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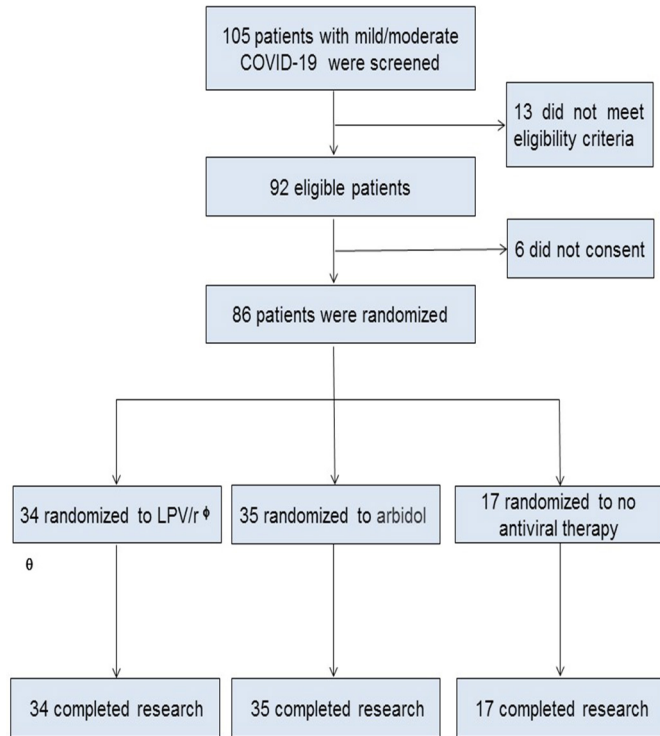
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**Efficacy and safety of lopinavir/ritonavir or arbidol in adult patients with mild/moderate COVID-19: an exploratory randomized controlled trial**

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**Abstract (249 words)**

**Background:** Antiviral therapies against the novel coronavirus SARS-CoV-2, which has caused a global pandemic of respiratory illness called COVID-19, are still lacking.

**Methods:** Our study (NCT04252885, named ELACOI), was an exploratory randomized (2:2:1) controlled trial assessing the efficacy and safety of lopinavir/ritonavir (LPV/r) or arbidol monotherapy for treating patients with mild/moderate COVID-19.

**Findings:** This study successfully enrolled 86 patients with mild/moderate COVID-19 with 34 randomly assigned to receive LPV/r, 35 to arbidol and 17 with no antiviral medication as control. Baseline characteristics of the three groups were comparable. The primary endpoint, the rate of positive-to-negative conversion of SARS-CoV-2 nucleic acid, was similar between groups (all  $P > 0.05$ ). There were no differences between groups in the secondary endpoints, the rates of antipyresis, cough alleviation, or improvement of chest CT at days 7 or 14 (all  $P > 0.05$ ). At day 7, eight (23.5%) patients in the LPV/r group, 3 (8.6%) in the arbidol group and 2 (11.8%) in the control group showed a deterioration in clinical status from moderate to severe/critical ( $P = 0.206$ ). Overall, 12 (35.3%) patients in the LPV/r group and 5 (14.3%) in the arbidol group experienced adverse events during the

follow-up period. No apparent adverse event occurred in the control group.

**Conclusions:** LPV/r or arbidol monotherapy present little benefit for improving the clinical outcome of patients hospitalized with mild/moderate COVID-19 over supportive care.

**Funding:** This study was supported by project 2018ZX10302103-002, 2017ZX10202102-003-004 and Infectious Disease Specialty of Guangzhou High-level Clinical Key Specialty (2019-2021).

**Key words:** COVID-19; SARS-CoV-2; Lopinavir/ritonavir; Arbidol; Efficacy

## Introduction

Since December 2019, a novel coronavirus disease (COVID-19) has rapidly spread throughout the world, with outbreaks in more than 200 countries and regions.<sup>1,2</sup> The pathogenic organism responsible was identified and termed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, previously named 2019 novel coronavirus or 2019-nCoV), belonging to the same family of viruses responsible for the severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS).<sup>3</sup> The World Health Organization (WHO) declared COVID-19 as a public health emergency of international concern and characterized the outbreak as a pandemic on March 12, 2020. As of March 31<sup>st</sup>, 2020, 750,890 confirmed cases and 36,405 death cases have been documented globally.<sup>2</sup> Despite its rapid global spread, little is known about the pathogenesis of the virus or its infectious host. Even worse, no vaccine or specific antiviral drugs have demonstrated efficacy in prevention or treatment of COVID-19, which has led to great difficulty in controlling the epidemic and decreasing the mortality rate.<sup>4</sup>

Based on "Diagnosis and treatment of pneumonitis caused by new coronavirus (trial version 6)" issued by the National Health Commission of China on February 19<sup>th</sup> 2020, several drugs, including lopinavir/ritonavir (LPV/r) and arbidol, were recommended as antiviral regimens for the treatment of COVID-19.<sup>5</sup> Lopinavir is a human immunodeficiency virus 1 (HIV-1) protease inhibitor, usually combined with ritonavir to inhibit cytochrome P450 in order to

increase the half-life of lopinavir.<sup>6</sup> In the past 10 years, LPV/r has been proven to have good efficacy and limited side effects for treating HIV-1.<sup>7</sup> Lopinavir was reported to have antiviral activity against MERS-CoV in Vero cells (concentration causing a 50% reduction in replication (EC<sub>50</sub>) = 8 µM).<sup>8</sup> Additionally, despite lacking a valid estimate of efficacy, the combination of LPV/r has been associated with significantly fewer adverse clinical outcomes (acute respiratory distress syndrome or death) in 41 patients with SARS compared with ribavirin alone in 111 historical controls (2.4% versus 28.8%,  $P=0.001$ ) in the 21 days after the onset of symptoms.<sup>9</sup> Thus, based on in vitro testing and previous clinical trials demonstrating its efficacy against other coronaviruses, LPV/r was regarded as an option for treating COVID-19.

Arbidol is a haemagglutinin inhibitor that can effectively block the fusion of influenza virus with its host cell. In addition, it can also induce the immune system to produce endogenous interferon against virus replication, enhance the phagocytic function of macrophages, and activate natural killer cells.<sup>10, 11</sup> Arbidol was reported to be effective against all strains of influenza viruses (A, B, C), especially influenza A viruses (H1N1, H2N2, H3N3), and to be safe with few side effects.<sup>12</sup> Arbidol was also shown to have direct antiviral effects by inhibiting the replication of SARS virus in vitro.<sup>13</sup>

Despite the above preliminary evidence, the actual clinical efficacy of LPV/r or arbidol against SARS-CoV-2 are unknown. Therefore, we were urgently in need of a randomized clinical trial (RCT) to evaluate the efficacy or adverse



outcomes of LPV/r or arbidol for treating COVID-19. Guangzhou Eighth People's Hospital is a designated hospital for the treatment of COVID-19 patients and over 80% of the patients confirmed with COVID-19 in Guangzhou were hospitalized at this facility. Here, we report an exploratory randomized and controlled study (ELACOI) at this hospital, aiming to provide a preliminary evaluation of the efficacy and safety of monotherapy with LPV/r or arbidol in the treatment of patients with mild/moderate COVID-19.

## **STAR Methods**

### **LEAD CONTACT**

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Linghua Li ([llheliza@126.com](mailto:llheliza@126.com)). This study did not generate new unique reagents and did not generate new datasets.

## **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

### **Study design and participants**

ELACOI was a single-center, randomized and controlled trial conducted at Guangzhou Eighth People's Hospital to preliminarily investigate the efficacy of LPV/r and arbidol in treating patients with COVID-19. This empirically exploratory study was initially designed to enroll 125 patients, based on the estimated number of patients admitted to the hospital. We ultimately recruited

86 patients, who were randomly assigned (2:2:1) into 3 groups as follows: In group A (LPV/r group), 34 patients were administered lopinavir (200mg) boosted by ritonavir (50mg) (orally administered, twice daily, 500 mg, each time for 7-14 days). In group B (arbidol group), 35 patients were given arbidol (100mg) (orally administered, 200mg three times daily for 7-14 days). In group C (control group), 17 patients were not given any antiviral therapy. All three groups were followed for up to 21 days. All three groups were treated with supportive care and effective oxygen therapy if in need.<sup>5</sup> Antiviral treatment was discontinued for patients who 1) had been treated for more than 7 days and tested negative for SARS-CoV-2 nucleic acid in two consecutive tests separated by more than 24 hours, or 2) were discharged from hospital, or 3) had intolerable side effects.

All participants met the following inclusion criteria: 1) age between 18 and 80 years; 2) SARS-CoV-2 infection confirmed by real-time PCR (RT-PCR) from pharyngeal swab; 3) mild clinical status, defined as having mild clinical symptoms but no signs of pneumonia on imaging or moderate clinical status, defined as having fever, respiratory symptoms and pneumonia on imaging;<sup>5</sup> 4) the following lab findings: creatinine  $\leq 110 \mu\text{mol/L}$ , creatinine clearance rate (eGFR)  $\geq 60 \text{ ml/min/1.73m}^2$ , aspartate aminotransferase (AST) and alanine aminotransferase (ALT)  $\leq 5 \times \text{ULN}$ , and total bilirubin (TBIL)  $\leq 2 \times \text{ULN}$ ; 5) willingness to participate in the study and provide informed consent. Patients were excluded based on the following criteria: 1) known or suspected to be

allergic to LPV/r or arbidol; 2) having severe nausea, vomiting, diarrhea or other complaints affecting oral intake or absorption in the digestive tract; 3) taking other drugs that may interact with LPV/r or arbidol; 4) having serious underlying diseases, including but not limited to heart, lung, or kidney disease, liver malfunction, or mental illnesses affecting treatment compliance; 5) complications with pancreatitis or hemophilia prior to the trial; 6) Pregnant or lactating women; 7) suspected or confirmed history of alcohol or substance use disorder; 8) participation in other drug trials within the past month; 9) deemed otherwise unsuitable for the study by researchers.

Before initiation of the trial, the protocol was approved by the ethics committee of Guangzhou Eighth People's Hospital (Approval No. 202002136) and registered on ClinicalTrials.gov (NCT04252885). The ethics committee agreed to set up the control group owing to a lack of reliable evidence about the benefit of present antiviral regimens for treating COVID-19. The trial was also performed in accordance with the International Conference on Harmonization's Guideline for Good Clinical Practice. Written informed consent was obtained from all screened patients after they fully understood the meaning of the trial and the potential risks involved.

## **METHOD DETAILS**

### **Randomization and masking**

All eligible participants were assigned a randomization number which

allocated them into one treatment group. The randomization numbers were computer-generated. Allocation concealment was achieved using a centralized web-based randomization system in which the participant identifier (hospitalization number) was entered before the allocation was revealed. The randomization numbers were used in case report form (CRF) pages. The study was blinded to participants and those physicians and radiologists who reviewed the data and radiological images but open-label to clinicians who recruited patients and research staff.

### **Procedures**

A standardized protocol was developed for collecting clinical data for all participants. The following data were collected: 1) important dates, including fever onset, admission, progression to severe clinical status, positive-to-negative conversion of SARS-CoV-2 nucleic acid, improvement of chest computerized tomography [CT] scan, discharge, or death; 2) presence of predefined comorbidities (hypertension, diabetes mellitus, etc.); 3) daily observation of clinical parameters (temperature, pulse, respiratory rate, oxygen saturation, Inhaled oxygen concentration if needed); 4) The conversion time of nucleic acid of SARS-CoV-2 from positive to negative and the clinical improvement including the rate of antipyresis, the rate of cough alleviation, the rate of improvement on chest CT at days 7 and 14; 5) details of drug treatment for supportive treatment and measures for oxygen therapy and 6) adverse events. The clinical information was merged with selected laboratory and

pharmacy information from the HIS and LIS database. All clinical, virological and laboratory data as well as adverse events were reviewed by two physicians, and all radiologic images were reviewed by two radiologists.

SARS-CoV-2 nucleic acid was detected by real-time fluorescence reverse transcriptional polymerase chain reaction (RT-PCR) using the platform of Da'an Gene Corporation, Sun Yat-sen University, Guangzhou, China. The specimens were obtained using pharyngeal swabs of patients. The nucleic acid detection of SARS-CoV-2 targeted the open reading frame 1a/b (ORF1a/b) and nucleocapsid protein (N) genes. Viral RNA was extracted with Nucleic Acid Isolation Kit on an automatic workstation Smart 32. A 200 µl sample was used for extraction following the standard protocol, and viral RNA was eluted with 60 µl elution buffer. Real-time reverse transcriptional polymerase chain reaction (RT-PCR) reagent was used following the RNA extraction. In brief, two PCR primer and probe sets, targeting ORF1ab (FAM reporter) and N (VIC reporter) genes separately, were added in the same reaction. Positive and negative controls were included for each batch. Samples were considered to be positive when either or both set(s) gave a reliable signal(s).<sup>14</sup>

## **Outcomes**

The primary outcome was the rate of positive-to-negative conversion of SARS-CoV-2 nucleic acid from the initiation of treatment to day 21, with the enrollment day as the first day of treatment. The secondary outcomes were: 1) the rate of positive-to-negative conversion of SARS-CoV-2 nucleic acid at day

14; 2) the rate of antipyresis (defined as axillary temperature  $\leq 37.3^{\circ}\text{C}$  for more than 72 hours) from the first day of treatment; 3) the rate of cough alleviation from initiation; 4) the improvement rate of chest CT at days 7 and 14; 5) the deterioration rate of clinical status from mild/moderate to severe/critical status during the study period. The severe status was defined as meeting any of the following criteria: experiencing respiratory distress,  $\text{RR} \geq 30$  times/minute; oxygen saturation  $\leq 93\%$  in the resting state; arterial blood oxygen partial pressure ( $\text{PaO}_2$ )/oxygen concentration ( $\text{FiO}_2$ )  $\leq 300$  mmHg (1mmHg = 0.133kPa).<sup>5</sup> The critical status was defined as meeting any of the following criteria: development of respiratory failure requiring mechanical ventilation; occurrence of shock; requirement for ICU monitoring and treatment because of complications with other organ failures.<sup>5</sup>

Specimens from pharyngeal swabs were tested every 2 to 3 days. Negative conversion of nucleic acid was defined as negative detection of SARS-CoV-2 nucleic acid for two consecutive instances separated by more than 24 hours. Criteria of chest CT improvement included: 1) no new exudative lesions; 2) decreasing size of exudative lesions; 3) decreasing densities of lesions.

All participants were monitored for adverse events. Safety outcomes were assessed from serious adverse event reports. Any unexpected medical occurrence resulting in death, prolonged hospitalization, persistent or significant disability or incapacity, which was judged to be causally related to the study intervention, would be reported as a serious adverse event to the

Institutional Review Board. Potential adverse events for the study were defined as follows (1) anaphylaxis; (2) elevation of ALT or AST to more than 2.5-fold the upper normal limit or elevation of TBIL to more than 1.5-fold the upper normal limit; (3) acute pancreatitis; and (4) diarrhea.

## **QUANTIFICATION AND STATISTICAL ANALYSIS**

The aim of this study is to explore the efficacy and safety of Lopinavir/ritonavir (LPV/r) monotherapy or arbidol monotherapy on the treatment of COVID-19 patients. However, COVID-19 is a new emerging disease without any data to provide a basis for calculating the sample size. In addition, the trend of the epidemic was not clear while we were designing the study. Based on the estimated number of patients admitted to the hospital at that time, we initially estimated that a maximum of 125 patients could meet the inclusion criteria, however, only 86 were ultimately recruited because few new cases developed in Guangzhou with the epidemic under control.

All statistical analyses were performed using SPSS (Statistical Package for SPSS, version 26.0). We presented continuous measurements as mean (SD) if the data were normally distributed or median (IQR) if they were not, and categorical variables as count (%). Means for continuous variables were compared using one-way ANOVA when the data were normally distributed; otherwise, the Mann-Whitney test was used. Proportions for categorical variables were compared using the  $\chi^2$  test or Fisher's exact tests. A two-sided

$\alpha$  of less than 0.05 was considered statistically significant.

### KEY RESOURCE TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological Samples		
Patients swabs and blood samples	Guangzhou Eighth People's Hospital	N.A.
Critical Commercial Assays		
Laboratory assessments of biochemistry were measured with the platform of Roche. ALT/AST was detected by rate assay, TBIL was detected by oxidase method.	Roche	Lab staff of Guangzhou Eighth People's Hospital
Nucleic Acid Isolation Kit (Da'an Gene Corporation, Cat: DA0630) Automatic workstation Smart 32 (Da'an Gene Corporation) RT-PCR reagent (Da'an Gene cooperation, Cat DA0930)	Da'an Gene Corporation, Sun Yat-sen University, Guangzhou, China	Lab staff of Guangzhou Eighth People's Hospital
Oligonucleotides		
<p>PCR primer and probe set target to ORF1ab (FAM reporter) and N (VIC reporter) genes separately.</p> <p>1. Primer sequences for the ORF1ab genes: Forward primer (F): CCCTGTGGGTTTTACTTAA. Reverse primer (R): ACGATTGTGCATCAGCTGA. Fluorescent probe (P): 5'-FAM-CCGTCTGCGGTATGTGGAAAGGTTATGG-BHQ1-3'</p> <p>2. Primer sequences for the ORF1ab genes: Forward primer (F): GGGGAAGTTCTCCTGCTAGAAT. Reverse primer (R): CAGACATTTTGCTCTCAAGCTG. Fluorescent probe (P): 5'-FAM-TTGCTGCTGCTTGACAGATT-TAMRA-3'</p> <p>Nucleic acid extraction and real-time fluorescence RT-PCR reaction system and reaction conditions refer to the relevant manufacturer kit instructions</p>	Da'an Gene Corporation, Sun Yat-sen University, Guangzhou, China	Lab staff of Guangzhou Eighth People's Hospital
Software and Algorithms		



SPSS (Statistical Package for SPSS, version 26.0)	IBM, Armonk, NY	
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## RESULTS

### Baseline data of patients

From Feb 1 to March 28, 2020, 105 patients with COVID-19 were screened for this study, among whom 86 patients (mean age of 49.4 years [SD 14.7, range 19-79]) including 40 men and 46 women were successful enrolled (figure 1). Patients were randomly assigned to receive LPV/r (n=34, arbidol (n=35), or control (n=17). All patients were followed up for 21 days.

None of the enrolled patients had chronic lung disease, chronic kidney disease, autoimmune disease or immunodeficiency disease. Twenty-seven (79.4%) patients in the LPV/r group, 22 (62.9%) in the arbidol group and 9 (52.9%) in the control group experienced fever. Twenty-one (61.8%) patients in the LPV/r group, 25 (71.4%) in the arbidol group and 9 (52.9%) in the control group developed cough. There were no significant differences in baseline demographic data, common clinical manifestations or pneumonia incidence seen on chest CT imaging between the three groups ( $P>0.05$ ). The baseline characteristics of the 86 patients in three groups are shown in table 1. None of the patients complained dyspnea, diarrhea, palpitation or headache on admission. The laboratory parameters of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL) and creatinine were normal when patients started the antiviral treatment. Other laboratory

parameters including white blood cell count, lymphocyte count, neutrophil count, C-reactive protein level and procalcitonin level did not show significant differences between the three groups ( $P > 0.05$ ).

During the study period, six patients used gamma globulin (10 g, once a day, for 2-3 days), 12 used glucocorticoids (methylprednisolone 40 mg, once a day, for 3-5 days) and 54 received oxygen supply therapy. The usage percentages of the above supportive treatment did not show statistical differences between the three groups ( $P = 0.865$ ,  $P = 0.153$ ,  $P = 0.677$  respectively).

### **Efficacy outcomes**

The mean time for positive-to-negative conversion of SARS-CoV-2 nucleic acid was 9.0 days (SD 5.0) in the LPV/r group, 9.1 (SD 4.4) in the arbidol group and 9.3 (SD 5.2) in the control group, with no statistical difference between them ( $P = 0.981$ ) (table 2 and figure 2). After 7 days of treatment, the positive-to-negative conversion rates of SARS-CoV-2 nucleic acid in pharyngeal swab in the LPV/r group, the arbidol group and the control group were 35.3% (12/34), 37.1% (13/35) and 41.2% (7/17) respectively and did not present statistical difference among the three groups ( $P = 0.966$ ) (table 2). After 14 days of treatment, the positive-to-negative conversion of SARS-CoV-2 nucleic acid was 85.3% (29/34), 91.4% (32/35) and 76.5% (13/17) respectively in the LPV/r group, the arbidol group and the control group, without significant statistical difference between them ( $P = 0.352$ ) (table 2). Over the 21-day follow-up, the cumulative incidence of positive-to-negative conversion of

SARS-CoV-2 nucleic acid in pharyngeal swabs did not show statistical difference between the three groups (figure 3).

With respect to other secondary outcomes, the rate of antipyresis, rate of cough resolution, and rate of improvement on chest CT imaging at day 7 and 14 did not show any statistical difference between the three groups ( $P > 0.05$ ). At day 7, eight (23.5%) patients in the LPV/r group, 3 (8.6%) in the arbidol group and 2(11.8%) in the control group deteriorated from mild/moderate clinical status to severe/critical clinical status, without statistical difference ( $P = 0.206$ ) (table 2).

In order to rule out the influence of the time from onset to treatment on the clinical status, we compared the time from onset to treatment in patients who deteriorated to severe/critical clinical status [5 (IQR 2, 8) days] with those who did not [4 (IQR 2, 7) days], and did not find any significant difference between them ( $P = 0.619$ ).

### **Safety outcomes**

During the follow-up period, 12 (35.3%) patients in the LPV/r group experienced adverse events including diarrhea (9/34, 26.5%), loss of appetite (5/34, 14.7%) and elevation of ALT over 2.5-fold above the normal limit (1/21, 4.8%). In addition, 5 (14.3%) patients in the arbidol group experienced adverse events including diarrhea (3/35, 8.6%) and nausea (2/34, 5.9%). No apparent adverse events occurred in the control group. Notably, one serious adverse event occurred in a 79-year-old man with underlying diseases including

diabetes and hypertension in the LPV/r group, characterized by severe diarrhea on day 3. The patient withdrew from this study and tested positive for SARS-CoV-2 nucleic acid lasting over 14 days of the follow-up period. This patient progressed to critical condition and received extracorporeal membrane oxygenation (ECMO). Fortunately, he recovered and stopped needing ECMO by the observation endpoint of this study.

### **Summary of cases with severe/critical clinical status**

A total of 13 (15.1%) patients (8 men, 5 women) progressed to severe/critical clinical status (containing 11 severe cases and 2 critical cases) during the study period including 8 receiving LPV/r, 3 receiving arbidol and 2 control. The two critical cases belonged to LPV/r group. The mean age of these 13 patients was 60.1 years [SD 13.8, range 37-79]. Ten (76.9%) patients came from Hubei province and 3 (23.1%) were local residents of Guangzhou. Two (15.4%) patients suffered from diabetes mellitus, 5 (38.5%) from hypertension and 1 (7.7%) from chronic liver disease. All patients complained of fever and 9 of them (69.2%) complained of cough, but none experienced diarrhea at the beginning of treatment. The SaO<sub>2</sub> at rest was  $\leq 93\%$  in 4 (30.8%) patients and PaO<sub>2</sub>/FiO<sub>2</sub> ratio was  $\leq 300$  in 5 (38.5%) patients. Among these patients, 2(15.4%) required mechanical ventilation due to respiratory failure. Five (38.5%) and 9 (69.2%) cases achieved positive-to-negative conversion of SARS-CoV-2 nucleic acid at days 7 and 14 respectively. At days 7 and 14 of follow-up, 6 (46.2%) and 10 (76.9%) patients had improvements in chest CT

imaging. At the follow-up endpoint of day 21, 12 patients had been discharged from hospital and only one case was still hospitalized. No deaths occurred.

## **Discussion**

Several clinical studies have reported the treatment of a large number of COVID-19 patients with antiviral and antibiotic therapy.<sup>15-17</sup> However, no specific medication has proven effective for suppressing or eliminating SARS-CoV-2 infection or for reducing complications and mortality. There are several ongoing clinical drug trials registered in the Chinese clinical trial registry.<sup>15,18</sup> Although the epidemic within China is now largely under control, epidemics in other countries are becoming increasingly severe.<sup>2</sup> Therefore, it is extremely important to find specific anti SARS-CoV-2 drugs and learn from the experience of Chinese health providers.

Our study was designed to be an empirical exploration intended to recruit 125 adult patients hospitalized with mild/moderate COVID-19; however, only 86 patients were involved in this study for the reasons previously mentioned. Through randomization, 34 patients were assigned to receive LPV/r, 35 to arbidol, and 17 to no antiviral medication as control. The results showed that LPV/r and arbidol did not shorten the time of positive-to-negative conversion of COVID-19 nucleic acid in respiratory specimens (9.0 vs. 9.1 vs. 9.3 days), nor did they improve the symptoms of COVID-19 or pneumonia on lung CT imaging at 7 days and 14 days. Moreover, more patients treated with LPV/r progressed from mild/moderate to severe/critical status than patients from the

other two groups

One reason behind the failure of LPV/r and arbidol to improve patient outcome could be that a higher dose is needed to successfully suppress SARS-CoV-2 in patients to achieve an effect comparable to in vitro cytotoxicity tests.<sup>8, 13</sup> However, this would be difficult to achieve clinically given the side effects caused by both drugs. In particular, it should be noted that patients treated with LPV/r had more gastrointestinal symptoms, which might affect the patient's recovery. Based on the drug instruction and previous experience in treating HIV-infected patients, the adverse reactions of the short-term use of LPV/r mainly include diarrhea, abnormal stools, abdominal pain, nausea, vomiting, and asthenia.<sup>6</sup> Since the above side effects may aggravate the disease, LPV/r treatment should be cautiously considered after weighing the risks and benefits.

The results in our study are consistent with findings from a recent clinical trial of LPV/r in adults hospitalized with severe COVID-19 conducted in Wuhan, which recruited 199 hospitalized adult patients with severe COVID-19 and concluded that no benefit was observed with LPV/r treatment beyond standard care.<sup>19</sup> In addition, another retrospective clinical research study conducted in Shanghai observed 134 patients with COVID-19 and did not find any effects of LPV/r and arbidol on relieving symptoms or accelerating virus clearance after treatment for 5 days.<sup>20</sup> Despite the small sample size, our study also suggests that monotherapy of LPV/r or arbidol might not improve the clinical outcome in

treating with mild/moderate COVID-19.

During the study period, a total of 13 (15.1%) patients progressed to severe/critical clinical status including 8 receiving LPV/r, 3 receiving arbidol and 2 in the control group, which indicates that disease condition could still worsen even after hospitalization, and thus urgently demands rigorous observation of illness and care. Fortunately, by the endpoint of this study twelve patients had been discharged from hospital after recovery, and only one patient remained hospitalized, albeit with significant clinical improvement. This gives us confidence that even in the absence of specific antiviral drugs, the vast majority of COVID-19 patients in severe/critical clinical status can still recover after comprehensive treatment.

### **Limitations of study**

Our study is not without its limitations. First, we recognize that our sample size was small. Second, the study did not enroll severely or critically ill patients, or patients at increased risk of poor outcome with many comorbidities and was conducted in only one center. Third, the study was not completely blinded, possibly influencing the outcome to some extent. We will continue to follow these patients to evaluate their long-term prognosis. Nevertheless, as a prospective randomized, controlled trial, this study could still provide meaningful suggestions for proper application of LPV/r and arbidol in the treatment of COVID-19.

In conclusion, our study found that LPV/r or arbidol monotherapy presents little benefit for improving the clinical outcome of hospitalized patients with mild/moderate COVID-19 beyond symptomatic and supportive care, causing instead more adverse events. Further work is needed to confirm these results.

### **Contributors**

Linghua Li, Xilong Deng and Yueping Li conceived the study and designed the protocol. Weiping Cai and Fuchun provided oversight. Chunyan Wen contributed to statistical analysis and interpretation of data. Linghua Li and Weiyin Lin drafted the manuscript. Feng Li and Fengyu Hu conducted of nuclear acid RT-PCR. Zhiwei Xie and Yujuan Guan reviewed the data independently. Jinxin Liu and Lieguang Zhang reviewed all radiologic images independently. Xiaoneng Mo, Jian Wang, Yaping Wang, Ping Peng, Xudan Chen, Wenxin Hong and Guangming Xiao contributed to conducting the study and collecting data.

### **Declaration of interests**

We declare no competing interests.

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## References

1. Wang Z, Yang B, Li Q, Wen L, and Zhang R. (2020). Clinical Features of 69 Cases with Coronavirus Disease 2019 in Wuhan, China. *Clin Infect Dis*. DOI: 10.1093/cid/ciaa272
2. Who Health Organization. Coronavirus disease 2019 (COVID-19) Situation Report -71.  
[https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200331-sitrep-71-covid-19.pdf?sfvrsn=4360e92b\\_8](https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200331-sitrep-71-covid-19.pdf?sfvrsn=4360e92b_8). Date last updated: 31 March 2020.
3. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, et al. (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 395, 565-574.
4. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, Wu Y, Zhang L, Yu Z, Fang M, et al.

- (2020). Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med*. DOI: 10.1016/S2213-2600(20)30079-5
5. National Health Commission of China. (2020). Diagnosis and treatment of pneumonia caused by new coronavirus (trial version 6). <http://www.nhc.gov.cn/yzygj/s7653p/202002/8334a8326dd94d329df351d7da8aefc2.shtml>. Date last updated: February 19th, 2020.
6. Mangum EM, and Graham KK. (2001). Lopinavir-Ritonavir: a new protease inhibitor. *Pharmacotherapy* 21, 1352-1363.
7. Pasquau J, Hidalgo-Tenorio C, Montes ML, Romero-Palacios A, Vergas J, Sanjoaquin I, Hernández-Quero J, Aguirrebengoa K, Orihuela F, Imaz A, et al. (2018). High quality of life, treatment tolerability, safety and efficacy in HIV patients switching from triple therapy to lopinavir/ritonavir monotherapy: A randomized clinical trial. *PLoS One* 13, e0195068.
8. Sheahan TP, Sims AC, Leist SR, Schäfer A, Won J, Brown AJ, Montgomery SA, Hogg A, Babusis D, Clarke MO, et al. (2020). Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. *Nat Commun* 11, 222.
9. Chan KS, Lai ST, Chu CM, Tsui E, Tam CY, Wong MM, Tse MW, Que TL, Peiris JS, Sung J, et al. (2003). Treatment of severe acute respiratory syndrome with lopinavir/ritonavir: a multicenter retrospective matched cohort study. *Hong Kong Med J* 9,399-406.

10. Brooks MJ, Burtseva EI, Ellery PJ, Marsh GA, Lew AM, Slepushkin AN, Crowe SM, and Tannock GA. (2012). Antiviral activity of arbidol, a broad-spectrum drug for use against respiratory viruses, varies according to test conditions. *J Med Virol* 84, 170-81.
11. Popov AF, Simakova AI, Dmitrenko KA, and Shchelkanov MY. (2017). Time course of changes in cytokines (IFN- $\gamma$ , IFN- $\alpha$ , IL-18, TNF- $\alpha$ ) in the treatment of moderate influenza A (H1N1) pdm09 (2013-2016) with oseltamivir (Tamiflu) and umifenovir (Arbidol) alone and in combination with Kagocel. *Ter Arkh* 89, 66-70.
12. Leneva IA, Falynskova IN, Makhmudova NR, Poromov AA, Yatsyshina SB, and Maleev VV. (2019). Umifenovir susceptibility monitoring and characterization of influenza viruses isolated during ARBITR clinical study. *J Med Virol* 91, 588-597.
13. Khamitov RA, Loginova Sla, Shchukina VN, Borisevich SV, Maksimov VA, and Shuster AM. (2008). Antiviral activity of arbidol and its derivatives against the pathogen of severe acute respiratory syndrome in the cell cultures. *Vopr Virusol* 53, 9-13.
14. Chen W, Lan Y, Yuan X, Deng X, Li Y, Cai X, Li L, He R, Tan Y, Deng X, et al. (2020). Detectable 2019-nCoV viral RNA in blood is a strong indicator for the further clinical severity. *Emerg Microbes Infect* 9, 469-473.
15. Zhang Q, Wang Y, Qi C, Shen L, and Li J. (2020). Clinical trial analysis of 2019-nCoV therapy registered in China. *J Med Virol*. DOI: 10.1002/jmv.25733

16. Jin YH, Cai L, Cheng ZS, Cheng H, Deng T, Fan YP, Fang C, Huang D, Huang LQ, Huang Q, et al. (2020). A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version). *Mil Med Res* 7, 4.
17. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, et al. (2020). clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-infected Pneumonia in Wuhan, China. *JAMA*. DOI: 10.1001/jama.2020.1585
18. Lai CC, Shih TP, Ko WC, Tang HJ, and Hsueh PR. (2020). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. *Int J Antimicrob Agents* 55, 105924.
19. Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, Ruan L, Song B, Cai Y, Wei M, et al. (2020). A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. *N Engl J Med*. DOI: 10.1056/NEJMoa2001282
20. Chen J, Ling Y, Xi X, Liu P, Li F, Li T, Shang Z, Wang M, Shen Y, and Lu H. (2020). Efficacies of lopinavir/ritonavir and abidol in the treatment of novel coronavirus pneumonia. (In Chinses). *Chin J Infect Dis*. DOI: 10.3760/cma.j.cn311365-20200210-00050

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**Table legends**

Table 1: Baseline characteristics of the three treatment groups  
(intention-to-treat population)

¶ list all the chronic diseases

Table 2: Outcomes of the three groups (intention-to-treat population)

**Figure legends:**

**Figure 1.** Trial profile

θ SAE: Severe Adverse Event

φ LPV/r: Lopinavir/ritonavir

**Figure 2.** Rate of positive-to-negative conversion of SARS-CoV-2 nucleic acid detected from pharyngeal swabs in each of the three treatment groups during the 21-day follow-up period.

LPV/r: lopinavir/ritonavir

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

**Figure 3.** Proportion of patients in each of the three treatment groups with positive SARS-CoV-2 nucleic acid during the 21-day follow-up period

LPV/r: lopinavir/ritonavir

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

Table 1: Baseline characteristics of the three treatment groups  
(intention-to-treat population)

Characteristics	LPV/r (n=34)	Arbidol (n=35)	Control (n=17)	P value
Gender (n, %)				0.831
Male	17(50.0%)	16(45.7%)	7(41.2%)	
Female	17(50.0%)	19(54.3%)	10(58.8%)	
Age, in years (mean, SD, range)	50.7(15.4,19-79)	50.5(14.6,20-74)	44.3(27-62)	0.288
Time from onset to treatment, in days (median, IQR)	3.5(2,6)	6(2,8)	5(2,8)	0.371
Underlying chronic diseases <sup>†</sup> (n, %)	7(20.6%)	5(14.3%)	6(35.3%)	0.236
Evidence of pneumonia based on chest CT imaging (n, %)	28(82.4%)	33(94.3%)	14(82.4%)	0.234
Clinical status (n, %)				0.234
mild	6(17.6%)	2(5.7%)	3(17.6%)	
moderate	28(82.4%)	33(94.3%)	14(82.4%)	
White blood cell count, 10 <sup>9</sup> /L				0.671
<4 (n, %)	8(23.5%)	11(31.4%)	3(17.6%)	
4-10 (n, %)	25(73.5%)	24(68.6%)	14(82.4%)	
>10 (n, %)	1(2.9%)	0		
Lymphocyte count, 10 <sup>9</sup> /L				0.974
<1.1 (n, %)	9(26.5%)	9(25.7%)	4(23.5%)	
1.1-3.2 (n, %)	25(73.5%)	26(74.3%)	13(76.5%)	
Neutrophil count, 10 <sup>9</sup> /L				0.981
<1.8 (n, %)	5(14.7%)	6(17.1%)	2(11.8%)	
1.8-6.3 (n, %)	26(76.5%)	27(77.1%)	14(82.4%)	
>6.3 (n, %)	3(8.8%)	2(5.7%)	1(5.9%)	
C-reactive protein, mg/L				0.364
<10 (n, %)	17(50.0%)	23(65.7%)	11(64.7%)	
>10 (n, %)	17(50.0%)	12(34.3%)	6(35.3%)	
Procalcitonin, ng/mL				0.308
<0.05 (n, %)	18(52.9%)	24(68.6%)	12(70.6%)	

>0.05(n, %)	16(47.1%)	11(31.4%)	5(29.4%)	
Use gamma globulin (%)	3/34(8.8%)	2/35(5.7%)	1/7(5.9%)	0.865
Use of glucocorticoids (%)	7/34(20.6%)	2/35(5.7%)	3/17(17.6%)	0.153
Oxygen therapy (%)				0.677
None	12/34(35.3%)	13/35(37.1%)	7/17(41.2%)	
Low flow oxygen supply	15/34(44.1%)	19/35(54.3%)	8/17(47.0%)	
High flow oxygen supply	7/34(20.6%)	3/35(8.6%)	2/17(11.8%)	

¶ Cases of chronic diseases were as follows: one patient with diabetes, 4 with hypertension and 2 with both in the LPV/r group; one patient with Coronary Heart Disease, 3 with hypertension and 1 with chronic liver disease in the arbidol group; and one patient with Coronary Heart Disease, 2 with hypertension, 2 with chronic liver disease and 1 with both diabetes and chronic liver disease in the control group.



Table 2: Outcomes of the three groups (intention-to-treat population)

Outcome	LPV/r	Arbidol	Control	P value
Rate of positive-to-negative conversion of SARS-CoV-2 nucleic acid by pharyngeal swab (%) at Day 7	12/34(35.3%)	13/35(37.1%)	7/17(41.2%)	0.966
Rate of positive-to-negative conversion of SARS-CoV-2 nucleic acid by pharyngeal swab (%) at Day 14	29/34(85.3%)	32/35(91.4%)	13/17(76.5%)	0.352
Time of positive-to-negative conversion of SARS-CoV-2 nucleic acid in pharyngeal swab, in days (mean/SD, 95%CI)	9.0(5.0), (7.2,10.8)	9.1(4.4), (7.6,10.6)	9.3(5.2), (6.7,11.9)	0.981
Conversion rate from moderate to severe/critical clinical status (%)	8/34(23.5%)	3/35(8.6%)	2/17(11.8%)	0.206
At 7 days after initiating treatment:				
Antipyresis rate (%)	20/27(74.1%)	18/22(81.8%)	8/9(88.9%)	0.579
Rate of cough alleviation (%)	9/21 (42.9%)	7/25(28.0%)	2/9(22.2%)	0.432
Rate of improvement on chest CT (%)	11/28(39.3%)	13/33(39.4%)	6/14 (42.9%)	0.971
At 14 days after initiating treatment:				
Antipyresis rate (%)	24/27 (88.9%)	21/22 (95.5%)	9/9 (100%)	0.343
Rate of cough alleviation (%)	16/21 (76.2%)	14/25 (56.0%)	4/9 (44.4%)	0.180
Rate of improvement on chest CT (%)	21/28(75.0%)	23/33(69.7%)	13/14 (92.9%)	0.089

**eTOC (50 words)**

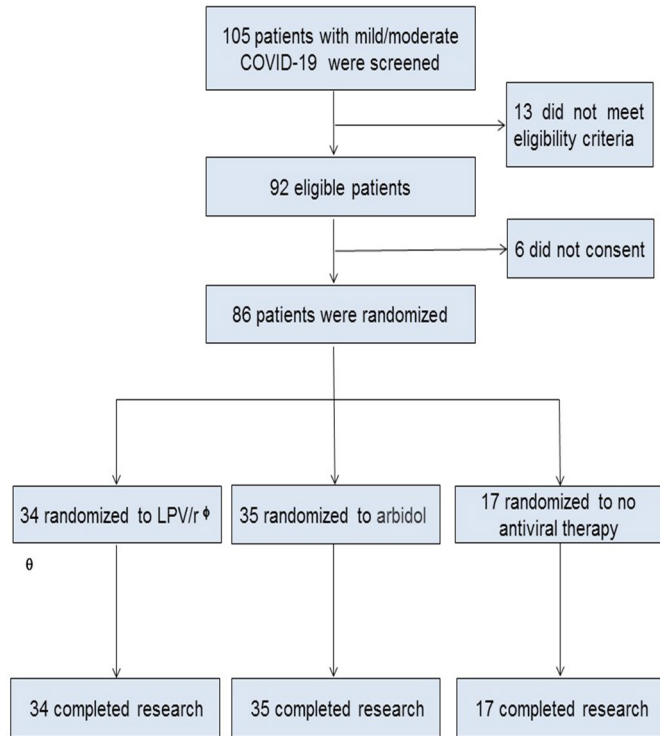
Several drugs are being tested against the novel coronavirus SARS-CoV-2, the pathogen responsible for the COVID-19 pandemic. Li et al show that the drugs lopinavir/ritonavir and arbidol, which are currently used against HIV-1 and influenza, respectively, show little benefit over supportive care in patients with mild and moderate COVID-19.

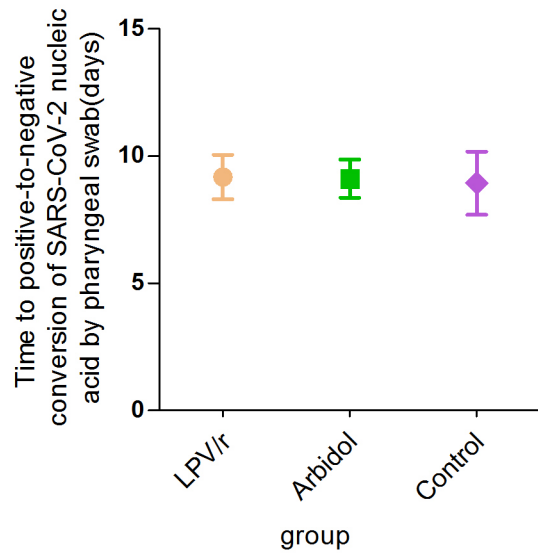
**Highlights (4x85 characters)**

- Effective therapies effective against COVID-19 are urgently needed
- Lopinavir/ritonavir and arbidol were tested in patients with mild/moderate COVID-19
- Neither treatment shows significant advantage over supportive care

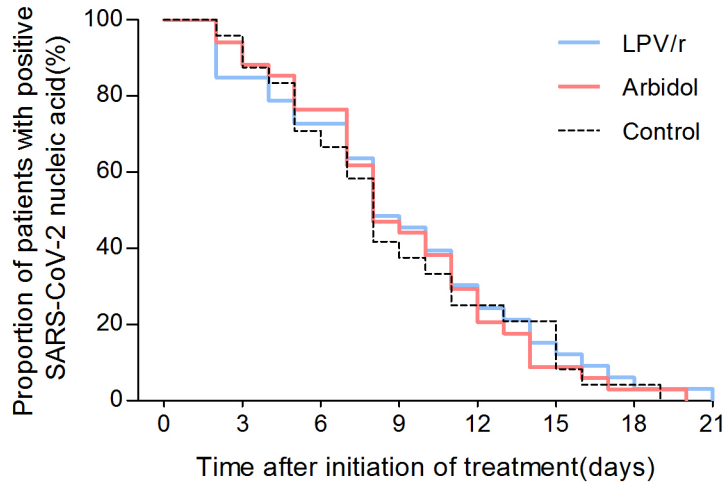
**C&S (120 words)**

The coronavirus SARS-CoV-2 is causing the current COVID-19 pandemic, which has affected over 1 million people worldwide. As the scientific community researches new treatments against the disease, drugs that have already been approved for other viruses are also being tested. Here, clinicians from Guangzhou Medical University here tested lopinavir/ritonavir and arbidol, which are currently used against HIV-1 and influenza, respectively, in patients with mild or moderate COVID-19. The authors show that neither drug improves the recovery compared to standard care, suggesting that treatment with either drug may not be beneficial against COVID-19 and other therapies may be a more effective choice.





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