitals

237 patients screened

2 did not meet eligibility criteria
2 did not have signed consent

233 enrolled and treated with Chloroquine

1 lost of follow-up
9 discontinued treatment
8 treated in combination with other anti-viral therapy
18 viral RNA undetectable on day 1

197 completed treatment and were analyzed

182 patients historical controls

182 treated with non-Chloroquine therapy

6 viral RNA undetectable on day 1

176 completed treatment and were analyzed
Figure 2. Kaplan-Meier curve for time to undetectable viral RNA comparing treatment groups.

![Kaplan-Meier curve](image)

Median difference = -6.0
(95% CI -6.0 to -4.0)
p < 0.0001
**Figure 3. Post hoc analysis on the effect of chloroquine on time to undetectable viral RNA by stratification.**

| Stratum       | chloroquine median(IQR) | non-chloroquine median(IQR) | N   | N   | Difference (95%CI) |
|---------------|--------------------------|-----------------------------|-----|-----|--------------------|
| Dosing        |                          |                             |     |     |                    |
| Full dose     | 4.0 (2.0, 8.0)           | 9.0 (5.8, 14.0)             | 89  | 96  | -5.0 (-7.0, -3.5)  |
| Half dose     | 5.0 (2.0, 8.0)           | 9.0 (5.8, 14.0)             | 29  | 96  | -4.0 (-8.0, -2.0)  |
| Clinical Manifestation |                 |                             |     |     |                    |
| Mild          | 4.0 (3.0, 5.0)           | 9.0 (7.0, 12.0)             | 9   | 5   | -5.0 (-9.0, -2.0)  |
| Moderate      | 3.0 (3.0, 5.0)           | 8.0 (6.0, 12.0)             | 184 | 158 | -5.0 (-6.0, -4.0)  |
| Severe        | 7.0 (4.5, 10.0)          | 10.5 (9.0, 14.0)            | 4   | 114 | -3.5 (-9.5, 2.5)   |
| Province * Time from onset to treatment initiation |           |                             |     |     |                    |
| GD <=3        | 4.5 (2.0, 7.3)           | 11.0 (7.0, 15.0)            | 32  | 37  | -6.5 (-9.5, -3.5)  |
| GD 3-7        | 5.0 (2.0, 8.0)           | 9.0 (7.0, 12.0)             | 32  | 36  | -4.0 (-7.0, -1.0)  |
| GD 7-14       | 4.0 (2.0, 6.0)           | 7.0 (4.3, 11.0)             | 45  | 18  | -3.0 (-6.0, 0.0)   |
| GD >14        | 3.0 (1.0, 5.0)           | 4.0 (3.0, 31.0)             | 9   | 5   | -1.0 (-3.0, 4.0)   |
| HB <=3        | -                        | 11.0 (11.0, 17.8)           | 0   | 6   |                    |
| HB 3-7        | 2.0 (2.0, 3.0)           | 8.5 (6.8, 12.3)             | 3   | 16  | -6.5 (-9.5, -4.0)  |
| HB 7-14       | 3.0 (3.0, 3.3)           | 9.0 (7.0, 10.5)             | 8   | 31  | -6.0 (-7.0, -4.0)  |
| HB >14        | 3.0 (3.0, 4.0)           | 7.0 (4.0, 9.5)              | 68  | 27  | -4.0 (-5.0, -2.0)  |
| Center        |                          |                             |     |     |                    |
| SYSU5         | 4.5 (2.0, 8.0)           | 8.0 (4.0, 11.0)             | 50  | 21  | -3.5 (-6.0, 1.0)   |

Abbreviations: GD, Guangdong; HB, Hubei.
95% CI are calculated by bootstrapping. The differences for all other variables are the absolute difference between chloroquine group and non-chloroquine group.
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