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Abstract We conducted a randomized, open-label, parallel-controlled, multicenter trial on the use of Shuanghuanglian (SHL), a traditional Chinese patent medicine, in treating cases of COVID-19. A total of 176 patients received SHL by three doses (56 in low dose, 61 in middle dose, and 59 in high dose) in addition to standard care. The control group was composed of 59 patients who received standard therapy alone. Treatment with SHL was not associated with a difference from standard care in the time to disease recovery. Patients with 14-day SHL treatment had significantly higher rate in negative conversion of SARS-CoV-2 in nucleic acid swab tests than the patients from the control group (93.4% vs. 73.9%, *P*=0.006). Analysis of chest computed tomography images showed that treatment with high-dose SHL significantly promoted absorption of inflammatory focus of pneumonia, which was evaluated by density reduction of inflammatory focus from baseline, at day 7 (mean difference (95% CI), −46.39 (−86.83 to −5.94) HU; *P*=0.025) and day 14 (mean difference (95% CI), −74.21 (−133.35 to

−15.08) HU; *P*=0.014). No serious adverse events occurred in the SHL groups. This study illustrated that SHL in combination with standard care was safe and partially effective for the treatment of COVID-19.

Keywords COVID-19; SARS-CoV-2; Shuanghuanglian oral liquid; clinical trial

Introduction

Since December 2019, an outbreak of novel coronavirus disease, namely, coronavirus disease 2019 (COVID-19), has spread rapidly around the world. The virus is officially named as SARS-CoV-2 by the World Health Organization because it is genetically linked to the coronavirus family, which is responsible for the outbreak of severe acute respiratory syndrome (SARS) in the spring of 2003 [1]. Patients with COVID-19 display various symptoms, ranging from no symptoms, mild symptoms with fever, cough, and shortness of breath to serious medical problems, including pneumonia, acute respiratory distress syndrome, septic shock, and multi-organ failure [2–4]. The mortality of critically ill patients with COVID-19 is alarmingly high [5–7], and at present, no specific therapeutic agents for treating or preventing COVID-19 are found [8].

Traditional Chinese medicines (TCMs) have attracted the attention of clinicians and researchers in China during the COVID-19 pandemic, but the use of TCMs for treating COVID-19 is controversial because of lack of data on the efficacy and safety of TCMs for COVID-19 [9]. Accordingly, well-controlled clinical trials are necessary to provide the proof of evidence-based medicine by showing the efficacy and safety of TCMs on COVID-19. Recently, the result of a multicenter, prospective, randomized controlled trial on the efficacy and safety of Lianhuaqingwen (LH) capsules, a repurposed Chinese herb, in patients with COVID-19, has been published by Hu *et al*. [10]. This clinical trial shows the application of TCM in the treatment of COVID-19. In the present study, we report the outcomes of a randomized, open-label, parallel-controlled, multicenter clinical trial of Shuanghuanglian (SHL) oral liquid for the treatment of COVID-19.

SHL oral liquid, a traditional Chinese patent medicine containing extracts of three herbs, including *Lonicera japonica* Thunb., *Scutellaria baicalensis* Georgi, and *Forsythia suspense* (Thunb.), has been used in clinical practice for a long time in China, particularly for patients with symptoms of colds, sore throat, and cough with fever [11]. SHL has been viewed as an effective broad-spectrum antiviral drugs [12–15], which plays an important role in preventing and controlling the epidemic of SARS, Ebola hemorrhagic fever, influenza A, and avian influenza in China [15–18].

For more than 17 years, a team led by Prof. Jianping Zuo (one of the co-authors of this paper) at Shanghai Institute of Materia Medica, Chinese Academy of Sciences (CAS), has been studying the anti-viral effects of SHL against SARS coronavirus, Middle East respiratory syndrome coronavirus, and influenza virus (H7N9, H1N1, and H5N1). Another team at CAS found that SHL and some of its ingredients have antiviral activities against SARS-CoV-2 in cultured cells with high potency [19]. Considering that no specific agents have been recommended for COVID-19 at the beginning of the COVID-19 pandemic, we conducted compassionate use of SHL in a family case of three patients (daughter and her parents) with COVID-19. We observed a positive result of SHL in treating all three patients who were fully recovered from COVID-19 after using SHL in combination with standard care [20]. Based on the abovementioned findings, we conducted a randomized, open-label, parallel-controlled, multicenter trial to evaluate the efficacy and safety of SHL (provided by Sanchine Phamaceutical, Harbin Pharmaceutical Group Co., Ltd.) for COVID-19 (registration number of Chinese clinical trial registration: ChiCTR2000029605).

Materials and methods

Design and oversight

We conducted a randomized, open-label, parallel-controlled, multicenter trial from February 8, 2020 through March 19, 2020 in patients with COVID-19 at Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei Province, China; the First Affiliated Hospital of China University of Science and Technology, Hefei, Anhui Province, China; Harbin Infectious Disease

Hospital, Harbin, Heilongjiang Province, China; Nanjing Second Hospital, Nanjing, Jiangsu Province, China; and the Central Theater General Hospital of the Chinese People's Liberation Army, Wuhan, Hubei Province, China. A total of 235 patients were randomized in a 1:1:1:1 ratio to receive either SHL (20 mL for the low-dose group, 40 mL for the middle-dose group, and 60 mL for the high-dose group, three times daily) in addition to standard therapy or standard therapy alone for 14 days. All patients received standard care, which consisted of supportive treatments, including supplemental oxygen therapy, daily symptom and vital sign monitoring, clinical laboratory testing, correction of water, electrolyte and acid base imbalances, and administration of antiviral agents and antibiotic agents if bacterial infection was found. The antiviral agents used in the standard care included lopinavir/ritonavir, ganciclovir, arbidol hydrochloride, oseltamivir phosphate, ribavirin, entecavir, and interferon. The antibiotics used in the standard care included cephalosporin, moxifloxacin, lavo-ofloxacin, and azithromycin. Standard care was used according to the "COVID-19 Diagnosis and Treatment Protocol (Trial Fifth Version or later updated versions)" released by the General Offices of National Health Committee and National Administration of Traditional Medicine of the People's Republic of China. The permuted block (eight patients per block) randomization sequence was prepared by an independent statistician in the trial, using SAS software, version 9.4 (SAS Institute). The study was approved by respective local ethics committees and performed according to the principles of the *Declaration of Helsinki* and the International Conference on Harmonization Guidelines for Good Clinical Practice. All patients in this study signed a written informed consent.

Study patients

Eligible patients were men or non-pregnant women aged 18 years or older with the diagnosis of confirmed cases or clinically diagnosed cases of COVID-19 in accordance with the Guidance in "COVID-19 Diagnosis and Treatment Protocol (Trial Fifth Version or later updated versions)" released by the General Offices of National Health Committee and National Administration of Traditional Medicine of the People's Republic of China. Confirmed cases had a positive test of SARS-CoV-2 by reverse transcription-polymerase chain reaction (RT-PCR) or clinical manifestations. Clinically diagnosed cases had imaging evidence of pneumonia combined with epidemiological history and clinical manifestations. The exclusion criteria were as follows: severe pneumonia with mechanical ventilation, death expected within 48 h, pregnant or breastfeeding women, respiratory infection caused by primary immunodeficiency disease, acquired immunodeficiency syndrome, congenital respiratory tract malformation, congenital heart disease, abnormal lung development, and any other clinically significant and coexisting condition.

Notably, in early February 2020, given the high false-negative rate of nucleic acid test, a large number of patients in Hubei met the clinical diagnosis but were negative for multiple nucleic acid tests. Therefore, in the "COVID-19 Diagnosis and Treatment Protocol (Trial Fifth Version)," Hubei Province added the classification of "clinical diagnosis," in particular antibody test and CT scan, to confirm the diagnosis of patients with COVID-19. Almost all the patients in this clinical trial received antibody tests and CT scan to confirm the diagnosis (Fig. S1 and Table S7). These procedures are in line with the policy of this special case in Hubei Province at that time.

According to "COVID-19 Diagnosis and Treatment Protocol" released by the General Offices of National Health Committee and National Administration of Traditional Medicine of the People's Republic of China, patients were classified into four categories: mild (without pneumonia), moderate, severe, and critical cases. Mild case showed mild clinical symptoms and no signs of pneumonia on chest imaging. Moderate case had clinical symptoms such as fever, cough, and imaging evidence of pneumonia. Severe case had any of the following criteria: respiratory distress with respiratory rate more than 30 times per minute, finger oxygen saturation lower than 93% at rest, or a ratio of the partial pressure of oxygen (PaO_2) to the fraction of inspired oxygen (FiO_2) of less than 300 mmHg. Critical case had any of the following conditions: respiratory failure requiring mechanical ventilation, shock, or other organ failure, which needed to be treated in ICU.

Laboratory measurements

Real-time RT-PCR assay for SARS-CoV-2

Serial oropharyngeal or nasopharyngeal swab samples were collected for extracting SARS-CoV-2 on day 0 (before SHL administration), day 7, and day 14 until discharged or another endpoint event had occurred.

Nucleic acid tests were performed with real-time RT-PCR assay, which were provided by DAAN Gene Co., Ltd. (Guangzhou, China). The detailed protocol was described in accordance with the previous study [7]. Our negative conversion results were confirmed by at least two successive tests.

Antibodies against SARS-CoV-2

The IgM and IgG antibodies against SARS-CoV-2 in serum specimens were detected using YHLO-CLIA-IgG and YHLO-CLIAIgM kits supplied by YHLO (YHLO Biotech Co. Ltd., Shenzhen, China), according to the manufacturer's instructions. The antibody levels were expressed as arbitrary unit per mL (AU/mL). The results ≥ 10 AU/mL were reactive (positive), and the results < 10 AU/mL were nonreactive (negative).

Quantitative analysis of chest CT images by using artificial intelligence (AI) technology

Pneumonia is the major injury and lesion in patients with COVID-19, as indicated by the increased density in chest CT imaging. The decreased density of infection focus on chest CT imaging could be considered as a sign of improvement. Hounsfield unit (HU) is a CT measurement unit for the density of a tissue or organ in the human body, where air is −1000 and bone cortex is +1000. The density of tissue infection on chest CT was evaluated by the HU value. The lower the density of imaging, the smaller the HU value. After quantitative analysis of the chest CT image using AI software recently developed by iFLYTEK, we calculated and compared the difference in HU values of infection degrees before and after treatment of SHL to evaluate the recovery of pneumonia.

Serial chest CT scans were performed on day 0, day 7, and 14 until discharge or another endpoint event had occurred. In the present study, a quantitative chest CT data analysis of each group was performed by using AI software developed by iFLYTEK. In particular, our system proposed a 3-dimensional (3D) patch-based segmentation model, which used a U-Net network with cross-layer connectivity, to segment lesions accurately. First, the input CT data were sliced into 3D patches. Then, according to the overlap between the 3D patches and true lesion, we can define a proper sampling probability for the training samples. Second, a coarse-to-fine segmentation pipeline was proposed. In the first stage, all the positive and negative patches participated in network training; meanwhile, the proportion of each batch was controlled during training. Therefore, the negative patches with large differences from the lesion characteristics can be easily filtered out in the first segmentation model, and the remaining negative patches will be fed into the second fine segmentation model together with all positive samples to obtain accurate segmentation results. Using the abovementioned coarse-to-fine segmentation method, we can perform accurate quantitative analysis of the volume and density (HU) of infection degree in chest CT of the participants.

Other laboratory measurements

We also collected other laboratory measurements, including blood routine (leukocytes, lymphocytes, eosinophils, platelets, etc.), liver function (alanine aminotransferase, ALT; aspartate aminotransferase, AST), renal function (uric acid, creatinine), total bilirubin, lactate dehydrogenase, and coagulation function on day 0, day 7, and day 14 until discharged or other endpoint event had occurred.

Study outcomes

The primary end point was the time to disease recovery, defined as the time from randomization to discharge. Secondary outcomes were SARS-CoV-2 nucleic acid negative conversion rate and time, and the time and rate of clinical symptom improvement was defined as the days of scoring of symptoms falling to zero. Score of symptoms, modified from previous study, has been used as endpoint in clinical trials in patients with respiratory infection [21]. The criteria for scoring primary symptoms (fever, fatigue, and cough) and secondary symptoms (diarrhea, nausea or vomiting, feeling cold, chest pain, polydipsia, hypohidrosis, chest tightness, and shortness of breath) are shown in Tables S2 and S3. Other outcomes included markers of inflammation and heart injury (NT-proBNP, cTNI). Pneumonia is the major damage in patients with COVID-19; thus, the quantification of infection degree on chest CT could be considered as an important outcome to evaluate the progress of pneumonia and effects of treatment according to the guideline in "COVID-19 Diagnosis and Treatment Protocol (Trial Fifth Version or later updated versions)."

Safety outcomes included adverse and serious adverse events. Adverse events were classified according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

Statistical analysis

This trial was initiated at the outbreak of COVID-19, which is a public health emergency. The information about COVID-19 and its related clinical outcome was limited. The original sample size of this trial was set at 400 cases. This sample size would provide 80% power to detect a difference, at a two-sided significance level of $\alpha = 0.05$, assuming that all three active-dose groups would have the mean time to disease recovery that was 4 days less than that of the standard-care group (20 days). However, given the rapid control of the epidemic in China, no more patients could be enrolled in clinical trial before April 1, 2020 (we planned to finish the trial before May 7, 2020). Finally, 235 patients were enrolled in the trial, and the assessment at that point was underpowered. All analyses were conducted in an intention-to-treat population, and all the patients who had undergone randomization were included. Categorical variables were presented as counts (percentages) and compared using the Chi-square test. Continuous variables were presented as mean and SD or median and interquartile range (IQR), and the Kolmogorov–Smirnov test was used to determine the distribution of continuous data. For continuous variables with normal distribution, the independent *t*-test or a one-way analysis of variance was used to test the differences between the two groups. Otherwise, the Wilcoxon rank-sum or the Kruskal–Wallis test was applied. The time to disease recovery was estimated using the Kaplan–Meier method and compared with the log-rank test. The Cox proportional-hazards model was used to calculate hazard ratios with 95% confidence intervals. Statistical analyses were conducted with SPSS (version 22.0, Armonk, USA), R (version 3.6.0, Vienna, Austria).

Results

Demographics and baseline characteristics

Of the 235 patients who underwent randomization, 176 patients were assigned to the SHL treatment groups (56 with low dose, 61 with middle dose, and 59 with high dose), and 59 patients were assigned to the control group with only standard care (Fig. 1 and Table 1). The standard care consisted of supportive treatments, including supplemental oxygen therapy, daily symptom and vital sign monitoring, clinical laboratory testing, correction of water, electrolyte and acid base imbalances, and administration of antiviral agents and antibiotic agents if bacterial infection evidence was provided. The standard care was used according to the "COVID-19 Diagnosis and Treatment Protocol (Trial Fifth Version or later updated versions)" released by the General Offices of National Health Committee and National Administration of Traditional Medicine of the People's Republic of China. The patients were classified as follows: 46 moderate cases and two mild cases accounting for 81.4% of the control group, 43 moderate cases accounting for 76.8% of the low-dose SHL group, 51 moderate cases and 1 mild case accounting for 85.2% of the middle-dose SHL group, and 49 moderate cases accounting for 83.1% of the high-dose SHL group. In addition, severe cases were included in this study, with 11 in the control group (18.6%), 13 in the low-dose SHL group (23.2%), 9 in the middle-dose SHL group (14.8%), and 10 in the high-dose SHL group (16.9%, Table 1).

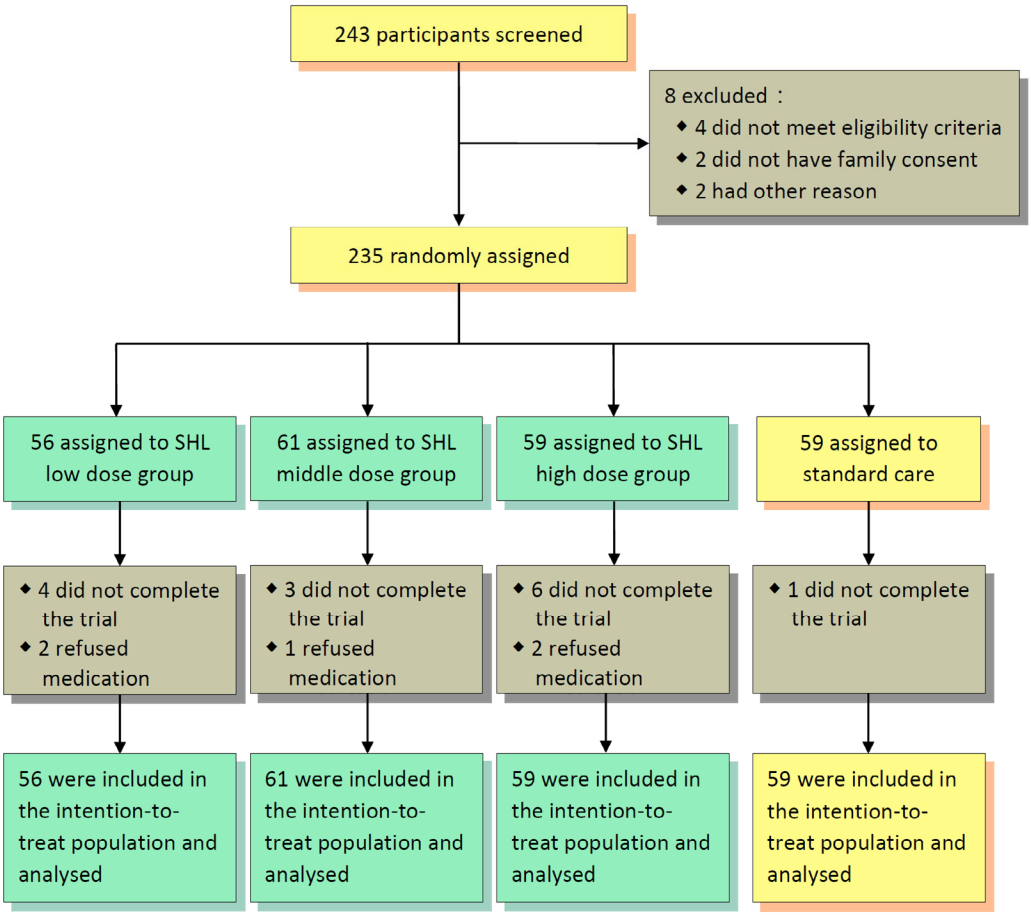

Fig. 1 Randomization and treatment assignment.

Table 1 Demographic and clinical characteristics of patients at baseline[a]

Characteristic	Standard care (*N* = 59)	Low-dose SHL (*N* = 56)	Middle-dose SHL (*N* = 61)	High-dose SHL (*N* = 59)	Total (*N* = 235)
Age, median (IQR), year	51.00 (38.50, 65.00)	54.00 (42.00, 62.25)	56.00 (44.00, 65.00)	53.00 (41.50, 63.00)	54.00 (42.00, 64.00)
Male sex, no. (%)	25 (42.4)	23 (41.1)	33 (54.1)	27 (45.8)	108 (46.0)
Classification, no. (%)					
Mild cases	2 (3.4)	0	1 (1.6)	0	3 (1.3)
Moderate cases	46 (78.0)	43 (76.8)	51 (83.6)	49 (83.1)	189 (80.4)
Severe cases	11 (18.6)	13 (23.2)	9 (14.8)	10 (16.9)	43 (18.3)
Days from illness onset to randomization, median (IQR)	22.00 (14.00, 31.50)	21.50 (16.00, 35.75)	23.00 (18.00, 34.00)	18.00 (14.00, 31.50)	22.00 (15.00, 33.00)
Body temperature (℃), median (IQR)	36.60 (36.30, 36.85)	36.50 (36.20, 36.80)	36.60 (36.30, 36.80)	36.60 (36.40, 36.85)	36.60 (36.30, 36.80)
Heart rate (beat/min), median (IQR)	80.00 (76.00, 89.50)	82.00 (78.75, 90.50)	84.00 (78.00, 94.00)	84.00 (72.00, 94.00)	83.00 (76.00, 92.00)
Respiratory rate (breath/min), median (IQR)	20.00 (20.00, 21.00)	20.00 (19.00, 21.00)	20.00 (19.00, 21.00)	20.00 (19.50, 20.00)	20.00 (19.50, 21.00)
Systolic blood pressure (mmHg), median (IQR)	128.00 (117.00, 146.00)	122.00 (113.75, 133.25)	127.00 (118.00, 139.00)	128.00 (120.00, 140.50)	125.00 (117.50, 141.00)
Fever, no. (%)	7 (11.9)	6 (10.7)	12 (19.7)	10 (16.9)	35 (14.9)
Cough, no. (%)	27 (45.8)	26 (46.4)	25 (41.0)	30 (50.8)	108 (46.0)
Fatigue, no. (%)	12 (20.3)	12 (21.4)	10 (16.4)	14 (23.7)	48 (20.4)
Diarrhea, no. (%)	2 (3.4)	5 (8.9)	5 (8.2)	6 (10.2)	18 (7.7)
White-cell count (× 10^9/L), median (IQR)	6.23 (4.94, 7.52)	5.77 (4.96, 6.96)	6.34 (5.01, 8.44)	5.41 (4.64, 6.90)	5.95 (4.89, 7.69)
4× 10^9/L −10 × 10^9/L, no. (%)	6 (10.2)	8 (14.5)	1 (1.6)	7 (12.1)	22 (9.4)
< 4 × 10^9/L, no. (%)	48 (81.4)	41 (74.5)	53 (86.9)	49 (84.5)	191 (82.0)
> 10 × 10^9/L, no. (%)	5 (8.5)	6 (10.9)	7 (11.5)	2 (3.4)	20 (8.6)
Lymphocyte count (× 10^9/L), median (IQR)	1.65 (1.21, 2.09)	1.41 (0.98, 1.89)	1.57 (1.16, 1.95)	1.47 (1.19, 1.73)	1.50 (1.14, 1.92)
< 1.0 × 10^9/L, no. (%)	9 (15.3)	16 (29.1)	10 (16.4)	8 (13.8)	43 (18.5)
≥ 1.0 × 10^9/L, no. (%)	50 (84.7)	39 (70.9)	51 (83.6)	50 (86.2)	190 (81.5)
Platelet count (× 10^9/L), median (IQR)	225.00 (179.50, 293.50)	220.00 (194.50, 278.50)	231.00 (192.00, 280.00)	233.50 (195.50, 286.00)	228.00 (192.00, 287.00)
< 100 × 10^9/L, no. (%)	0	1 (1.8)	0	1 (1.7)	2 (0.9)
≥ 100 × 10^9/L, no. (%)	59 (100.0)	54 (98.2)	61 (100.0)	57 (98.3)	231 (99.1)
Alanine aminotransferase (U/L), median (IQR)	24.00 (15.50, 44.50)	22.00 (14.00, 36.00)	26.00 (16.50, 42.50)	21.00 (14.00, 39.00)	23.00 (15.00, 40.00)

< 40 U/L	42 (71.2)	42 (77.8)	42 (71.2)	46 (80.7)	172 (75.1)
≥ 40 U/L	17 (28.8)	12 (22.2)	17 (28.8)	11 (19.3)	57 (24.9)
Aspartate aminotransferase (U/L), median (IQR)	22.00 (17.00, 30.50)	19.00 (15.25, 27.75)	20.00 (17.00, 28.00)	21.00 (18.00, 30.00)	21.00 (17.00, 29.00)
< 40 U/L	50 (84.7)	47 (87.0)	53 (89.8)	50 (87.7)	200 (87.3)
≥ 40 U/L	9 (15.3)	7 (13.0)	6 (10.2)	7 (12.3)	29 (12.7)
Serum creatinine (µmol/L), median (IQR)	60.50 (54.00, 73.75)	61.00 (53.25, 73.75)	65.50 (57.75, 79.25)	67.00 (60.00, 77.00)	64.00 (56.00, 77.00)
< 133 µmol/L	57 (98.3)	54 (100.0)	60 (100.0)	57 (100.0)	228 (99.6)
≥ 133 µmol/L	1 (1.7)	0	0	0	1 (0.4)
Lactate dehydrogenase (U/L), median (IQR)	201.00 (169.00, 240.50)	198.00 (160.25, 232.75)	200.00 (153.50, 242.00)	203.00 (181.25, 233.25)	200.00 (163.00, 237.00)
< 245 U/L	45 (76.3)	43 (79.6)	45 (75.0)	47 (81.0)	180 (77.9)
≥ 245 U/L	14 (23.7)	11 (20.4)	15 (25.0)	11 (19.0)	51 (22.1)
Total bilirubin (µmol/L), median (IQR)	9.50 (6.50, 14.05)	9.40 (6.42, 13.17)	9.90 (7.50, 15.10)	9.45 (6.53, 11.80)	9.55 (6.60, 13.10)
< 21 µmol/L	56 (94.9)	50 (92.6)	57 (93.4)	55 (94.8)	218 (94.0)
≥ 21 µmol/L	3 (5.1)	4 (7.4)	4 (6.6)	3 (5.2)	14 (6.0)
Prothrombin time (s), median (IQR)	13.30 (12.97, 13.90)	13.60 (13.20, 14.10)	13.60 (12.80, 14.22)	13.70 (13.20, 14.10)	13.60 (13.00, 14.10)
< 16 s	56 (100.0)	50 (94.3)	59 (98.3)	54 (96.4)	219 (97.3)
≥ 16 s	0	3 (5.7)	1 (1.7)	2 (3.6)	6 (2.7)

[a]The values shown are based on available data. To convert the values for creatinine to milligrams per deciliter, divide by 88.4. IQR denotes interquartile range.

The baseline characteristics and clinical presentation parameters of patients were well balanced among different groups, which are comparable in days from illness onset to randomization, systolic blood pressures, body temperatures, heart rates, and respiratory rates among the four groups. In addition, the blood routine items, including leukocytes, lymphocytes, eosinophils, and platelets, are similar among different groups. Liver functions (ALT and AST), renal functions (creatinine), total bilirubin, lactate dehydrogenase, and coagulation function (prothrombin time) were also comparable among different groups (Table 1).

The coexisting conditions and combined medication of the participants are shown in Table 2. The incidence of hypertension, diabetes, coronary heart disease, and non-severe respiratory diseases was similar in the low-dose, middle-dose, and high-dose groups of SHL and the control group. The use of other combined drugs was comparable among different groups (Table 2).

Table 2 Coexisting conditions and combined medication[a]

	Standard care (*N* = 59)	Low-dose SHL (*N* = 56)	Middle-dose SHL (*N* = 61)	High-dose SHL (*N* = 59)	Total (*N* = 235)
Coexisting conditions, no. (%)					
Hypertension	14 (23.7)	10 (17.9)	20 (32.8)	15 (25.4)	59 (25.1)
Diabetes	8 (13.6)	7 (12.5)	13 (21.3)	9 (15.3)	37 (15.7)
Coronary heart disease	2 (3.4)	3 (5.4)	1 (1.6)	4 (6.8)	10 (4.3)
Respiratory disease	2 (3.4)	0	5 (8.2)	2 (3.4)	9 (3.8)
Treatment, no. (%)					
Angiotensin II receptor blocker	0	3 (5.4)	5 (8.2)	1 (1.7)	9 (3.8)
β-blocker	4 (6.8)	4 (7.1)	8 (13.1)	4 (6.8)	20 (8.5)
Calcium channel blockers	10 (16.9)	8 (14.3)	16 (26.2)	14 (23.7)	48 (20.4)
Diuretic	1 (1.7)	2 (3.6)	1 (1.6)	1 (1.7)	5 (2.1)
Antiplatelet drugs	2 (3.4)	3 (5.4)	6 (9.8)	2 (3.4)	13 (5.5)
Chinese herb	0	0	0	1 (1.7)	1 (0.4)
Lianhuaqingwen capsule	22 (37.3)	17 (30.4)	14 (23.0)	13 (22.0)	66 (28.1)
Other Chinese patent medicine	18 (30.5)	9 (16.1)	15 (24.6)	12 (20.3)	54 (23.0)
Lopinavir–ritonavir	2 (3.4)	4 (7.1)	3 (4.9)	1 (1.7)	10 (4.3)
Oseltamivir	12 (20.3)	8 (14.3)	21 (34.4)	16 (27.1)	57 (24.3)
Arbidol	3 (5.1)	6 (10.7)	0	3 (5.1)	12 (5.1)
Other anti-viral drugs	31 (52.5)	25 (44.6)	22 (36.1)	26 (44.1)	104 (44.3)
Hydroxychloroquine	4 (6.8)	3 (5.4)	2 (3.3)	3 (5.1)	12 (5.1)
Glucocorticoid therapy	14 (23.7)	6 (10.7)	9 (14.8)	8 (13.6)	37 (15.7)
Intravenous immunoglobulin	4 (6.8)	5 (8.9)	6 (9.8)	3 (5.1)	18 (7.7)
Antibiotics	24 (40.7)	27 (48.2)	30 (49.2)	16 (27.1)	97 (41.3)
Interferon	6 (10.2)	6 (10.7)	5 (8.2)	6 (10.2)	23 (9.8)

[a]Data are presented by the number of frequencies (percentages), and respiratory diseases are defined as non-severe chronic obstructive pulmonary disease. ARB, angiotensin receptor antagonist; other proprietary Chinese medicines include Gold Leaf Poison particles; other antiviral drugs include ganciclovir and ribavirin.

Primary outcome

Time to disease recovery

Patients who received SHL did not have a difference in time to disease recovery from that of patients assigned to standard care only in the intention-to-treat population (median, 13 days vs. 13 days; hazard ratio (HR) for recovery, 1.16; 95% confidence interval (CI), 0.85 to 1.57; $P = 0.864$) (Fig. 2 and Table S1). No significant effect, as compared with standard care only, was observed with regard to the primary outcome in the low dose group (HR (95% CI), 1.16 (0.79−1.68); $P = 0.866$), middle dose group (HR (95% CI), 1.27 (0.87−1.83); $P = 0.79$) and high dose group (HR (95% CI), 1.07 (0.74−1.55); $P = 0.934$) (Table S1).

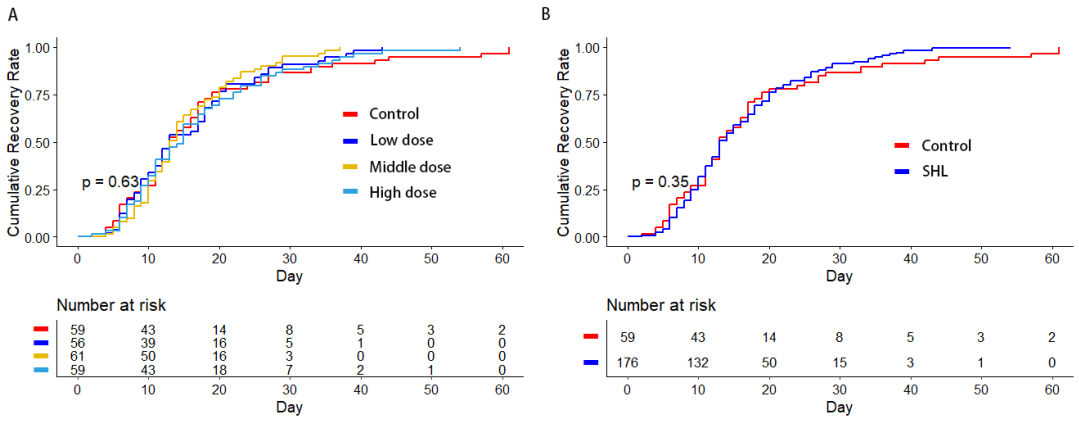

Fig. 2 Kaplan–Meier Curve for time to disease recovery. (A) Time to disease recovery in different doses of the SHL treatment groups and control group. (B) Time to disease recovery in combined dose of the SHL treatment groups and control group.

Secondary outcomes

Rate of negative conversion of viral tests

On day 14, the negative conversion rate of viral tests in groups with combined SHL doses (93.4%) was significantly higher than that in the control group (73.9%, $P = 0.006$; Table 3 and Fig. S1). The P value trend across the SHL groups as compared with the control group was 0.043 (Table 3 and Fig. S1).

Table 3 Secondary outcomes

	Standard care	SHL				*P* value	
		Low-dose SHL	Middle-dose SHL	High-dose SHL	Combined dose groups	For trend across doses vs. standard care	For combined dose groups vs. standard care
Conversion rate of virus[a]	*N* = 23	*N* = 30	*N* = 31	*N* = 30	*N* = 91	0.043	0.006
	17 (73.9)	28 (93.3)	30 (96.8)	27 (90.0)	85 (93.4)		
Clinical improvement[b]	*N* = 32	*N* = 29	*N* = 28	*N* = 33	*N* = 90		
Primary symptoms improvement, day	4.00 (2.00, 7.00)	2.00 (2.00, 7.00)	4.00 (2.00, 5.50)	4.00 (2.00, 7.00)	4.00 (2.00, 7.00)	0.914	0.782
Clinical improvement rate, day 7	24 (75.0)	25 (86.2)	24 (85.7)	26 (78.8)	75 (83.3)	0.62	0.301
Hazard ratio (95% CI)	–	1.31 (0.75–2.29)	1.28 (0.72–2.25)	1.06 (0.61–1.84)	1.20 (0.76–1.90)		
P	–	0.765	0.783	0.948	0.835		
Clinical improvement rate, day 14	30 (93.8)	29 (100.0)	25 (89.3)	31 (93.9)	85 (94.4)	0.381	0.885
Hazard ratio (95% CI)	–	1.34 (0.80–2.24)	1.09 (0.64–1.86)	1.02 (0.62–1.68)	1.13 (0.74–1.72)		
P	–	0.749	0.92	0.98	0.213		

Data are median (IQR) or *n* (%). [a]The values shown are based on patients whose viral test was positive on day 0. Conversion rate of virus means viral nucleic acid negative conversion in 14 days from day 0. [b]The values shown are based on patients who have any primary symptom (fever, fatigue, and cough) on day 0, and patients without any symptom were excluded.

Symptom improvement

No significant effect, as compared with standard care only, was observed with regard to the time to improvement of primary symptoms in the combined SHL groups (P = 0.782, Table 3). No statistical differences were detected with regard to the percentage of improvement rates at day 7 and day 14 among different groups (Table 3). Fig. 3 shows the symptoms evaluated by the combined score of primary symptoms (fever, fatigue, and cough) and secondary symptoms (diarrhea, nausea or vomiting, feeling cold, chest pain, polydipsia, hypohidrosis, chest tightness, and shortness of breath) during 21 days after randomization (scoring system is shown in Tables S2 and S3).

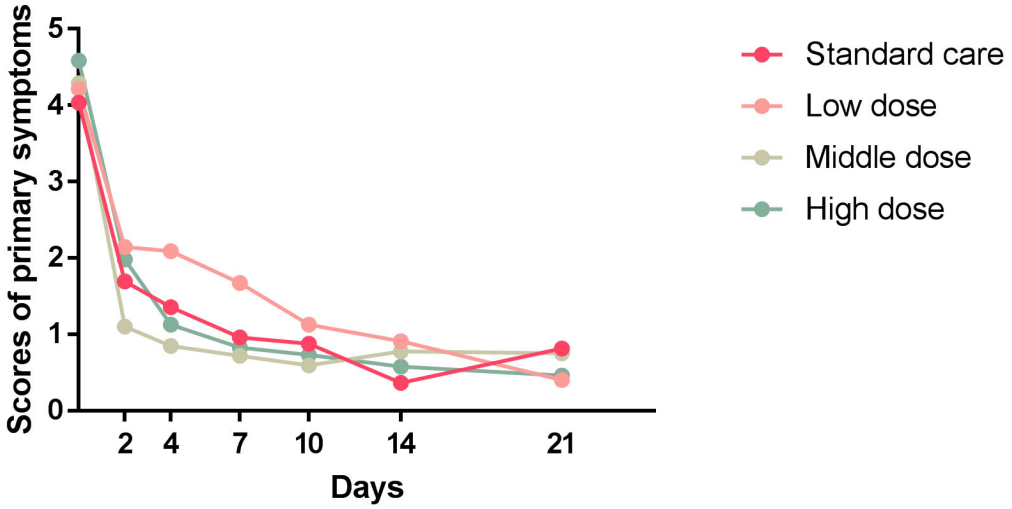

Fig. 3 Effects of SHL treatment on the symptoms scores as compared with standard care. Shown are the median symptoms scores. Control, standard care; Low dose, low dose of SHL; Mid dose, middle dose of SHL; High dose, high dose of SHL.

Other outcomes

Quantitative analysis of chest computed tomography (CT) images by AI

Pneumonia is the major injury in patients with COVID-19, as indicated by the increased density of infection focus in chest CT imaging. The decreased density of infection focus on chest CT imaging could be considered as a sign of improvement. The reduction in density of infection focus on CT imaging from baseline in the SHL group was numerically more than that in the control group on day 7 (SHL vs. control, 26.14 ± 87.36 HU vs. 10.79 ± 81.86 HU; mean difference (95% CI), −15.35 (−47.90–17.20); P = 0.353) (Fig. 4A) and day 14 (SHL vs. control, 51.57 ± 81.71 HU vs. 6.62 ± 148.49 HU; mean difference (95% CI), 24.32 (−93.25–3.36); P = 0.068) (Fig. 4B). In particular, the amplitude of reduction of density of the infection focus on CT imaging in the high-dose SHL group was significantly more than that in the control group on day 7 (high dose SHL vs. control, 57.17 ± 72.47 HU vs. 10.79 ± 81.86 HU; mean difference (95% CI), −46.39 (−86.83 to −5.94); P = 0.025) (Fig. 4C) and day 14 (high dose SHL vs. control, 80.84 ± 78.18 HU vs. 6.62 ± 148.49 HU, = 0.014; mean difference (95% CI), −74.21(−133.35 to −15.08) (Fig. 4D). In the middle-dose group and low-dose group, the density reduction was also numerically more than that in the control group on day 7 and day 14, but the P value did not meet the statistical significance (Fig. 4C and 4D). These data suggested that

SHL promoted the absorption of lung inflammation in the SHL treatment group, and the efficacy of high dose was better than that of middle and low doses in patients with COVID-19.

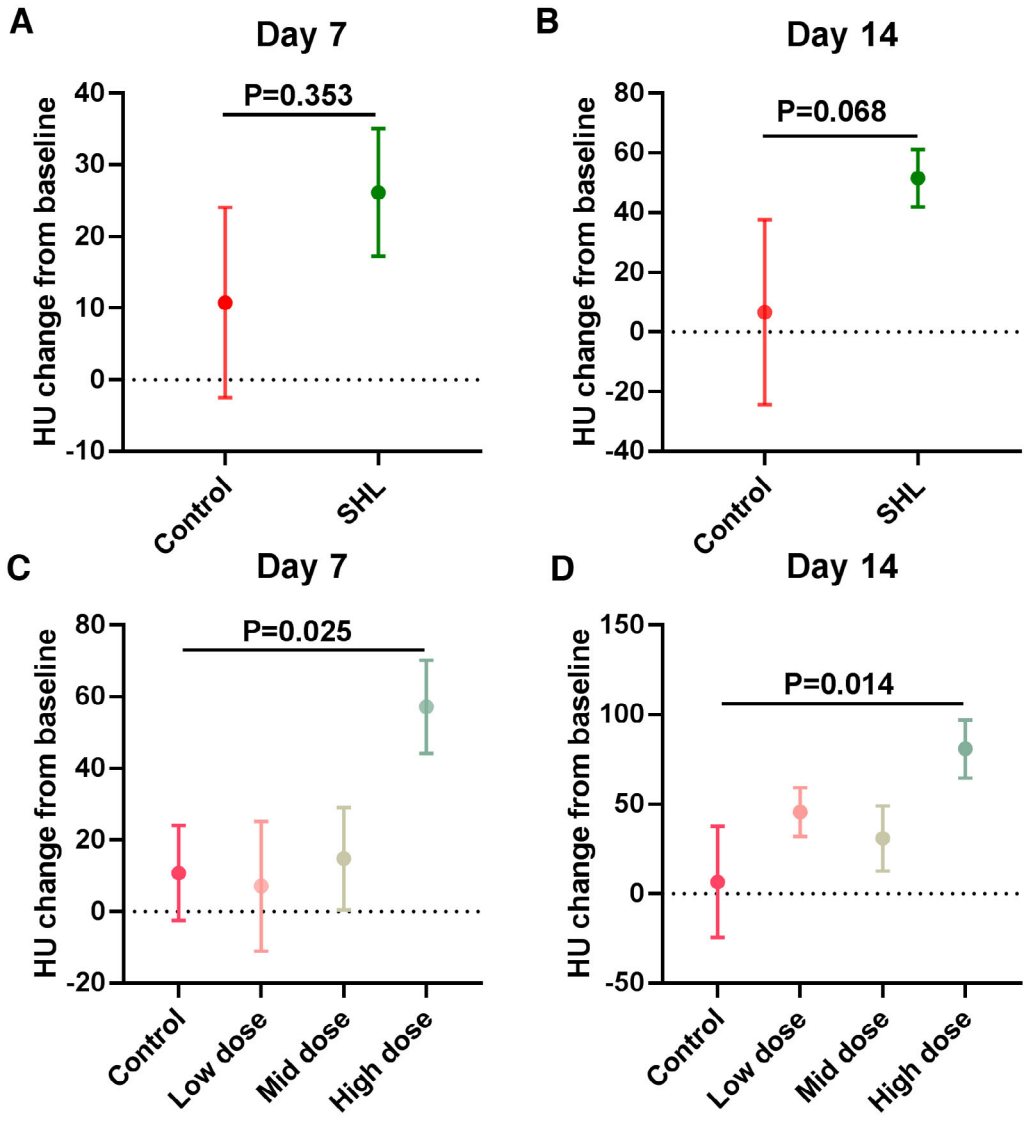

Fig. 4 Reduction in density of infection focus on CT imaging from baseline. (A, B) The reduction in density of infection focus on CT imaging from baseline in combined dose of the SHL treatment groups and control group on day 7 (A) and day 14 (B). (C, D) The reduction in density of infection focus on CT imaging from baseline in different doses of the SHL treatment groups and control group on day 7 (C) and day 14 (D). Shown are the mean (standard error of mean, SEM) changes from baseline. Control, standard care; Low dose, low dose of SHL; Mid dose, middle dose of SHL; High dose, high dose of SHL; HU, Hounsfield unit.

Analyses of serum inflammatory factors and plasma markers of myocardial injury

Regarding the changes of serum inflammatory factors, no evident difference was found among the SHL groups and control group (Table S4). Table S5 shows the changes of plasma markers of myocardial injury in the SHL treatment groups and control group. The levels of N-terminal brain natriuretic peptide (NT-proBNP) were numerically lower in the SHL groups on day 7 than that in the control group, with *P* value of 0.063. NT-proBNP is an important marker of heart failure [22]. Given the

insufficient understanding of this novel disease at the beginning of the outbreak and the limitation of medical resources in Wuhan, only portions of patients were examined for serum inflammatory factors and markers of myocardial injury. The size of tested patients was not big enough to perform subgroup analyses for the different SHL dosing groups. Nevertheless, SHL showed a beneficial trend on the hearts of patients with COVID-19.

Safety

No serious adverse events occurred in patients randomly enrolled in this project who were treated with SHL. Only 20 people had mild adverse reactions, among them 12 people experienced an adverse reaction once; 4 people experienced adverse reactions two times, and 4 people experienced adverse reactions three times. A total of 12 cases were included in the low-dose group, 8 cases in the middle-dose group, and 12 cases in the high-dose group. The types of mild adverse reaction are as follows: gastrointestinal discomfort (3 cases in the middle-dose group, 2 cases in the high-dose group), increased ALT (3 cases in the low-dose group, 1 case in the high-dose group), increased γ-glutamyl transpeptidase (3 cases in the low-dose group, 1 case in the high-dose group), increased AST (2 cases in the low-dose group, 1 case in the high-dose group), rash (1 case in the middle-dose group, 2 cases in the high-dose group), nausea (1 case in the low-dose group, 1 case in the high-dose group) and vomiting (2 cases in the high-dose group), diarrhea (1 case in the low-dose group, 1 case in the high-dose group), hyperlipidemia (2 cases in the middle-dose group), abdominal distension and pain (1 case in the high-dose group), poor appetite (1 case in the middle-dose group), hypokalemia (1 case in the middle-dose group), urinary tract infection (1 case in the low-dose group), and skin allergy (1 case in the low-dose group). In addition, two cases of adverse reactions were reported in the standard care group, namely, constipation and increased ALT (Table 4). No statistical difference in adverse effects was found between the control group and SHL groups.

Table 4 Summary of adverse events

		SHL				*P* value	
	Standard care (*N* = 59)	Low-dose SHL (*N* = 56)	Middle-dose SHL (*N* = 61)	High-dose SHL (*N* = 59)	Combined dose groups (*N* = 176)	For trend across doses vs. standard care	For combined dose groups vs. standard care
Rash	0	0	1 (1.6)	2 (3.4)	3 (1.7)	0.303	0.313
Skin allergies	0	1 (1.8)	0	0	1 (0.6)	0.36	0.562
Gastrointestinal discomfort	0	0	3 (4.9)	2 (3.4)	5 (2.8)	0.156	0.191
Nausea	0	1 (1.8)	0	1 (1.7)	2 (1.1)	0.55	0.411
Vomiting	0	0	0	2 (3.4)	2 (1.1)	0.111	0.411
Diarrhea	0	1 (1.8)	0	1 (1.7)	2 (1.1)	0.55	0.411
Abdominal distension and pain	0	0	0	1 (1.7)	1 (0.6)	0.392	0.562
Poor appetite	0	0	1 (1.6)	0	1 (0.6)	0.413	0.562
Hypokalemia	0	0	1 (1.6)	0	1 (0.6)	0.413	0.562
Constipation	1 (1.7)	0	0	0	0	0.392	0.083
Urinary tract infection	0	1 (1.8)	0	0	1 (0.6)	0.36	0.562
Increased aspartate aminotransferase	0	2 (3.6)	0	1 (1.7)	3 (1.7)	0.264	0.313
Increased alanine aminotransferase	1 (1.7)	3 (5.4)	0	1 (1.7)	4 (2.3)	0.237	0.79
Increased γ-glutamyl transpeptidase	0	3 (5.4)	0	1 (1.7)	4 (2.3)	0.088	0.243
Hyperlipidemia	0	0	2 (3.3)	0	2 (1.1)	0.124	0.411

Discussion

In this study, we conducted a randomized, open-label, parallel-controlled, multicenter trial to evaluate the efficacy and safety of SHL on COVID-19. Although this trial did not reach the primary endpoint of shortening the disease time to recovery by treating with SHL from standard care. However, three favorable outcomes have been observed in the trial: (1) patients with 14-day SHL treatment had significantly higher rate in negative conversion of SARS-CoV-2 in nucleic acid swab tests than the patients from the control group with standard care (Table 3), implying that SHL has antiviral efficacy in treating patients with COVID-19; (2) another important result was from the analysis of chest CT images, which showed that treatment with high-dose SHL might promote absorption of inflammatory focus of pneumonia at day 7 and day 14 (Fig. 4); (3) SHL may be beneficial to the heart of patients with COVID-19 through lowering NT-proBNP, an important marker of heart failure (Table S5). In this study, the standard care in the SHL and control groups is in accordance with the Guidance in "COVID-19 Diagnosis and Treatment Protocol" released by the General Offices of National Health Committee and National Administration of Traditional Medicine of the People's Republic of China (Table 2). Based on such a standard treatment, the SHL groups showed several beneficial clinical outcomes compared with the control group, illustrating the effect of SHL on the treatment of patients with COVID-19.

Our previous pharmacological study indicated that SHL and several pure ingredients from SHL had potent antiviral activities against SARS-CoV-2 in enzymatic and cultured cell assays on a clinical isolate of SARS-CoV-2 in Vero E6 cells. The enzymatic and cultured cell inhibition activities of SHL oral liquid are IC_{50} = 0.090 μL/mL and EC_{50} = 1.20 μL/mL, respectively [19]. The main mechanism of SHL to combat SARS-CoV-2 is inhibiting the enzymatic activity of the 3C-like protease (3CLpro) of the virus, which is essential for viral transcription and replication. Further studies on SHL compositions indicated that baicalin and baicalein, two active components of *S. baicalensis* Georgi, are potent inhibitors of 3CLpro. The enzymatic activities (IC_{50} values) of these two natural products are 6.4 and 0.94 μmol/L, respectively. The antiviral activities of baicalin and baicalein against a clinical isolate of SARS-CoV-2 in Vero E6 cells were also determined, and the resulting EC_{50} values were 27.87 and 2.94 μmol/L, respectively [19]. We had also determined the X-ray crystal structure of baicalein with 3CLpro [19]. Baicalein has been developed as a neuroprotective agent to treat Parkinson's disease. Two phase 1 clinic trials indicated that this natural product is safe; a single oral dose of 100–2800 mg of baicalein was well tolerated by healthy subjects, and it had favorable pharmacokinetic (PK) properties [23,24]. These studies also revealed that baicalin was a metabolite of baicalein in the human body. In addition, the total cumulative amounts of baicalin and its glucuronide metabolites in bile were c.a. 54% and 40% of the doses after oral administration of baicalin and baicalein in rats, respectively [25]. The high biliary excretion indicated that baicalin and baicalein had good oral absorption. Another PK study of anti-viral components from SHL oral liquid showed that C_{max} of baicalin in the preparation of SHL in rat plasma was 14 672 ng/mL (oral administration**)**, equivalent to 32.9 μmol/L, which was higher than the value of EC_{50} (27.87 μmol/L) for baicalin against SARS-CoV-2 in Vero E6 cells [26]. These results combined with this trial provide evidence that baicalein or baicalein–baicalin combination may be developed as an anti-SARS-CoV-2 drug, but its efficacy needs to be verified by additional clinical trial.

TCM and the combined treatment of western medicine with TCM played an important role in combating COVID-19 in Wuhan and other cities in China in 2020. However, only the clinical trial result on the efficacy and safety of LH capsules, a repurposed Chinese herb, in patients with COVID-19, has been recently published by Hu *et al.* [10], although many herbal medicines or TCM have been used in treating patients with COVID-19 during the pandemic. This multicenter, prospective, randomized controlled trial revealed that the recovery rate was higher in the treatment group as compared with the control group, and the median time to symptom recovery was markedly shorter in the treatment group. However, both groups did not differ in the rate of conversion to severe cases or viral assay findings. These two TCMs contain *L. japonica* Thunb. and *F. suspense* (Thunb.), but LH does not contain *S. baicalensis* Georgi, which is a main component of SHL. This result indirectly indicated that the activity and efficacy of SHL for anti-SARS-CoV-2 might be due to some ingredients in *S. baicalensis* Georgi because LH treatment did not alter the rate of conversion in viral assays.

Pneumonia is a major manifestation in patients with COVID-19, where the decreased density of chest CT imaging in infection focus can be considered as a sign of symptom improvement. In obtaining a sophisticated analysis result, we used AI software to quantitatively calculate the infection density of inflammatory focus of the lungs rather than to evaluate the infection area. The results indicated that the reduction in density of pneumonia infection focus from the baseline after treatment in the SHL groups was more than that in the control group. In particular, the reduction in imaging density of infection focus in the high-dose SHL group was significantly more than that in the control group on day 7 and day 14. In addition, the reduction of lung inflammation enhanced by SHL showed a dose–effect relationship, in which the high-dose SHL treatment achieved better pneumonia recovery than the low- and middle-dose treatments. As for the detailed mechanism of SHL to speed up the absorption of pneumonia focus, further pharmacological research and clinical experiments are necessary.

Considerable evidence has shown that inflammation storm plays an important role in the progress of COVID-19, particularly for critically ill patients [27,28]. Considering that all patients we recruited were non-critically ill patients, their inflammatory factors remained at normal levels during the trial for either the SHL groups or the control group. As previously mentioned, in the middle phase of our clinical trial, the role of inflammatory factors in COVID-19 had attracted considerable attention. Accordingly, we only investigated inflammatory cytokines for a portion of participants, including 117 from the SHL groups and 43 from the control group on day 0, 52 from the SHL groups and 15 from the control group on day 7, and 57 from the SHL groups and 20 from the control group on day 14. Therefore, we did not observe the effects of SHL on inhibiting inflammatory cytokines in the trial (Table S4), although other studies indicated that SHL and some of its ingredients such as baicalin could inhibit the expression levels of TNF-α, IL-6, and IL-8 in MRC5 cells induced by LPS [13,29,30].

Cardiac injury also contributed to the system damage in patients with COVID-19 [7]. Our clinical trial revealed a phenomenon that SHL might affect the hearts of patients with COVID-19 because the trial observed a trend that N-terminal brain natriuretic peptide (NT-proBNP), a biomarker for heart failure, was lower in the SHL groups compared with the control group (Table S5). Similar to the absorption of pneumonia focus, the mechanism of SHL lowering the NT-proBNP of patients should be further studied.

The dose–response (effect) relationship in clinical trials is important for the safe and effective use of drugs in patients [31]. The trial of SHL revealed dose–effect relationships for inflammation focus absorption of pneumonia, that is, the effects of middle or high-dose SHL were better than that of low dose. Although we did not study the dose–effect relationship systematically in this trial because of the emergency situation of COVID-19, we were still able to obtain useful information from this trial: (1) SHL was effective in anti-virus as indicated by the result of negative conversion in nucleic acid swab tests; (2) high-dose SHL was more efficacious than low-dose SHL in promoting absorption of inflammatory focus of pneumonia. These observations are important for the treatment of patients with COVID-19 by using SHL in the future.

Our clinical trial has several limitations. First, the trial was not double blinded and placebo controlled because of the emergency situation in Wuhan in early February 2020, which may cause an interpretive bias by influencing clinical decision making. Second, half of the viral tests of patients (116 cases) at randomization were negative, some with previous positive tests and some cases of “clinical diagnosis” without any viral positive tests before randomization (Fig. S2 and Table S7). In early February 2020, given the high false-negative rate of nucleic acid test, a large number of patients in Hubei met the clinical diagnosis but were negative for multiple nucleic acid tests. Therefore, in the “COVID-19 Diagnosis and Treatment Protocol (Trial fifth Version),” Hubei Province added the classification of “clinical diagnosis.” Suspected cases in Hubei Province were clinically diagnosed when imaging characteristics of pneumonia (CT scan) and positive antibody tests were presented. Therefore, a CT scan of a suspected case showing COVID-19 compliance, in the absence of a nucleic acid test, is a clinically diagnosed case, and the clinical diagnosis refers to the treatment of the confirmed case. All the patients in this clinical trial received antibody tests to confirm the diagnosis when the assays were available (Fig. S2, Tables S6 and S7). Accordingly, negative conversion of viral tests in this trial was based on the 114 patients who had positive tests on viral nucleic acids at baseline. The false-positive rate was low because of the additional antibody test, and the false-negative rates dropped in the later testing. Therefore, the statistical analysis of the rate in the negative conversion of SARS-CoV-2 in nucleic acid swab tests was not affected by the number of negative patients before randomization. Third, about half of the patients at randomization had no any

symptoms, and the time of illness onset to randomization was long. Considering that Tongji Hospital was a designated hospital for severe and critical cases of COVID-19 in Hubei Province, most of the patients were transferred from other general hospitals or mobile cabin hospitals; thus, a considerable number of patients in this clinical trial had already waited for a long time before randomization in this trial. This limitation is possibly one of the reasons that SHL did not improve the symptom and shorten the time of recovery. Therefore, a further clinical study with larger samples and appropriate clinical design is necessary to investigate the detailed effects of SHL on patients with COVID-19.

Treatment with SHL oral liquids could accelerate negative conversion rate in SARS-CoV-2 nucleic acid tests and promote the absorption of inflammatory focus of pneumonia in mild, moderate, and severe patients with COVID-19. These findings indicated that combining SHL with standard care could enhance antiviral effects and improve clinical outcomes in patients with COVID-19. This trial provided direct evidence for the efficacy and safety of SHL for COVID-19 by using evidence-based medicine.

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Compliance with ethics guidelines

Li Ni, Zheng Wen, Xiaowen Hu, Wei Tang, Haisheng Wang, Ling Zhou, Lujin Wu, Hong Wang, Chang Xu, Xizhen Xu, Zhichao Xiao, Zongzhe Li, Chenze Li, Yujian Liu, Jialin Duan, Chen Chen, Dan Li, Runhua Zhang, Jinliang Li, Yongxiang Yi, Wei Huang, Yanyan Chen, Jianping Zhao, Jianping Zuo, Jianping Weng, Hualiang Jiang, and Dao Wen Wang declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. Informed consent was obtained from all the patients, in which their identifying information is included in this article. Other ethical board approval is not applicable in this manuscript.

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