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Nebulized inhalation of plasma-activated water in the treatment of progressive moderate COVID-19 patients with antiviral treatment failure: a randomized controlled pilot trial

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Abstract

Background Antiviral drugs show significant efficacy in non-severe COVID-19 cases, yet there remains a subset of moderate COVID-19 patients whose pneumonia continues to progress post a complete course of treatment. Plasma-activated water (PAW) possesses anti-SARS-CoV-2 properties. To explore the potential of PAW in improving pneumonia in COVID-19 patients following antiviral treatment failure, we conducted this study.

Methods This was a randomized, controlled trial. Moderate COVID-19 patients with antiviral treatment failure were randomly assigned to the experimental group or the control group. They inhaled nebulized PAW or saline respectively. This was done twice daily for four consecutive days. We assessed improvement in chest CT on day 5, the rate of symptom resolution within 10 days, and safety.

Results A total of 23 participants were included, with 11 receiving PAW and 12 receiving saline. The baseline characteristics of both groups were comparable. The experimental group showed a higher improvement rate in chest CT on day 5 (81.8% vs. 33.3%, $p=0.036$). The cumulative disappearance rate of cough within 10 days was higher in the experimental group. Within 28 days, 4 patients in each group progressed to severe illness, and no patients died. No adverse reactions were reported from inhaling nebulized PAW.

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Conclusion This pilot trial preliminarily confirmed that nebulized inhalation of PAW can alleviate pneumonia in moderate COVID-19 patients with antiviral treatment failure, with no adverse reactions observed. This still needs to be verified by large-scale studies.

Trial registration Chinese Clinical Trial Registry; No.: ChiCTR2300078706 (retrospectively registered, 12/15/2023); URL: www.chictr.org.cn.

Keywords Plasma-activated water, COVID-19, Chest computed tomography

Introduction

Coronavirus disease 2019 (COVID-19) continues to be pandemic globally, and a variety of antiviral drugs have been widely used to treat COVID-19 pneumonia. Commonly used oral antiviral drugs such as Nirmatrelvir/Ritonavir and Molnupiravir are small molecules that suppress viral replication and propagation by acting on key enzymes in the replication process of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1–3]. These drugs can reduce the hospitalization and mortality rates of non-severe COVID-19 patients [4, 5], but a portion of patients still progress to severe disease and death after treatment. For example, a retrospective study by Wai et al. found that among high-risk mild to moderate COVID-19 hospitalized patients in Hong Kong, the 28-day all-cause mortality rates for Nirmatrelvir/Ritonavir and Molnupiravir were 4.3% and 8.5%, respectively [6]. Additionally, the aforementioned drugs also have adverse reactions, such as disease recurrence, dysgeusia, diarrhea, and rash [7, 8]. Therefore, the search for new, more effective, and safer antiviral drugs is imperative.

Plasma-activated water (PAW) is a category of aqueous solution activated by discharge plasma, containing abundant reactive species with various biological effects such as anti-microbial, anti-cancer, and stopping bleeding [9, 10]. Generally, PAW is produced by the reaction between plasma-generated gaseous reactive species and aqueous solutions via diffusion, bubbling, or jetting to produce aqueous species [11]. Plasma can inactivate SARS-CoV-2 in the environment [12, 13] and degrade its RNA [14]. Guo et al. found that PAW can disrupt the receptor-binding domain (RBD) of SARS-CoV-2 spike protein, preventing its binding to human angiotensin-converting enzyme 2 (ACE2) [15]. Acute and chronic toxicity tests in animals confirmed the biosafety of PAW [16, 17]. In addition, we have observed no adverse events in C57 mice after nebulizing PAW for 1 week. Blood indicators and histopathological examinations of important organs were normal. C57 mice infected with influenza A virus H1N1 PR8 were given aerosolized PAW for 3 days, and we observed its effectiveness. The results showed that the experimental group was superior to the control group in terms of survival status, body weight, and decreased inflammatory factors. Two clinical studies have confirmed the safety of PAW in humans [18, 19]. Guo et al. found that gargling with PAW can accelerate the conversion of viral RNA from positive to negative in COVID-19 patients with persistently positive oropharyngeal swabs, improve sore throat, and without adverse reactions [20]. However, for COVID-19 patients with pneumonia, gargling treatments may be less effective. Inhalation administration has the advantages of rapid onset, high local drug concentration, low dosage, and fewer systemic adverse reactions [21]. Several studies suggest that inhaled antiviral drugs may be effective in treating COVID-19 [22–25]. Cortázar et al. found in vitro that nebulized PAW can reduce the infectivity of SARS-CoV-2 [26]. In December 2022, we provided remedial nebulized inhalation of PAW to 5 hospitalized COVID-19 patients whose chest computed tomography (CT) showed worsening pneumonia after 5 days of oral antiviral drug treatment. Worsening pneumonia refers to an increase in the number and/or size of pneumonia lesions on chest CT. Results showed significant improvement in chest CT on day 5 in 4 patients, and 1 patient with slight worsening on day 5 improved on day 12 after one week of standard treatment. Their symptoms gradually improved, with no adverse events occurring.

Based on the aforementioned findings, to further explore the effectiveness and safety of nebulized inhalation of PAW in COVID-19 patients, we selected moderate COVID-19 patients with high risk of severity and worsening pulmonary imaging post-oral antiviral treatment, and administered nebulized PAW. This is the first study of nebulized inhalation of PAW for the treatment of pulmonary infections and COVID-19.

Methods

Study design and randomization

This is a single-center, single-blind, randomized, controlled trial. All subjects were randomly assigned in a 1:1 ratio to the experimental and control groups by simple randomization. Random numbers were generated by a separate researcher using computer software (SPSS software version 27), and then secured in sequentially numbered opaque sealed envelopes to conceal allocation. The researcher in charge of enrollment contacted this independent researcher to obtain random numbers and corresponding groups when the subjects were enrolled.

Ethical review

The study follows international scientific research standards and the Declaration of Helsinki, approved by the Medical Ethics Committee of the First Affiliated Hospital of the Air Force Medical University (Approval No.: KY20232004-C-1), registered with the Chinese Clinical Trial Registry (Registration No.: ChiCTR2300078706), and written informed consent was obtained from all participants.

Subjects

The eligible population consists of moderate COVID-19 patients hospitalized in Xijing Hospital from January to July 2023. The COVID-19 classification is based on the "Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 9)" issued by China's National Health Commission. The criteria for moderate COVID-19 are as follows: presence of fever and/or respiratory symptoms and other clinical manifestations related to COVID-19, with radiological evidence of pneumonia. Additionally, the following criteria must be met: aged 18–85 years; presence of high-risk factors for severe disease; positive SARS-CoV-2 polymerase chain reaction or rapid antigen test within the past week; chest CT after 5 days of oral Nirmatrelvir/Ritonavir treatment showing increased number and/or size of pneumonia lesions. Patients with severe or critical COVID-19, allergic constitution, bacterial or fungal co-infection of the lungs, pregnant or lactating women, and those planning to conceive were excluded. Detailed COVID-19 classification criteria and high-risk factors for severe disease are provided in the Supplementary Materials.

Preparation of PAW

The equipment and preparation method used in the study of PAW gargling treatment for COVID-19 were used [20]. A hybrid plasma discharge configuration using ambient air was used for the PAW preparation, where a dielectric barrier discharge reactor and a gliding arc discharge reactor generated air plasmas in O_3 mode and NO_x mode, respectively. The dielectric barrier discharge reactor was powered by a sinusoidal high-voltage power supply (Xiaozhong Environmental Ltd., XZ-10GKT) with an output voltage (peak-to-peak value) of about 12 kV and a frequency of about 10 kHz, while the gliding arc discharge reactor was driven by a high-voltage transformer (Hongba Electronics Ltd., MXP-12KV-40MA) with an output voltage (peak-to-peak value) of about 15 kV and a frequency of about 50 Hz. In brief, gas plasma was introduced into 200 ml of saline (0.9%NaCl) and reacted for 10 min to prepare PAW.

Interventions and blinding

All subjects received standard treatment according to COVID-19 treatment guidelines. On this basis, the experimental group inhaled nebulized PAW twice daily (interval>4 h). Using an oxygen-driven jet nebulizer (oxygen flow rate 6 L/min) to nebulize 5 ml of PAW for 10 min each time, for 4 consecutive days. PAW was nebulized immediately after preparation for treatment of the subjects. The control group inhaled nebulized 5 ml saline using the same method. Subjects were blinded in the study. There was no difference in appearance between PAW and saline, so the subjects did not know what they received. Subjects gargled and washed their faces with water after the nebulization treatment.

Efficacy

The primary efficacy indicator was the improvement rate of chest CT on day 5. Each chest CT was evaluated by two radiologists with over 15 years of experience in chest imaging diagnosis and a respiratory physician with over 20 years of pulmonary medicine experience. All three evaluators were blinded. This qualitative assessment was classified as improvement, stability, or aggravation, with the following specific criteria: improvement: ① lesion range decreased; ② lesion range unchanged, density reduced. Stability: lesion range and density basically unchanged. Aggravation: ① lesion range increased; ② lesion range unchanged, density increased; ③ new lesions appeared. Stability and aggravation were classified as non-improvement. Scans were performed using a 64-slice spiral CT (GE, LightSpeed VCT). In addition, we conducted quantitative analysis of chest CT using a computerized quantitative analysis method that was utilized in the study by Shen et al. [27]. The volume and density of lung lesions were automatically measured in the Digital Lung software (DEXIN, China). The results of lesion segmentation obtained by the software were inspected by another radiologist. If there were any inaccuracies, they were manually rectified by the radiologist.

Secondary efficacy indicators included the rate of symptom disappearance within 10 days, the rate of symptom relief within 10 days, hospitalization duration, levels of inflammatory factors on day 5 (procalcitonin [PCT], interleukin 6 [IL-6]), the proportion of moderate to severe cases within 28 days, and all-cause death within 28 days. Symptom scores were assessed and reported by patients according to the severity of their symptoms. We collected three symptoms: cough, sputum production, and shortness of breath. The scoring criteria from the U.S. FDA COVID-19-related symptom questionnaire were used [28]. Specific scoring criteria are as follows: none=0 points, mild=1 point, moderate=2 points, severe=3 points. The aforementioned symptoms were recorded at the same time every day, for 10 consecutive

days, starting from enrollment. Face-to-face interviews during hospitalization and follow-up calls after discharge. The changes of each symptom were recorded in detail. Symptom disappearance was defined as the symptom being absent for two consecutive days, with the first day of absence recorded as the time of symptom disappearance. Symptom relief was defined as a decrease of 1 point or more in the patient's symptom score compared to baseline, maintained for at least two days, with the first day of relief recorded as the time of symptom relief. Symptom disappearance and symptom relief were both for each symptom. Venous blood from patients was collected at the study baseline and at the end of the trial, with PCT and IL-6 levels measured using a fluorescence immunoassay analyzer (Getein1600, GeteinBiotech).

Safety

Potential adverse reactions such as dry mouth, nausea, palpitations, cough, chest tightness, dyspnea, difficulty breathing, and skin/mucosal damage were monitored in patients during the study, as well as vital signs before and after the intervention. Venous blood was collected from patients at the study baseline and at the end of the trial, and a complete blood count was performed using a hematology analyzer (XN-3000, SYSMEX). Biochemical markers (alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, and creatinine) were measured using a dry chemistry analyzer (FS 5.1, Johnson & Johnson MedTech).

Statistical analysis

Data were analyzed in the per-protocol analysis population. The Shapiro-Wilke test was used to check whether the continuous data were normally distributed. Continuous data adhering to a normal distribution were expressed as mean ± standard deviation and compared between groups using the independent samples t-test. Continuous data following a skewed distribution were expressed as median (interquartile range) and compared between groups using the Mann-Whitney U test. Categorical data were expressed as frequency (percentage) and compared between groups using Fisher's exact test. Ordinal data were expressed as frequency (percentage) and compared between groups using the Mann-Whitney U test. The rate of symptom disappearance and symptom relief within 10 days were displayed using Kaplan-Meier curves and compared using the Log-Rank test. The Kaplan-Meier curves were generated and the Log-Rank test was conducted using R software (version 4.3.2). All other statistical analyses were performed in SPSS software version 27. A P-value < 0.05 (two-sided) was considered statistically significant.

Sample size

Before this study was conducted, no literature reported the improvement rate of chest CT after Nirmatrelvir/Ritonavir treatment in moderate COVID-19. Therefore, we preliminarily analyzed the data of hospitalized COVID-19 patients in our hospital before the study. Among 152 moderate COVID-19 patients treated with Nirmatrelvir/Ritonavir, 16 cases showed worsening pneumonia on chest CT. Those with worsening pneumonia continued to receive standard treatment according to COVID-19 treatment guidelines. Of the 11 patients who received only standard treatment, 3 showed improvement in chest CT, an improvement rate of approximately 27%. Among the 5 patients who received both standard treatment and PAW, 4 showed improvement in chest CT, an improvement rate of approximately 80%. Conservatively estimating the improvement rate of PAW at 60%, with a two-sided alpha of 0.05, a power of 0.8, and a dropout rate of 20%, the sample size was calculated to be 80 cases, with 40 cases in each group.

Results

Baseline characteristics

From January to July 2023, a total of 335 subjects were screened. 311 individuals were excluded, of which 301 patients did not meet the inclusion criteria and 10 eligible patients refused to participate. Subjects who withdrew consent after randomization were excluded. Due to slow recruitment, patient enrollment was stopped on August 31, 2023, as recommended by the independent data and safety monitoring board. A total of 24 subjects were involved in the randomization, with 11 in the experimental group and 13 in the control group. One patient in the control group withdrew from the study due to an acute myocardial infarction before the first intervention on the day of randomization. Ultimately, 23 subjects received the intervention and completed follow-up (Fig. 1). According to data from the Chinese Center for Disease Control and Prevention, the prevalent strains of SARS-CoV-2 in China during this period were all Omicron variants, mainly BA.5.2, BF.7, XBB, and EG.5. There were no significant differences between the experimental and control groups in terms of age, sex, body mass index, smoking history, comorbidities, disease duration, and vaccination status (Table 1).

Primary outcomes

After 4 days of intervention, qualitative assessment of follow-up chest CT showed that the experimental group was superior to the control group. The improvement rate of chest CT in the experimental group was significantly higher than that in the control group (81.8% vs. 33.3%, estimate difference [95%CI], 48.48% [13.40–83.57%], p = 0.036) (Table 2). The images from the quantitative

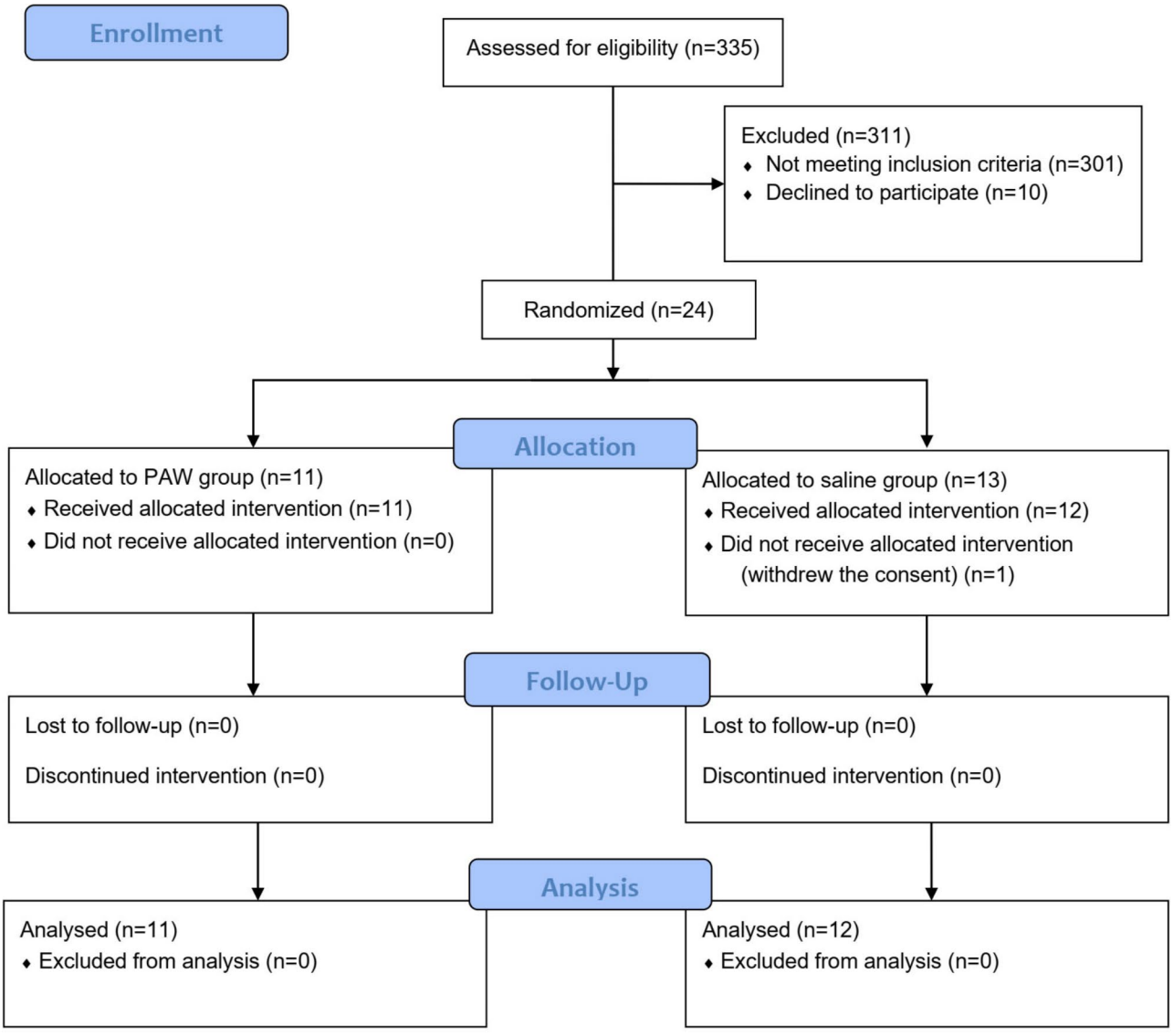

Fig. 1 Randomization, treatment assignments, and follow-up

analysis of chest CT are shown in Fig. 2. Quantitative analysis showed similar results: the experimental group's $D_{LeV\%}$ (percentage of lung volume occupied by lesions before treatment - percentage of lung volume occupied by lesions after treatment) and D_{LW} (mass of lesions before treatment - mass of lesions after treatment) were significantly higher than those of the control group ($p=0.010$, $p=0.006$). The experimental group's D_{MleD} (mean density of lesions before treatment - mean density of lesions after treatment) was also higher than that of the control group ($p=0.055$).

Secondary outcomes

During the 10-day observation period, the cumulative disappearance rate of cough in the experimental group was higher than in the control group ($p=0.029$) (Fig. 3-A). No significant differences were observed between groups in the cumulative disappearance rates and cumulative relief rates of other symptoms (Fig. 3-B, C, D, E, F).

The average hospital stay for the experimental group was approximately 5 days shorter than the control group, and the median time from the first intervention to discharge was 3 days shorter for the experimental group, although these differences were not statistically significant (Table 3). There were no significant differences in inflammatory markers (PCT, IL-6) between the two groups on day 5. Within a 28-day follow-up period, 4 cases in each group progressed to severe COVID-19, with no significant difference in the proportion of moderate-to-severe cases between groups. No patients died, and there was no difference in 28-day all-cause mortality.

Table 1 Demographic and clinical characteristics of the subjects

Characteristics	Experimental group (*n* = 11)	Control group (*n* = 12)	*P* value*
Age (years), mean ± SD	70.4 ± 10.0	64.0 ± 14.9	0.247
Body mass index (kg/m^2), mean ± SD	23.05 ± 1.38	21.59 ± 2.94	0.142
Time since first symptom (days), median (IQR)	11.0 (11.0, 14.0)	14.5 (10.5, 17.5)	0.239
Sex			0.414
Male, n (%)	6 (54.5)	4 (33.3)	
Female, n (%)	5 (45.5)	8 (66.7)	
Smoking history, n (%)	2 (18.2)	2 (16.7)	> 0.999
Comorbidities, n (%)	11 (100.0)	11 (91.7)	> 0.999
Chronic lung disease, n (%)	1 (9.1)	1 (8.3)	> 0.999
Diabetes, n (%)	3 (27.3)	4 (33.3)	> 0.999
Hypertension, n (%)	9 (81.8)	5 (41.7)	0.089
Coronary heart disease, n (%)	5 (45.5)	2 (16.7)	0.193
Chronic kidney disease, n (%)	2 (18.2)	0 (0.0)	0.217
Cancer, n (%)	1 (9.1)	3 (25.0)	0.590
Transplant recipient, n (%)	1 (9.1)	0 (0.0)	0.478
COVID-19 vaccination, n (%)	8 (72.7)	6 (50.0)	0.400
Previous SARS-CoV-2 infection, n (%)	0 (0.0)	1 (8.3)	> 0.999

SD: standard deviation; IQR: interquartile range; COVID-19: coronavirus disease 2019; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2. * Independent samples t-test, Mann-Whitney U test, Fisher's exact test

Safety

Throughout the study, no patients reported discomfort or adverse reactions. No new instances of dry mouth, nausea, palpitations, cough, chest tightness, dyspnea, difficulty breathing, or skin/mucosal damage were observed after the intervention, nor did any existing symptoms worsen. There were no significant differences in complete blood count or hepatic and renal function indicators between the groups after the intervention (Table 4).

Discussion

In moderate COVID-19 patients with high risk of severity and progression of pneumonia on radiological imaging following oral antiviral medication, we administered nebulized PAW or saline. The results showed that the PAW group had significantly higher rates of improvement in chest CT and cough disappearance compared to the saline group, with a trend toward shorter hospital stays in the PAW group. No patient died within 28 days. No adverse reactions occurred.

Chest imaging is a commonly used and objective indicator for clinicians to evaluate whether pneumonia is controlled by medication. Chest CT allows for a direct visual assessment of the severity of pneumonia lesions in COVID-19 patients [29–31]. This study found that PAW could halt or even reverse the deterioration of chest CT

Table 2 Primary outcomes: qualitative and quantitative analysis of chest CT on day 5

	Experimental group (*n* = 11)	Control group (*n* = 12)	Estimate difference (95%CI)	*P* value*
Qualitative assessment of chest CT on day 5				0.036
Improvement, n (%)	9 (81.8)	4 (33.3)	48.48 (13.40 to 83.57)	
Non-improvement, n (%)	2 (18.2)	8 (66.7)		
Quantitative analysis of chest CT				
LeV (ml), median (IQR)				
Baseline	334.35 (262.42, 429.20)	306.06 (168.18, 433.92)	59.06 (-116.60 to 260.30)	0.538
Day 5	191.87 (64.98, 256.80)	250.10 (175.58, 400.41)	-80.37 (-208.18 to 51.69)	0.218
LV (ml), mean ± SD				
Baseline	3445.44 ± 835.71	3648.11 ± 1099.88	-202.66 (-1055.96 to 650.64)	0.626
Day 5	3823.25 ± 810.26	3684.54 ± 907.04	138.71 (-609.85 to 887.27)	0.704
LeV%, median (IQR)				
Baseline	11.12 (7.37, 18.16)	8.81 (4.40, 11.95)	1.89 (-3.15 to 8.36)	0.460
Day 5	4.78 (1.94, 6.45)	7.53 (4.55, 9.75)	-2.63 (-6.41 to 0.82)	0.124
MLeD (HU), mean ± SD				
Baseline	-443.98 ± 100.56	-454.66 ± 91.63	10.68 (-72.64 to 94.01)	0.792
Day 5	-496.62 ± 60.62	-453.06 ± 76.51	-43.56 (-103.80 to 16.69)	0.148
LW, median (IQR)				
Baseline	247.28 (161.01, 284.67)	201.85 (100.74, 263.00)	46.57 (-62.70 to 184.04)	0.295
Day 5	103.43 (34.12, 176.61)	172.87 (93.28, 241.54)	-57.90 (-144.75 to 24.66)	0.268
$D_{LeV\%}$, mean ± SD	6.73 ± 5.18	1.74 ± 3.05	4.98 (1.33 to 8.63)	0.010
D_{MLeD} (HU), mean ± SD	52.64 ± 67.04	-1.60 ± 61.06	54.24 (-1.30 to 109.78)	0.055
D_{LW}, mean ± SD	145.50 ± 123.74	25.65 ± 56.09	119.85 (37.78 to 201.92)	0.006

CI: Confidence interval; CT: computed tomography; IQR: interquartile range; SD: standard deviation; LeV: lesion volume; LV: lung volume; LeV%: the ratio of lesion volume to lung volume; MLeD: mean lesion density; LW: lesion weight; $D_{LeV\%}$: LeV% at baseline minus LeV% on day 5; D_{MLeD}: MLeD at baseline minus MLeD on day 5; D_{LW}: LW at baseline minus LW on day 5. * Fisher's exact test, Mann-Whitney U test, Independent samples t-test

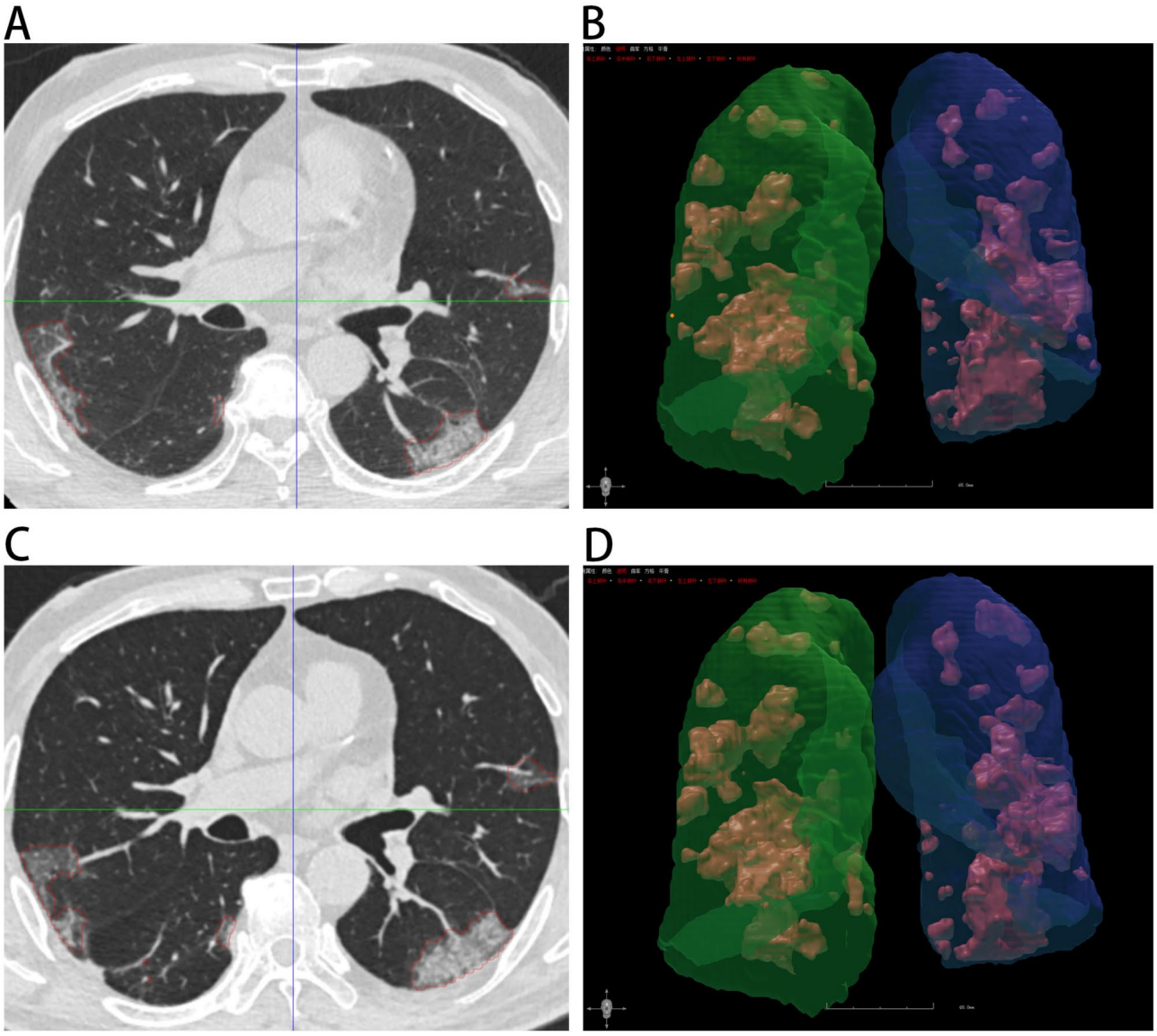

Fig. 2 Illustration of lesion segmentation and dynamic changes. As shown in **A** and **C**, all lesions were segmented and marked by red lines. **B** and **D** represent the three-dimensional reconstruction of lesions. **A** and **B** are pre-intervention images, while **C** and **D** are post-intervention images

in moderate COVID-19 patients post-antiviral treatment. Possible reasons for PAW facilitating radiological improvement in COVID-19 patients include: on one hand, PAW can disrupt the structure of SARS-CoV-2 and inhibit its replication. PAW contains a large number of reactive oxygen species and reactive nitrogen species, such as O_2•−, O_3, •OH, ONOOH, H_2O_2, NO•, etc [10, 11, 32]. Guo et al. found that the short-lived reactive species such as ONOO− in PAW play a significant role in damaging the RBD of the SARS-CoV-2 spike protein [15]. Wang et al. confirmed that •OH play a dominant role in triggering ACE2 nucleus translocation [33]. Khanikar et al. demonstrated that H_2O_2 can inactivate the SARS-CoV-2 spike protein and reduce its gene abundance [34]. Guo et al. have verified that gargling with PAW can accelerate the viral RNA clearance in COVID-19 patients with prolonged positive oropharyngeal swabs [20]. On the other hand, we postulate that PAW has anti-inflammatory and immunosuppressive effects on pulmonary inflammatory lesions. Pulmonary pathology in COVID-19 patients reveals substantial immune cell accumulation [35, 36]. Immune cells, cytokines, and chemokines are significantly elevated in bronchoalveolar lavage fluid [37]. Numerous animal experiments and human studies have confirmed that plasma has local immunosuppressive and anti-inflammatory effects [38–41]. Therefore, we speculate that PAW might reduce the infiltration of immune cells and the levels of inflammatory cytokines in the lungs of COVID-19 patients, thus avoiding an excessive immune response and promoting the improvement of

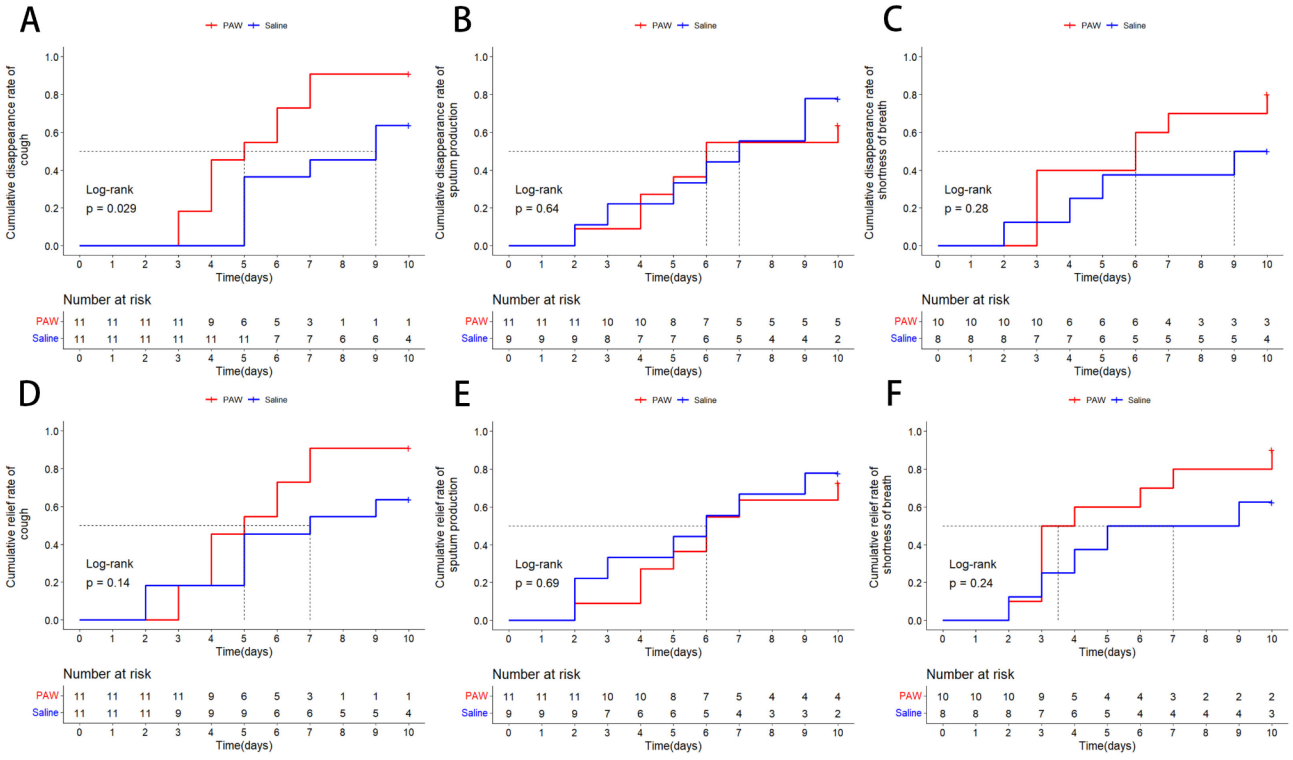

Fig. 3 The cumulative disappearance rate and cumulative relief rate of symptoms within 10 days. **A**: The cumulative disappearance rate of cough within 10 days. **B**: The cumulative disappearance rate of sputum production within 10 days. **C**: The cumulative disappearance rate of shortness of breath within 10 days. **D**: The cumulative relief rate of cough within 10 days. **E**: The cumulative relief rate of sputum production within 10 days. **F**: The cumulative relief rate of shortness of breath within 10 days

Table 3 Secondary outcomes

	Experimental group (*n* = 11)	Control group (*n* = 12)	Estimate difference (95%CI)	*P* value*
Length of hospital stay (days), mean ± SD	13 ± 3	18 ± 10	-4 (-11 to 2)	0.163
Days from the first intervention to discharge, median (IQR)	7 (5, 13)	10 (6, 21)	-2 (-9 to 1)	0.239
Aggravated to severe COVID-19 within 28 days, n (%)	4 (36.4)	4 (33.3)	3.03 (-35.95 to 42.01)	> 0.999
Death within 28 days, n (%)	0 (0.0)	0 (0.0)	-	> 0.999
Procalcitonin (ng/ml), median (IQR)				
Baseline	0.062 (0.052, 0.277)	0.071 (0.043, 0.196)	0.011 (-0.110 to 0.141)	0.460
Day 5	0.051 (0.046, 0.065)	0.056 (0.043, 0.093)	0.001 (-0.032 to 0.026)	0.926
Interleukin-6 (pg/ml), median (IQR)				
Baseline	27.05 (3.60, 43.71)	7.30 (1.59, 42.38)	2.10 (-12.04 to 29.42)	0.478
Day 5	3.48 (1.50, 9.27)	7.09 (1.64, 26.95)	-1.19 (-10.94 to 2.54)	0.402

CI: Confidence interval; SD: standard deviation; IQR: interquartile range; COVID-19: coronavirus disease 2019. * Independent samples t-test, Mann-Whitney U test, Fisher's exact test

pneumonia. There was no significant difference between the two groups in terms of improvement in serum IL-6. This may be because all patients included in this study were moderate COVID-19 cases, with overall low and comparable baseline serum IL-6 levels. Additionally, the samples used for IL-6 measurement were venous blood, not from the lungs or bronchoalveolar lavage fluid. Cytokine levels in the blood may not match those in the lungs [42]. Whether PAW can reduce pulmonary IL-6 and other cytokine levels remains to be explored.

Common clinical manifestations in COVID-19 patients include fever, fatigue, myalgia, cough, and sputum production [43, 44]. Our study results indicate that nebulized inhalation of PAW can accelerate the resolution of cough in COVID-19 patients. According to the literature, the potential mechanism of COVID-19-related cough may involve accumulation of inflammatory cells and release of inflammatory mediators in the respiratory tract, epithelial cell damage, and increased secretions following infection with SARS-CoV-2. These stimuli can

Table 4 Safety of plasma-activated water

	Experimental group (*n* = 11)	Control group (*n* = 12)	Estimate difference (95%CI)	*P* value*
Adverse Events, n (%)	0 (0.0)	0 (0.0)	-	> 0.999
Biosafety Parameters				
White blood cell ($\times 10^9$/L), mean ± SD				
Baseline	7.48 ± 3.08	8.42 ± 4.38	-0.95 (-4.26 to 2.36)	0.558
Day 5	7.61 ± 3.53	7.74 ± 3.84	-0.12 (-3.33 to 3.09)	0.937
Neutrophils ($\times 10^9$/L), median (IQR)				
Baseline	6.03 (3.82, 8.27)	5.60 (3.54, 10.11)	-0.28 (-3.49 to 2.66)	0.902
Day 5	5.30 (3.22, 9.70)	4.38 (3.60, 8.37)	-0.06 (-2.70 to 2.88)	0.926
Lymphocyte ($\times 10^9$/L), mean ± SD				
Baseline	0.79 ± 0.48	0.78 ± 0.28	0.02 (-0.32 to 0.35)	0.927
Day 5	1.22 ± 0.67	1.15 ± 0.58	0.07 (-0.47 to 0.61)	0.797
Red blood cell ($\times 10^{12}$/L), mean ± SD				
Baseline	3.85 ± 0.61	4.02 ± 0.53	-0.17 (-0.67 to 0.32)	0.469
Day 5	3.72 ± 0.67	3.96 ± 0.59	-0.24 (-0.79 to 0.30)	0.366
Hemoglobin (g/L), median (IQR)				
Baseline	121 (115, 134)	119 (108, 138)	1 (-15 to 16)	0.926
Day 5	111 (101, 130)	115 (105, 138)	-4 (-21 to 13)	0.518
Platelet ($\times 10^9$/L), mean ± SD				
Baseline	194 ± 111	240 ± 114	-45 (-143 to 53)	0.347
Day 5	232 ± 124	238 ± 66	-6 (-96 to 83)	0.882
Alanine transaminase (IU/L), median (IQR)				
Baseline	32 (20, 48)	22 (14, 47)	3 (-8 to 19)	0.579
Day 5	44 (22, 55)	33 (21, 75)	4 (-25 to 24)	0.821
Aspartate transaminase (IU/L), median (IQR)				
Baseline	28 (23, 38)	26(15, 32)	4 (-4 to 14)	0.339
Day 5	29 (21, 34)	23(16, 36)	4 (-9 to 14)	0.384
Blood urea nitrogen (mmol/L), median (IQR)				
Baseline	6.89 (4.79, 8.94)	4.95 (3.73, 6.99)	1.77 (-0.21 to 4.62)	0.074
Day 5	7.06 (5.40, 11.80)	5.34 (4.38, 8.70)	1.77 (-0.69 to 4.14)	0.205
Creatinine (μmol/L), median (IQR)				
Baseline	62 (53, 78)	60 (44, 67)	5 (-8 to 23)	0.388
Day 5	59 (50, 82)	51 (43, 65)	13 (-4 to 33)	0.098

CI: Confidence interval; SD: standard deviation; IQR: interquartile range. * Fisher's exact test, Independent samples t-test, Mann-Whitney U test

induce a cough hypersensitivity state through neuroinflammatory or neuroimmune processes [45–47]. This is a complex process that may involve factors such as viral invasion, inflammation, immunity, neuroreflex, and hypersensitivity. The improvement in cough observed in COVID-19 patients treated with PAW may result from the combined effects of multiple pathways. First, PAW can block the binding of SARS-CoV-2 Spike protein RBD to the respiratory tract ACE2, preventing viral invasion. Second, PAW can suppress the infiltration of immune cells and reduce the levels of inflammatory mediators in the respiratory tract. Finally, PAW may have anti-allergic effects. In vitro studies have found that liquid-type plasma inhibits mast cell activation [48]. Animal experiments suggest that plasma can suppress the recruitment of mast cells and eosinophils in the skin lesions of mice with atopic dermatitis and reduce IgE levels in the lesions [48, 49]. Interestingly, an improvement in cough was also observed in another randomized controlled study where we used nebulized inhalation of PAW to treat acute upper respiratory infections caused by the influenza A virus.

There was no difference in the rate of progression to severe disease between the two groups in this study, which may be related to the small number of cases. This study suggests a trend toward reduced hospital stay duration with PAW treatment, which could help save medical costs and alleviate medical congestion during epidemic outbreaks.

During the study, no local or systemic adverse reactions occurred in patients in the PAW group. There were no significant differences in routine blood tests, liver function, or kidney function laboratory indicators between the groups. A large number of animal studies have confirmed the safety of PAW [16, 17]. Studies by Hwang et al. and Jang et al. confirmed that PAW spray treatment for bacterial vaginosis did not cause adverse reactions [18,

19]. PAW gargle treatment for COVID-19 patients did not cause adverse reactions [20].

This study is a preliminary investigation into the efficacy and safety of nebulized inhalation of PAW for the treatment of COVID-19 and has some limitations. First, the sample size of this study was small. The main reason is the significant reduction in the incidence of COVID-19 in China, especially the scarcity of moderate COVID-19 patients with oral antiviral treatment failure at the hospital where the researchers are located. Second, this trial was a pilot study and used a single-blind design. Finally, studies of this nature often use mortality as a primary endpoint. However, due to the small sample size and the low mortality rate among moderate COVID-19 patients, it was not appropriate to use mortality as the primary endpoint.

Currently, there is a lack of effective treatment methods for COVID-19 pneumonia that fails to respond to antiviral medication. We innovatively used nebulized inhalation of PAW to treat such COVID-19 patients and preliminarily confirmed its efficacy and safety. Further multicenter, large-sample studies are needed for validation. We are researching the use of nebulized inhalation of PAW for the initial treatment of COVID-19 and the treatment of acute upper respiratory infections caused by the influenza A virus. Additionally, we will explore the therapeutic effects of PAW on other pathogen infections.

Conclusion

This pilot trial preliminarily confirmed that for moderate COVID-19 patients with worsening pneumonia radiologically after antiviral treatment and high-risk factors for severe illness, nebulized inhalation of PAW can alleviate pneumonia, with no adverse reactions observed. The clinical efficacy of nebulized inhalation of PAW in the treatment of COVID-19 still needs to be confirmed by large-scale, multicenter clinical studies.

Abbreviations

ACE2	Angiotensin-converting enzyme 2
COVID-19	Coronavirus disease 2019
CT	Computed tomography
$D_{LeV\%}$	LeV% at baseline minus LeV% on day 5
D_{LW}	LW at baseline minus LW on day 5
D_{MLeD}	MLeD at baseline minus MLeD on day 5
IL-6	Interleukin 6
LeV%	The ratio of lesion volume to lung volume
LeV	Lesion volume
LV	Lung volume
LW	Lesion weight
MLeD	Mean lesion density
PAW	Plasma-activated water
PCT	Procalcitonin
RBD	Receptor-binding domain
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-024-09886-w.

Supplementary Material 1: COVID-19 classification criteria and high-risk factors for severe disease. Table S1 Detailed qualitative assessment of chest CT on day 5

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Author contributions

Conceptualization, L.S. and D.L.; Methodology, L.S., X.L. and H.Z.; Software, C.S. and H.Z.; Validation, L.S., H.Z. and W.M.; Formal Analysis, L.S. and H.Z.; Investigation, X.L. and L.S.; Resources, D.L., Z.W., L.G., M.R., H.Z., W.M., J.L. and C.S.; Data Curation, H.Z and W.M.; Writing – Original Draft Preparation, H.Z. and W.M.; Writing – Review & Editing, L.S., D.L. and X.L.; Visualization, H.Z.; Supervision, J.L., X.G. and Z.C.; Project Administration, L.S. and X.L.; Funding Acquisition, L.S. All authors have read and agreed to the published version of the manuscript.

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Data availability

Data supporting the findings of this study are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Medical Ethics Committee of the First Affiliated Hospital of the Air Force Medical University (Approval No.: KY20232004-C-1, January 5, 2023), registered with the Chinese Clinical Trial Registry (Registration No.: ChiCTR2300078706). Informed consent was obtained from all subjects involved in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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