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## Chansu improves the respiratory function of severe COVID-19 patients

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## ABSTRACT

Targeted therapeutics for SARS-CoV-2 virus caused COVID-19 are in urgent need. Chansu has been reported to have broad-spectrum antiviral effects and widely used in Southeast Asian countries. This study aims to assess the efficacy of Chansu injection in treating patients with severe COVID-19. A randomized preliminary clinical trial was conducted and eligible patients were allocated to receive general treatment plus Chansu injection or only general treatment as control for 7 days. The primary outcomes of the oxygenation index  $\text{PaO}_2/\text{FiO}_2$  and ROX, secondary outcomes of white blood cell count, respiratory support step-down time (RSST), safety indicators, etc were monitored. After 7 days of treatment, the oxygenation index was improved in 95.2% patients in the treatment group compared with 68.4% in the control group. The  $\text{PaO}_2/\text{FiO}_2$  and ROX indices in the treatment group (mean,  $226.27 \pm 67.35$  and  $14.01 \pm 3.99$  respectively) were significantly higher than the control group (mean,  $143.23 \pm 51.29$  and  $9.64 \pm 5.54$  respectively). The RSST was 1 day shorter in the treatment group. Multivariate regression analysis suggested that Chansu injection contributed the most to the outcome of  $\text{PaO}_2/\text{FiO}_2$ . No obvious adverse effects were observed. The preliminary data showed that Chansu injection had apparent efficacy in improving the respiratory function of patients with severe COVID-19.

## 1. Introduction

The current pandemic caused by SARS-CoV-2 represents one of the greatest threats to both human health and socioeconomic development globally. Known as coronavirus disease 2019 (COVID-19), this disease has plagued over 196.5 million cases and claimed about 4.2 million lives as of 30 July, 2021 (data released by the World Health Organization [1]). Fever, dry cough, and runny nose are common characteristics of COVID-19. Severe cases usually have dyspnea and/or hypoxemia one week after the onset of symptoms, which could quickly progress to acute respiratory distress syndrome, septic shock, metabolic acidosis that is difficult to correct, coagulation dysfunction and multiple organ failure [2-5]. In severely affected areas, the overall mortality rate is over 10% according to the data released by WHO, while the mortality rate of severe COVID-19 patients is much higher. Therefore, a critical goal of

COVID-19 treatment is reducing the mortality of severe patients. Current treatment recommendation is largely empirical and targeted therapeutics are in urgent need [6]. The Lopinavi-Ritonavir combination has been proved to have little effects on severe COVID-19 patients in clinical trials [7]. However, China's anti-epidemic experience during the outbreak in Wuhan suggested that traditional Chinese medicine might play an important role in the treatment of COVID-19.

Chansu, aqueous extracts of venoms from the parotid and skin glands of the toad *Bufo bufo gargarizans Cantor*, has been widely used in China, Japan and other Southeast Asian countries as a cardiogenic, antimicrobial, anodyne, antineoplastic, and local anesthetic agent for thousands of years, and it has been used to treat plague in history [8,9]. Recently, Chansu, in dosage forms of injection, capsules, oral solution and tablets, has been approved by the Chinese State Food and Drug Administration (SFDA). In clinical practice, Chansu injection (the ethanol extract of dry

**Abbreviations:** ALT, alanine aminotransferase; AST, aspartate aminotransferase; ATP1A1,  $\text{Na}^+/\text{K}^+$ -ATPase  $\alpha 1$  subunit; BMI, body mass index; CK-MB, cardiac marker creatine kinase isoenzyme MB; COVID-19, coronavirus disease 2019; Cr, creatinine; FIPV, feline infectious peritonitis virus; MERS-CoV, Middle East respiratory syndrome; MHV, murine hepatitis virus;  $\text{PaO}_2/\text{FiO}_2$ , a ratio of the arterial partial pressure of oxygen to the fraction of inspiration oxygen; PBML, peripheral blood mononuclear lymphocyte; PLT, platelets; ROX, index ( $\text{ROX} = \text{SpO}_2 / (\text{FiO}_2 \cdot \text{RR})$ ); RR, respiratory rate; RSST, respiratory support step-down time; RSV, respiratory syncytial virus; RT-PCR, reverse-transcriptase-polymerase-chain-reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2;  $\text{SpO}_2$ , pulse oxygen saturation; TB, total bilirubin; VSV, vesicular stomatitis virus; WBC, white blood cell; WHO, World Health Organization.

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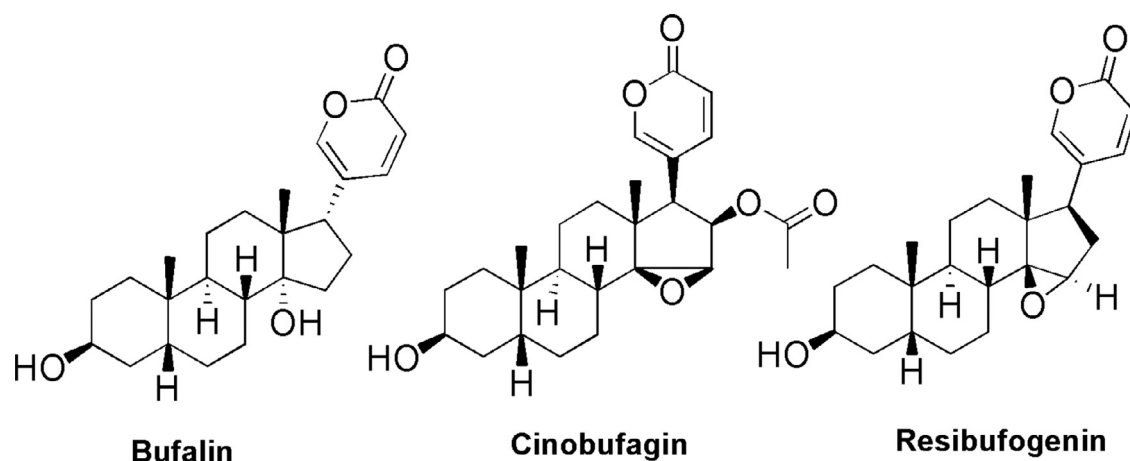


Fig. 1. Chemical structures of the main constituents of Chansu injection: bufalin, cinobufagin and resibufogenin.

secretions from the parotid and skin glands of *Bufo bufo gargarizans Cantor* processed into injection) is used to treat severe upper and lower respiratory tract infections, chronic hepatitis B therapy and cancer, and the incidence of adverse reactions is "occasionally", with good clinical safety [10-13]. The main active constituents of Chansu are bufadienolides, including bufalin, resibufogenin, and cinobufagin etc. Our preliminary *in vitro* studies have shown that Chansu or its main constituents have anti-H1N1, HIV and other RNA viruses activities (data have not been published). Reported studies also showed that, bufalin, the major active constituent of Chansu, can inhibit the infection of cells by murine hepatitis virus (MHV), feline infectious peritonitis virus (FIPV), Middle East respiratory syndrome coronavirus (MERS-CoV), and vesicular stomatitis virus (VSV) via targeting the  $\text{Na}^+/\text{K}^+$ -ATPase  $\alpha 1$  subunit (ATP1A1)-mediated Src signaling pathway [14,16], which also plays a critical role in the cell entry of Ebola virus and respiratory syncytial virus (RSV) [15,21]. ATP1A1 was shown to be required for macropinocytic entry and replication of a series of coronaviruses [15,16]. In addition, cardiac glycosides targeting ATP1A1 have been reported to be effective against both DNA and RNA viruses, emerging as potential broad-spectrum antiviral drugs [17-20].

SARS-CoV-2 is a positive-sense RNA virus. Recently, using affinity-purification mass spectrometry and CRISPR methods, researchers identified ATPase as one of the targets of SARS-CoV-2 [22,23]. Based on these findings, Chansu injection was empirically applied to patients with severe COVID-19. The HPLC fingerprint of Chansu injection and the chemical structures of its main constituents (bufalin, cinobufagin and resibufogenin) are presented in Supplementary Figure S1 and Fig. 1.

Of note, adequate and well-controlled clinical studies are difficult to perform in the condition of such a life threatening epidemic. Herein, we present the results of a randomized, preliminary clinical study that evaluated the effect of Chansu injection plus the general treatment in improving the respiratory function of patients with severe COVID-19, using the general treatment group as control.

## 2. Methods

### 2.1. Study design and participants

From February 5, 2020 to March 5, 2020, a total of 50 patients with severe COVID-19 admitted to the Department of Respiratory and Critical Care Medicine of the First People's Hospital of Jiangxia District, Wuhan, China were enrolled in the trial after approval by the ethics committees. All patients were confirmed as COVID-19 positive by either a reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assay or clinical diagnosis according to the Guidelines for the Prevention, Diagnosis, and Treatment of Pneumonia Caused by COVID-19 (version 5) issued by the

National Health Commission of the People's Republic of China [24]. Patients meeting any of the following diagnostic criteria were considered as severe patients: respiratory rate (RR)  $\geq 30$  bpm in calm state, pulse oxygen saturation ( $\text{SpO}_2$ )  $\leq 93\%$  while breathing ambient air in resting state, oxygenation index (a ratio of the arterial partial pressure of oxygen to the fraction of inspiration oxygen,  $\text{PaO}_2/\text{FiO}_2$ )  $\leq 300$  mmHg, or respiratory failure ( $\text{PaO}_2 \leq 60$  mmHg with or without carbon dioxide retention under standard conditions).

Main exclusion criteria included age  $< 18$  years old, pregnancy, history of arrhythmia, chronic respiratory failure caused by other diseases such as heart failure, thoracic deformity, structural lung disease, hemodynamic instability, severe immunodeficiency, recent use of immunosuppressants, allergies, estimated survival time  $< 3$  days, withdrawal from research or return visits. Severe COVID-19 patients admitted to the hospital mainly presented with hypoxemia, and very few were hemodynamically unstable. Hemodynamic instability in patients with end-stage COVID-19 is mostly caused by factors such as high-dose sedation, muscle relaxation, and high-strength mechanical ventilation etc. Good stability of the blood pressure of the enrolled patients was observed in this study. Survival time was estimated by two experienced clinicians. Patients with persistent severe hypoxemia that was difficult to correct, recurring malignant arrhythmia, coma, severe gastrointestinal dysfunction, and uncontrollable gastrointestinal bleeding were excluded to avoid difficulty in the observation of drug efficacy. Written informed consent was obtained from all patients or their legal representatives. The trial was registered at ChiCTR.org.cn (registration number: ChiCTR2000030704) and conducted in accordance with the principles of the Declaration of Helsinki and the Good Clinical Practice guidelines of the International Conference on Harmonisation.

### 2.2. Randomization and trial procedures

Using a random-numbers table, the enrolled patients were randomly assigned in a 1:1 ratio to receive general treatment (the control group) and general treatment plus Chansu injection (20 mL/day, containing about 60  $\mu\text{g}$  dry extract of Chansu, produced by Jiangsu Pujin Pharmaceutical Co., Ltd., Nanjing, China. Batch number: 191002, SFDA approval number: Z32020694). General treatment includes, as necessary, empirical antiviral therapy with peramivir, arbidol and interferon  $\alpha$ ,  $\gamma$ -globulin, supplemental oxygen and standard glucocorticoid therapy, nutritional support, etc (Supplementary Table S1). Besides general treatment, the treatment group was also given Chansu injection (20 mL formulated into 250 mL 0.9% physiological saline) intravenously at a rate of 125 mL/h every day, while the control group was given 250 mL 0.9% physiological saline. According to the instructions of Chansu injection, the treatment cycle is 7 days for anti-infective therapy. So this study

**Table 1**  
Baseline characteristics of the participants.

	All patients (n=40)	Treatment Group (n=21)	Control Group (n=19)	p value
<b>Gender (Male, n, %)</b>	24 (60)	11 (52.38)	13 (68.42)	0.301
<b>Age (yr), median (IQR)</b>	61.5 (51.0-71.0)	58.0 (52.5-66.0)	70.0 (51.0-77.0)	0.283
<b>BMI (kg/m<sup>2</sup>), median (IQR)</b>	26 (23.6-27.2)	26.4 (24.6-27.3)	25.2 (22.3-26.9)	0.391
<b>Time from onset to treatment (day), median (IQR)</b>	6.0 (4.0-7.8)	7.0 (4.0-7.5)	5.0 (3.0-8.0)	0.685
<b>Basic Diseases (n, %)</b>				
Hypertension	12 (30.00)	5 (23.81)	7 (36.84)	0.369
Diabetes	9 (22.50)	5 (23.81)	4 (21.05)	0.835
Chronic Obstructive Pulmonary Disease	1 (2.50)	0	1 (5.26)	0.475
Coronary Heart Disease	2 (5.00)	0	2 (10.53)	0.127
Chronic Cardiac Insufficiency	1 (2.50)	0	1 (5.26)	0.475
<b>Vital signs at enrollment<sup>†</sup></b>				
Temperature (°C)	38.01±0.93	38.18±0.94	37.82±0.90	0.230
RR (bpm)	24.95±4.30	25.48±4.59	24.37±4.00	0.423
HR (bpm)	94.88±19.62	99.67±13.54	89.58±23.96	0.105
SpO <sub>2</sub> (%)	88.73±6.12	88.05±7.65	89.47±23.96	0.469

BMI: body mass index, RR: respiratory rate, HR: heart rate, SpO<sub>2</sub>: pulse oxygen saturation

p value: statistical significance between the treatment group and control group

<sup>†</sup>: Plus-minus values are means ±SD

treatment course was set as 7 days and would be terminated in the event of intolerable adverse events, withdrawal of consent, or transferring to another hospital for further treatment.

### 2.3. Baseline and outcomes

Baseline characteristics include gender, age, body mass index (BMI), time from onset to treatment, basic diseases, and vital signs including body temperature, RR, heart rate, and SpO<sub>2</sub> etc. at the time of enrollment.

Primary outcomes include PaO<sub>2</sub>/FiO<sub>2</sub> and ROX index (ROX=SpO<sub>2</sub>/(FiO<sub>2</sub>\*RR)). Secondary outcomes include the white blood cell (WBC) count, peripheral blood mononuclear lymphocyte (PBML) count and respiratory support step-down time (RSST). The supporting intensity of the four respiratory supports of non-invasive mechanical ventilation, high-flow oxygen therapy, oxygen mask, and nasal cannula oxygen therapy, gradually weakens. Therefore, RSST is defined as the transition time from advanced respiratory support to low respiratory support after enrollment, or the time required for FiO<sub>2</sub> to decrease by 50%. The respiratory support transition was deemed successful for patients when their SpO<sub>2</sub> stayed above 94% and the increase of their respiratory rates was less than 20%.

Safety outcomes include liver function indicators of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and total bilirubin (TB), kidney function indicator of creatinine (Cr), cardiac marker creatine kinase isoenzyme MB (CK-MB), platelets (PLT) and adverse events such as systemic or local rash without other explanations, gastrointestinal symptoms such as nausea, vomiting, abdominal pain and diarrhea, new arrhythmia, etc. Adverse events were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

### 2.4. Statistical analysis

SPSS software version 22 (SPSS Inc., Chicago, IL, USA) was used to perform data analyses. Continuous variables were summarized as either medians and ranges or arithmetic means with standard deviations. Student's *t* test was used for assessing statistical significance of continuous variables between the control group and treatment group. Categorical variables were summarized as percentages (%), with their statistical significance between groups assessed by the  $\chi^2$  test. Multiple linear regression analysis was performed to evaluate the effect of the variables on the primary outcome by adjustment of imbalance for important baseline variables due to the withdrawal of some patients. Univariate linear regression analysis was performed to identify variables with regression

coefficients statistically significant ( $p < 0.05$ ), which were further included in the multivariate linear regression to evaluate their effects on the primary outcome.

## 3. Results

### 3.1. Patient characteristics

This prospective study preliminarily evaluated the efficacy and safety of Chansu injection for severe COVID-19 patients infected by SARS-CoV-2. Following eligibility assessment, a cohort of 50 patients were randomly allocated into general treatment group (the control group) and general treatment plus Chansu injection group (the treatment group) in a 1:1 ratio. Ten patients were excluded from the final analysis, including 3 patients in the treatment group and 6 patients in the control group transferring to another hospital for further treatment, and 1 patient in the treatment group failed to undergo re-examination on schedule. The remaining 40 patients, including 21 in the treatment group and 19 in the control group, were included for statistical analysis (Fig. 2).

Of the 40 participants, 24 (60%) were male. The median age was 61.5 years old (interquartile range [IQR], 51.0 to 71.0 years) and most patients (82.5%) were > 50 years old. At least one third of the patients had one or more basic diseases. The median time from onset to treatment was 6 days (IQR, 4.0 to 7.8 days). 36 patients (90%) had a body temperature > 37°C at enrollment. The mean SpO<sub>2</sub> was 88.73%. No significant differences were found between the treatment group and control group in gender, age, BMI, time from onset to treatment, basic diseases and vital signs at enrollment ( $p > 0.05$ , Table 1).

### 3.2. Chansu improved the respiratory function of severe COVID-19 patients

General treatments included peramivir, arbidol and interferon  $\alpha$ , glucocorticoid therapy,  $\gamma$ -globulin and respiratory support as necessary, which had no significant difference between the two groups ( $p > 0.05$ , Supplementary Table S1). Compared with CT imaging characteristics and various biochemical indicators, the patients' respiratory function indicators such as PaO<sub>2</sub>/FiO<sub>2</sub>, SpO<sub>2</sub> and RR related ROX index are more direct indicators of disease outcome. Therefore, PaO<sub>2</sub>/FiO<sub>2</sub> and ROX index were used as the primary outcomes in this study. No significant difference was found in the initial PaO<sub>2</sub>/FiO<sub>2</sub> and ROX index on Day 1 between the treatment group and control group ( $p = 0.118$ , 95% CI, -6.46 to 55.18 for PaO<sub>2</sub>/FiO<sub>2</sub>;  $p = 0.716$ , 95% CI, -1.89 to 2.73 for ROX) (Table 2). After 7 days of treatment, patients receiving general treatment plus Chansu injection (20 mL/day) improved significantly in

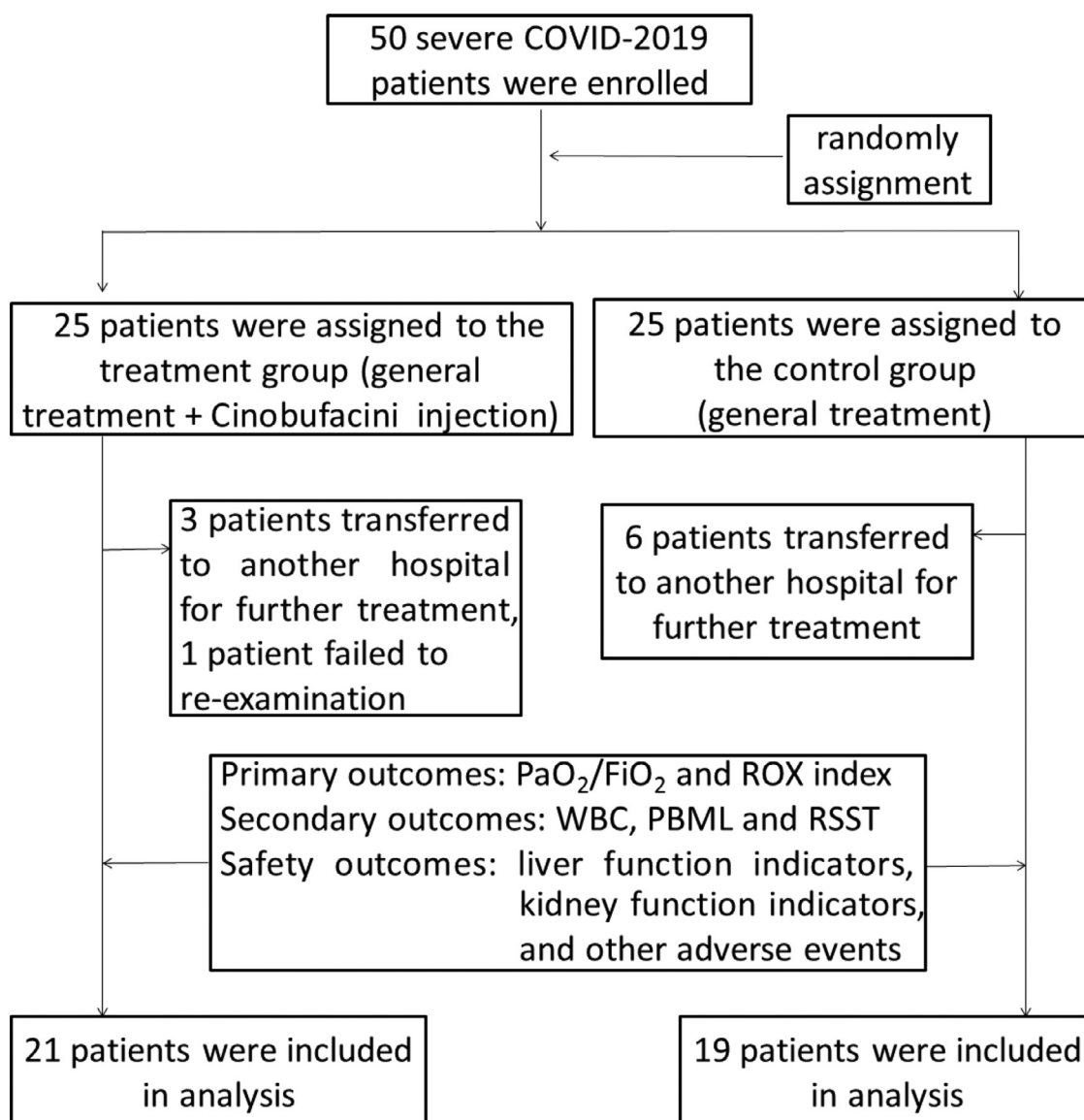


Fig. 2. Randomization and treatment assignment.

both  $\text{PaO}_2/\text{FiO}_2$  and ROX index ( $p < 0.001$ , 95% CI, -111.30 to -35.90 for  $\text{PaO}_2/\text{FiO}_2$ ;  $p < 0.001$ , 95% CI, -7.56 to -2.94 for ROX), while no significant differences were found between Day 1 and Day 7 in the control group receiving only general treatment ( $p > 0.05$ ). The  $\text{PaO}_2/\text{FiO}_2$  and ROX index were improved in 20 patients (95.2%) in the treatment group, as compared with 13 (68.4%) and 14 (73.7%) patients in the control group, while the mean values were significantly higher in the treatment group than in the control group on Day 7 ( $p < 0.001$ , 95% confidence interval [CI], 44.39 to 121.70 for  $\text{PaO}_2/\text{FiO}_2$  and  $p = 0.006$ , 95% CI, 1.31 to 7.44 for ROX) (Table 2). These results indicated that most severe COVID-19 patients benefited from Chansu injection, while general treatment with paramivir, arbidol and interferon  $\alpha$ , standard glucocorticoid therapy or other symptom relievers and oxygen therapy had little effect on improving the respiratory function of severe COVID-19 patients.

COVID-19 patients usually have normal or decreased WBC and decreased PBML. For the secondary outcomes of WBC and PBML, no obvious improvement was observed after 7 days treatment in both groups ( $p > 0.05$ ). RSST is the time needed for the patient to transform from advanced respiratory support to low respiratory support. The shorter RSST is associated with the faster prognosis of patients' respiratory function.

The median RSST was 5.0 (IQR, 4.0 to 6.0) days in the treatment group, 1 day shorter than that in the control group (median: 6.0 days, IQR: 5.0 to 8.0 days) (Table 2).

### 3.3. Chansu injection contributed the most to the primary outcome of $\text{PaO}_2/\text{FiO}_2$

Univariate linear regression analyses were performed on the treatment interventions, patients' baseline characteristics (including age, gender and basic diseases),  $\text{PaO}_2/\text{FiO}_2$ , ROX index, WBC and PBML on Day 1 to screen out variables with regression coefficients statistically significant. As a result, four characteristics, the  $\text{PaO}_2/\text{FiO}_2$  and PBML on Day 1, the initial respiratory support mode and Chansu injection affected the primary outcome of  $\text{PaO}_2/\text{FiO}_2$  on Day 7 statistically ( $p < 0.05$ ). To determine the main variables affecting the primary outcome of  $\text{PaO}_2/\text{FiO}_2$  on Day 7, multivariate regression analysis was applied. The  $\text{PaO}_2/\text{FiO}_2$  on Day 1, the initial respiratory support mode and Chansu injection were further confirmed to affect the primary outcome of  $\text{PaO}_2/\text{FiO}_2$  on Day 7 statistically ( $p < 0.05$ ). Among them, Chansu injection contributed the most to the regression model (Beta = 0.486) (Table 3).



**Table 2**  
Comparison of the outcomes between the treatment group and control group.

		Treatment Group (n=21)	Control Group (n=19)	p value*	Difference (95% CI)
<b>PaO<sub>2</sub>/FiO<sub>2</sub></b> ( $\bar{x}\pm s, mmHg$ )	Day 1	152.67±52.63 <sup>†</sup>	128.31±42.42	0.118	24.36 (-6.46~55.18)
	Day 7	226.27±67.35	143.23±51.29	<0.001	83.04 (44.39~121.70)
	p value*	<0.001	0.335		
	Difference (95% CI)	-73.60 (-111.30~-35.90)	-14.92 (-45.91~16.07)		
	No. (%) of patients improved	20 (95.2)	13 (68.4)	0.026	
<b>ROX</b> ( $\bar{x}\pm s$ )	Day 1	8.76±3.38	8.34±3.82	0.716	0.42 (-1.89~2.73)
	Day 7	14.01±3.99	9.64±5.54	0.006	4.37 (1.31~7.44)
	p value*	<0.001	0.407		
	Difference (95% CI)	-5.25 (-7.56~-2.94)	-1.30 (-4.43~1.84)		
	No. (%) of patients improved	20 (95.2)	14 (73.7)	0.057	
<b>WBC</b> ( $\bar{x}\pm s, 10^9/L$ )	Day 1	10.72±4.34	8.75±2.54	0.093	1.97 (-0.34~4.28)
	Day 7	8.96±3.57	7.22±2.20	0.074	1.74 (-0.18~3.67)
	p value*	0.160	0.055		
	Difference (95% CI)	1.75 (-0.72~4.23)	1.53 (-0.04~3.10)		
<b>PBML</b> ( $\bar{x}\pm s, 10^9/L$ )	Day 1	0.91±0.54	0.78±0.33	0.371	0.13 (-0.16~0.42)
	Day 7	1.24±0.67	0.80±0.40	0.018	0.44 (0.08~0.80)
	p value*	0.090	0.872		
	Difference (95% CI)	-0.33 (-0.71~0.05)	-0.02 (-0.26~0.22)		
<b>RSST</b> (days), median (IQR)	5.0 (4.0-6.0)	6.0 (5.0-8.0) <sup>*</sup>	0.016	-1.17 (-2.12~-0.23)	

\*: Statistical significance between Day 1 and Day 7 in the same group.

†: Plus-minus values are means  $\pm$ SD.

\*: Statistical significance between the treatment group and control group.

95% CI: 95% confidence intervals.

**Table 3**  
Multivariate regression analysis to determine the most contributing factor to the primary outcome of PaO<sub>2</sub>/FiO<sub>2</sub>.

Model	Unstandardized Coefficients		Standardized Coefficients		t	p value	Collinearity Statistics	
	B	Std. Error	Beta				Tolerance	VIF
Constant	113.317	32.888			3.446	0.001		
PaO <sub>2</sub> /FiO <sub>2</sub> (Day 1)	0.513	0.170	0.346	0.346	3.019	0.005	0.744	1.344
Initial respiratory support mode	-19.093	6.666	-0.314	-0.314	-2.864	0.007	0.812	1.231
Chansu injection	69.969	15.321	0.486	0.486	4.567	0.000	0.863	1.159
PBML (Day 1)	9.137	13.525	0.676	0.676	0.676	0.504	0.843	1.186

### 3.4. Safety

No obvious adverse events, such as systemic and local rash, gastrointestinal symptoms, abdominal pain, diarrhea, and new arrhythmia, occurred during the treatment with Chansu injection. No significant difference in other safety outcomes including liver function indicators (AST, ALT, TB), kidney function indicators (Cr), cardiac marker CK-MB and PLT was found after treatment with Chansu injection ( $p > 0.05$ ). There was also no significant difference between the treatment group and control group in these safety indicators (Table 4). All these indicated that the dose of Chansu injection used in this study is safe for severe COVID-19 patients.

### 4. Discussion

Except for the vaccines, only remdesivir is approved by FDA as targeted therapeutics for COVID-19 at present [25], whereas other treatment recommendations such as chloroquine, hydroxychloroquine, favipiravir, corticosteroids and convalescent plasma are largely empirical [26]. Chansu is a type of traditional Chinese medicine extracted from the skin and parotid glands of the toad, and it has been widely used for antiviral therapies in China and other Southeast Asian countries. To evaluate the effect of Chansu plus general treatment in patients with severe COVID-19, we conducted a randomized, controlled clinical trial in this study.

From clinical experience, the patients' respiratory function status such as SpO<sub>2</sub>, PaO<sub>2</sub>/FiO<sub>2</sub>, and ROX index were found to be more direct indicators to evaluate pulmonary function [27,28]. This preliminary trial found that, compared with the general treatment alone, Chansu injection combined with general treatment significantly improved the primary outcomes of PaO<sub>2</sub>/FiO<sub>2</sub> and ROX index in patients with severe COVID-19 after 7 days of treatment. General treatment with empirical antiviral therapy such as paramivir, arbidol and interferon  $\alpha$ , standard glucocorticoid therapy or other symptom relievers and oxygen therapy had little effect on improving the respiratory function of severe COVID-19 patients. In addition, the median RSST needed for the patients in the treatment group is 5.0 days, about one day shorter than the control group. Multivariate regression analysis suggested that, among various variables, Chansu injection contributed the most to the primary outcomes of the patients in the treatment group.

The main active ingredients of Chansu include bufalin, resibufogenin and cinobufagin, etc. Researchers have found that Chansu has various activities including anti-inflammatory, anti-infection and immunomodulatory [29-31]. Previous studies reported that Chansu or its active ingredient bufalin shows obvious anti-HBV activities and can inhibit infection of cells with MERS-CoV, Ebola, RSV, MHV, FIPV, and VSV [11-12,15]. The mechanism of Chansu inhibiting coronavirus entry into host cells might be related to ATP1A1-mediated Src signaling pathway. A recent study identified ATPases as one of the targets of SARS-CoV-2 by interacting with Nsp6 [22,23,32]. However, how the coronavirus interacts with ATPases needs further investigation.

**Table 4**  
Comparison of safety indicators between the treatment group and control group.

		Treatment Group (n=21)	Control Group (n=19)	p value*
AST ( $\bar{x}\pm s$ , U/L)	Day 1	34.68 $\pm$ 24.58 <sup>ˆ</sup>	45.57 $\pm$ 29.73	0.213
	Day 7	26.46 $\pm$ 12.46	30.79 $\pm$ 16.07	0.345
	p value*	0.179	0.065	
ALT ( $\bar{x}\pm s$ , U/L)	Day 1	39.39 $\pm$ 34.19	35.90 $\pm$ 37.55	0.760
	Day 7	37.23 $\pm$ 23.14	46.58 $\pm$ 30.55	0.279
	p value*	0.812	0.342	
TB ( $\bar{x}\pm s$ , U/L)	Day 1	12.56 $\pm$ 7.23	11.42 $\pm$ 5.40	0.578
	Day 7	12.98 $\pm$ 5.97	12.61 $\pm$ 5.95	0.843
	p value*	0.839	0.525	
Cr ( $\bar{x}\pm s$ , mmol/L)	Day 1	59.66 $\pm$ 14.12	76.54 $\pm$ 30.31	0.051
	Day 7	55.32 $\pm$ 20.36	67.97 $\pm$ 33.12	0.126
	p value*	0.427	0.411	
CK-MB ( $\bar{x}\pm s$ , U/L)	Day 1	32.32 $\pm$ 64.14	18.07 $\pm$ 8.31	0.343
	Day 7	36.91 $\pm$ 62.99	16.67 $\pm$ 11.23	0.162
	p value*	0.816	0.665	
PLT ( $\bar{x}\pm s$ , 10 <sup>9</sup> /L)	Day 1	195.52 $\pm$ 57.75	174.00 $\pm$ 48.40	0.212
	Day 7	202.52 $\pm$ 62.26	201.10 $\pm$ 95.70	0.921
	p value*	0.708	0.298	

\*: Statistical significance between Day 1 and Day 7 in the same group,  $p > 0.05$ .

ˆ: Plus-minus values are means  $\pm$ SD.

ˆ: Statistical significance between the treatment group and control group,  $p > 0.05$ .

Known side effects of Chansu are mainly related to its cardiac glycoside property [33]. However, Chansu injection shows good clinical safety at the treatment dosage [34,35]. In this clinical trial, no obvious adverse events occurred in the patients receiving Chansu injection. Our preliminary results suggest that the dose of Chansu injection (20 mL/day, containing about 60  $\mu$ g dry extract of Chansu) used in this study is safe and effective in treating patients with severe COVID-19. Such results are promising, offering an alternative strategy to treat the emerging SARS-CoV-2 infection in real time. Given the urgent need for effective drugs against SARS-CoV-2 in the current pandemic, we recommend treatment consisting of Chansu for severe COVID-19 patients to improve their respiratory function.

This clinical trial conducted in such hastiness has several limitations. Firstly, the trial was not blind, which could influence clinical decision-making and expose the study to observer bias. Secondly, owing to limited conditions, virologic clearance was not assessed after 7 days of treatment. In addition, the effect of different interventions on the final outcome of patients was difficult to evaluate, as some patients were transferred to other hospitals for follow-up treatment. Herein, we use the outcomes of respiratory function such as PaO<sub>2</sub>/FiO<sub>2</sub> and ROX index on the 7<sup>th</sup> day after treatment to evaluate the efficacy of Chansu injection. Thirdly, some patients had a history of out-of-hospital treatment, leading to inconsistencies of disease course. Interventions were not carried out at the same time after the patients transformed from mild symptoms to severe symptoms, which may lead to biased results. Other limitations include a small sample size and limited follow-up assessment of long-term outcome.

In conclusion, we found that Chansu might have a therapeutic effect on severe COVID-19 in this preliminary study. It can improve the patients' respiratory function significantly. These promising early data provide a reference for the treatment of severe COVID-19, although the effects need further confirmation by clinical trials with larger sample sizes.

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## Author Contributions

X. Hu and P. Cao conceived the presented idea, F. Hu, J. Chen, X. Hu and P. Cao designed the research; F. Hu, H. Chen, J. Zhu, C. Wang, and H. Ni performed research; F. Hu, J. Chen, J. Cheng, and X. Hu analyzed data; and J. Chen, P. Cao and X. Hu wrote the paper. F. Hu and J. Chen contributed equally to this work.

## Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.prmcm.2021.100007.

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