

Efficacy of low-intensity pulsed ultrasound in the treatment of COVID-19 pneumonia

Wirksamkeit von niedrigenergetischem gepulstem Ultraschall bei der Behandlung von COVID-19-Pneumonien



Authors

Wen Li^{1†}, Xiao Li^{2†}, Zhibin Kong^{3†}, Bin Chen^{4†}, Hongsheng Zhou⁵, Yimin Jiang¹, Weimei Li¹, Lichang Zhong¹, Xinyu Zhang¹, Kaihua Zhang⁶, Lili Zhang⁴, Xiangyun Zong⁷, Wenkun Bai⁸, Yuanyi Zheng¹

Affiliations

- 1 Department of Ultrasound, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China
- 2 Department of Ultrasound, Institute of Ultrasound in Medicine and Engineering, Zhongshan Hospital, Fudan University, Shanghai, China
- 3 Respiratory Medicine, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China
- 4 Cardiovascular Medicine, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China
- 5 Shanghai Acoustics Jiao Tong University, Shanghai, China
- 6 Department of Radiology, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China
- 7 Breast Surgery, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China
- 8 Department of Ultrasound, Tong Ji Hospital Affiliated to Tong Ji University, Shanghai, China

Key words

COVID-19, SARS-CoV-2, Ultrasonic therapy, Low-intensity pulsed ultrasound, viral pneumonia

received 28.09.2022

accepted after revision 13.07.2023

accepted manuscript online 19.07.2023

published online 2023

Bibliography

Ultraschall in Med 2023; 44: e274–e283

DOI 10.1055/a-2133-0835

ISSN 0172-4614

© 2023. The Author(s).

This is an open access article published by Thieme under the terms of the Creative Commons Attribution-NonDerivative-NonCommercial-License, permitting copying and reproduction so long as the original work is given appropriate credit. Contents may not be used for commercial purposes, or adapted, remixed, transformed or built upon. (<https://creativecommons.org/licenses/by-nc-nd/4.0/>).

Georg Thieme Verlag KG, Rüdigerstraße 14,
70469 Stuttgart, Germany

Correspondence

Prof. Yuanyi Zheng

Department of Ultrasound, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Yishan road 600, 200030 Shanghai, China
zhengyuanyi@sjtu.edu.cn

Dr. Xiangyun Zong

Breast Surgery, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Yishan road 600, 200030 Shanghai, China
tigerzong@msn.com

Dr. Wenkun Bai

Department of Ultrasound, Tong Ji Hospital Affiliated to Tong Ji University, Xincun Road 389, 200065 Shanghai, China
doctor505@alumni.sjtu.edu.cn

Additional material is available at <https://doi.org/10.1055/a-2133-0835>.

ABSTRACT

Purpose As a public health emergency of international concern, coronavirus disease 2019 (COVID-19) still lacks specific antiviral drugs, and symptomatic treatment is currently the mainstay. The overactivated inflammatory response in COVID-19 patients is associated with a high risk of critical illness or even death. Low-intensity pulsed ultrasound (LIPUS) can mitigate inflammation and inhibit edema formation. We

† These authors contributed equally.

aimed to investigate the efficacy of LIPUS therapy for COVID-19 pneumonia.

Materials and Methods 62 patients were randomly assigned to a treatment group (LIPUS treatment area – Group 1; self-control area – Group 2) and an external control group (Group 3). The primary outcomes were the volume absorption rate (VAR) and the area absorption rate (AAR) of lung inflammation in CT images.

Results After an average duration of treatment 7.2 days, there were significant differences in AAR and VAR between Group 1 and Group 2 (AAR 0.25 vs 0.12, $p = 0.013$; VAR 0.35 vs 0.11, $p = 0.005$), and between Group 1 and Group 3 (AAR 0.25 vs 0.11, $p = 0.047$; VAR 0.35 vs 0.19, $p = 0.042$). Neither AAR nor VAR was statistically different between Group 2 and Group 3. After treatment, C-reactive protein, interleukin-6, leukocyte, and fingertip arterial oxygen saturation (SaO₂) improved in Group 1, while in Group 3 only fingertip SaO₂ increased.

Conclusion LIPUS therapy reduced lung inflammation and serum inflammatory factor levels in hospitalized COVID-19 patients, which might be a major advancement in COVID-19 pneumonia therapy.

ZUSAMMENFASSUNG

Ziel Für die Coronavirus-Erkrankung (COVID-19), die einen internationalen Gesundheitsnotstand darstellt, gibt es immer noch keine spezifischen antiviralen Medikamente, und die Hauptstütze ist derzeit die symptomatische Behandlung. Die überaktivierte Entzündungsreaktion bei COVID-19-Patienten

ist mit einem hohen Risiko für einen schweren Erkrankungsverlauf und Mortalität verbunden. Niedrigenergetischer gepulster Ultraschall (LIPUS) kann die Entzündung lindern und die Ödembildung hemmen. Unser Ziel war es, die Wirksamkeit der LIPUS-Therapie bei COVID-19-Pneumonie zu untersuchen.

Material und Methoden 62 Patienten wurden nach dem Zufallsprinzip einer Behandlungsgruppe (LIPUS-Bereich – Gruppe 1; Selbstkontroll-Bereich – Gruppe 2) und einer externen Kontrollgruppe (Gruppe 3) zugewiesen. Das primäre Outcome waren die Volumenabsorptionsrate (VAR) und die Flächenabsorptionsrate (AAR) der Pneumonie in CT-Aufnahmen.

Ergebnisse Nach einer durchschnittlichen Behandlungsdauer von 7,2 Tagen gab es signifikante Unterschiede bei AAR und VAR zwischen Gruppe 1 und Gruppe 2 (AAR 0,25 vs. 0,12, $p = 0,013$; VAR 0,35 vs. 0,11, $p = 0,005$) sowie zwischen Gruppe 1 und Gruppe 3 (AAR 0,25 vs. 0,11, $p = 0,047$; VAR 0,35 vs. 0,19, $p = 0,042$). Weder AAR noch VAR unterschieden sich statistisch zwischen Gruppe 2 und Gruppe 3. Nach der Behandlung verbesserten sich in Gruppe 1 die Werte für C-reaktives Protein, Interleukin-6, Leukozyten und die arterielle Sauerstoffsättigung (SaO₂) an der Fingerspitze, während in Gruppe 3 nur die SaO₂ an der Fingerspitze anstieg.

Schlussfolgerung Die LIPUS-Therapie verringerte die Pneumonie und die Konzentration von Entzündungsfaktoren im Serum bei hospitalisierten COVID-19-Patienten, was einen wichtigen Fortschritt in der Therapie der COVID-19-Pneumonie darstellen könnte.

Introduction

Coronavirus disease 2019 (COVID-19) is an acute respiratory infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-COV-2). SARS-COV-2 and its variants are highly infectious, highly transmissible, and have gradually enhanced immune escape ability [1]. Although the current clinical severity and case fatality rate are lower, the absolute number is likely to be much higher due to the sheer incidence of infection and the sizable number of patients [2].

The main clinical manifestations of COVID-19 patients include headache, cough, fever, generalized myalgia and fatigue [3]. The pathological mechanism is mainly based on alveolar exudative inflammation and pulmonary interstitial inflammation. The virus invades alveolar epithelial cells, mediates cellular damage, induces inflammatory response [4], and promotes the release of various pro-inflammatory cytokines, such as interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)- α , inducing fever and cough symptoms [5]. At the same time, the massive release of inflammatory mediators leads to increased vascular permeability, and some patients experience increased sputum, and in severe cases, pulmonary edema, dyspnea, or hypoxemia [6, 7]. In addition, virus-induced immune complexes induce local microthrombosis, leading to stasis of pulmonary circulation, and further affecting ventila-

tion functions [8]. Patients with advanced age or underlying diseases may experience more severe symptoms and are prone to secondary infection and severe ventilation dysfunction [9].

Different from bacterial pneumonia, specific antiviral drugs are still lacking, and symptomatic treatment is the main method. Reducing pulmonary inflammation and improving clinical symptoms is the focus of current treatment.

Low-intensity pulsed ultrasound (LIPUS), a therapeutic ultrasound technique that has emerged in recent years, has been approved by the US Food and Drug Administration (FDA) for the treatment of soft tissue and musculoskeletal injuries [10]. It is delivered with low intensity ($< 3 \text{ W/cm}^2$) in the mode of a medium frequency (0.7–3.0 MHz) pulsed wave, which can not only exert physical stimulation but also carry out biological therapy [11]. In many preclinical studies, LIPUS has been shown to help reduce local inflammation and promote tissue repair, and its anti-inflammatory effects involve multiple mechanisms, including inhibiting the expression of inflammatory factors, up-regulating the expression of anti-inflammatory genes and the regulatory factors of immunosuppressive cells, and reducing inflammatory cell infiltration, etc. [12].

The mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF- κ B) signaling pathways are classic inflammatory response pathways. It has been observed in many animal models

such as acute viral myocarditis [13], synovitis [14], and nerve cell mechanical injury [15] that the above-mentioned inflammatory pathways were significantly inhibited after LIPUS sonication, and inflammatory cytokines such as TNF- α , IL-1 β and IL-6 decreased accordingly. The potential of LIPUS to modulate the inflammatory cell phenotype and reduce the number of neutrophils and M1 inflammatory macrophages was also observed in musculoskeletal injury models [16, 17]. Similarly, in human clinical trials, repair of damaged bone tissue and absorption of local edema [18], healing of diabetic ulcers, and reduction of the inflammatory response [19] were also observed after LIPUS treatment. In addition, in the case of acute myocardial infarction, ultrasound can remove microthrombosis, improve myocardial blood perfusion, and thereby reduce the size of the infarct area [20]. It is suggested that LIPUS therapy may also play a certain role in reducing the microthrombotic state and improving ventilatory function in patients with COVID-19 pneumonia. Although the treatment principle has not been fully clarified, the comprehensive and synergistic effects of LIPUS in alleviating inflammation, promoting tissue repair, inhibiting edema formation, and stimulating angiogenesis are of great significance in the field of clinical treatment.

At present, the positive effect of LIPUS in animal experiments and clinical treatment has been confirmed, but the application in the treatment of pneumonia has not been reported, so we aimed to study the therapeutic effect of wearable LIPUS on COVID-19 pneumonia.

Methods

Study design and objectives

This study was an individually randomized and controlled trial to evaluate the efficacy of LIPUS therapy in patients hospitalized with COVID-19 pneumonia and was approved by the ethics committee in our hospital.

Participants

Adult patients from ages 20 to 85 with COVID-19 pneumonia confirmed by pharyngeal swab polymerase chain reaction (PCR) and lung CT were included in the study. The inclusion criteria were as follows: (1) diagnosis of COVID-19 based on a positive PCR test; (2) aged 20 to 85 years; (3) pneumonia confirmed by chest CT; (4) willingness to cooperate with all examinations during the clinical study and signed informed consent; (5) no participation in other clinical trials during the study period.

The exclusion criteria were as follows: (1) bronchiectasis, COPD, or lung malignancies; (2) local skin injury on the chest cannot wear the LIPUS device; (3) pregnancy or lactation; (4) contraindications to therapeutic US, including dermatological conditions, local abnormal sensations in the chest or back, epilepsy, etc.; (5) mental illness or inability to communicate properly.

Randomization

62 patients were randomly assigned to a treatment group and an external control group (Group 3) with a computer random number generator (1:1). The treatment group was then divided into

two areas according to the location of inflammation on lung CT images, and one area was randomly selected as the LIPUS sonication area (Group 1), and the other area was used as the self-control area (Group 2). Inflammatory lesions in Group 3 close to the diseased lung lobes in Group 1 were used as external control areas.

Two members of the research team were responsible for recruiting and registering subgroup information. The patients and clinicians who performed LIPUS therapy were not blinded to the grouping results and the course of treatment. Researchers performed the image processing and statistics were masked to the grouping result.

Procedures

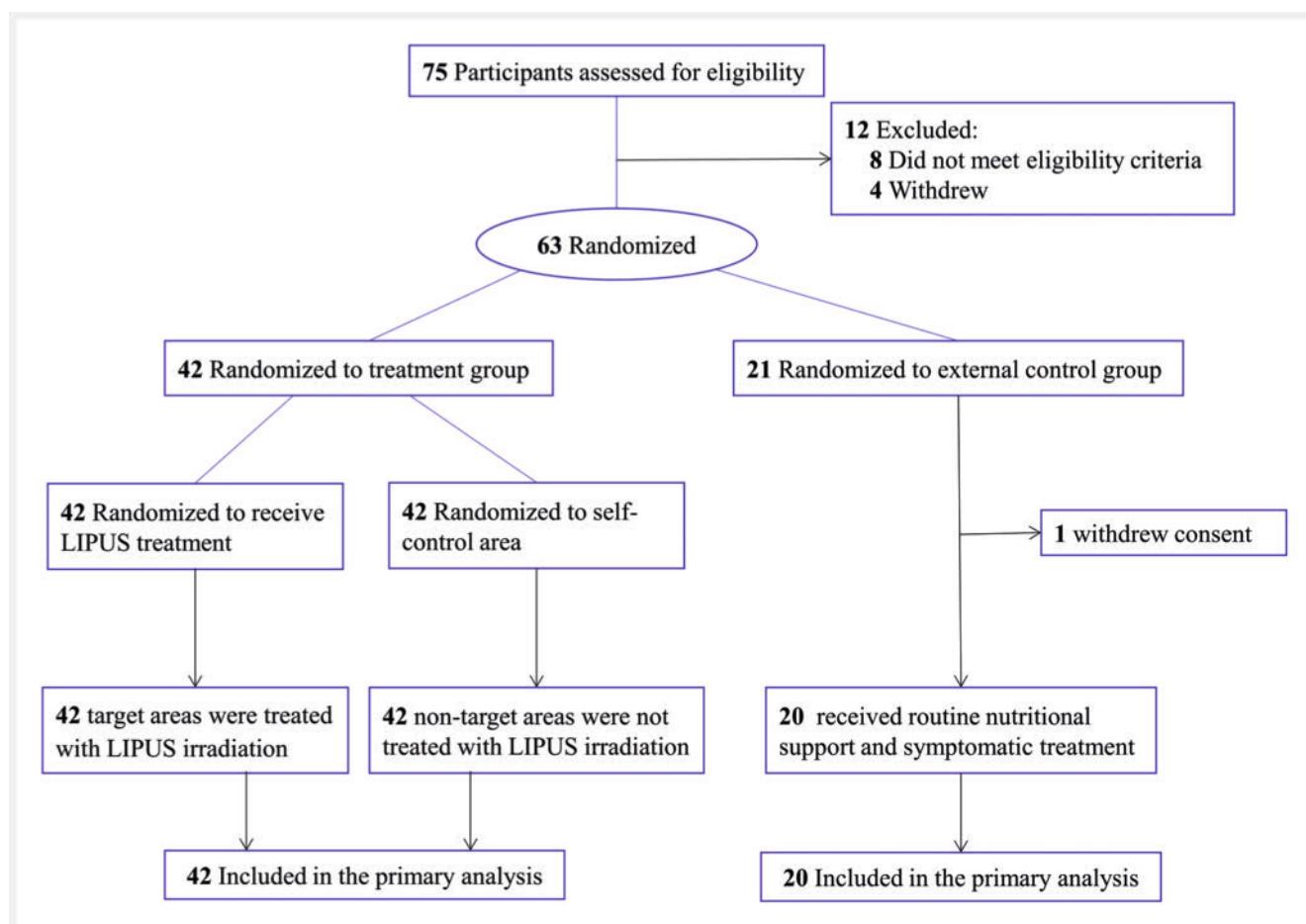
LIPUS sonication

The wearable LIPUS therapeutic apparatus (UT – HP 05 21, Shanghai, China) consists of two movable ultrasound probes and a central device (**Supplementary Figure 1**). The operator attached the ultrasound probes to the patient's chest or back and adjusted the position of the probes according to the shape and scope of the lung lesion to perform vertical or parallel sonication therapy (**Supplementary Figure 2**). The operating parameters of the ultrasound probes were chosen to be a pulse frequency of 572 kHz, a pulse repetition frequency of 50 Hz, an intensity of 820 mW/cm², and a duty cycle of 50 % (1 s inter-stimulus interval), and the probe alternately transmitted sound waves (**► Video 1**). During hospitalization, patients received 30-minute treatments at 8:00 am and 2:00 pm daily.

Treatment group

Patients allocated to this group were to receive localized LIPUS sonication. If the lesions were located in bilateral lungs, LIPUS treatment was randomly performed on one side, namely Group 1, and the other side was considered as self-control (Group 2); if the lesions were confined to one side, one area was randomly selected for LIPUS sonication (Group 1), and the other area was defined as self-control (Group 2). First, CT examination was performed to locate the body surface of the inflammatory exudative area. Then





► Fig. 1 Flowchart of the study population.

operators attached the wearable LIPUS therapeutic apparatus to the chest or back of the patient and conducted ultrasonic therapy.

External control group

Patients allocated to this group did not receive LIPUS treatment. Other symptomatic and supportive treatment methods were the same as in the LIPUS group. All patients underwent chest CT (Lia-nying-uCT 510 scanner) examinations before and after treatment. The standard lung window (window position 600HU, window width 1255HU) was adopted and Pair software was used to mark and 3D reconstruct the target area to record the changes in pulmonary inflammation. Two fellowship-trained radiologists independently interpreted the lung images. In the case of any non-concurrence, interpretation was finalized by consensus.

All participants underwent a standardized interview to obtain demographic and clinical information, including age, sex, disease history, symptoms (e.g., cough, sputum, muscle pain, etc.), and laboratory results. Temperature, blood pressure, blood sugar, and other indicators were obtained from the patient's medical records. Hypertension was defined as a history of hypertension, systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or the use of antihypertensive medications. Diabetes was diagnosed if the subject had a history and was currently using

insulin or oral hypoglycemic agents or had a fasting blood glucose level ≥ 126 mg/dL.

Outcomes

The primary outcomes were the volume absorption rate (VAR) and the area absorption rate (AAR) of lung inflammation on CT images. VAR is defined as $[(\text{target site inflammation volume before treatment} - \text{target site inflammation volume after treatment}) / \text{target site inflammation volume before treatment}] \times 100$ (%). AAR is defined as $[(\text{target site inflammation area before treatment} - \text{target site inflammation area after treatment}) / \text{target site inflammation area before treatment}] \times 100$ (%).

Secondary outcomes included serum leukocyte and inflammatory factor levels, arterial partial pressure of oxygen (PaO_2), arterial partial pressure of carbon dioxide (PaCO_2), fingertip arterial oxygen saturation (SaO_2), and symptom improvement before and after treatment.

During treatment, researchers also recorded whether the patients had adverse effects, including local swelling, spotting bleeding, enhanced local pain response, hemoptysis, aggravation of symptoms, etc., until the end of the study.

► **Table 1** Baseline demographics and clinical characteristics of all patients with COVID-19.

Variable	Total	LIPUS treatment area (n = 42)	External control group (n = 20)	p-value
Age, median (IQR), y	68.5 (60.8–75.3)	67.0 (59.8–76.0)	70.0 (61.8–75.0)	0.729
Sex, n (%)				
Male	38 (61.2 %)	26 (61.9 %)	12 (60.0 %)	0.886
Female	24 (38.7 %)	16 (38.1 %)	8 (40.0 %)	
Target site of inflammation, n (%)				
The right lung	40 (64.5 %)	28 (66.7 %)	12 (60.0 %)	0.608
The left lung	22 (35.5 %)	14 (33.3 %)	8 (30.0 %)	
Area of lung inflammation, median (IQR), mm ²	632.9 (305.7–1643.1)	543.4 (302.6–1643.1)	927.0 (308.6–1643.1)	0.436
Volume of lung inflammation, median (IQR), mm ³	36.2 (13.5–58.2)	37.6 (17.1–58.6)	29.3 (8.6–49.2)	0.233
Smoking, n (%)	14 (22.5 %)	12 (28.5 %)	2 (10.0 %)	0.102
Vaccination	23 (37.0 %)	19 (45.2 %)	4 (20.0 %)	0.054
Previous diseases, n (%)				
Hypertension	39 (62.9 %)	27 (64.2 %)	12 (60.0 %)	0.744
Diabetes	25 (40.3 %)	17 (40.4 %)	8 (40.0 %)	0.971
Chronic kidney disease	17 (27.4 %)	10 (23.8 %)	7 (35.0 %)	0.753
Coronary heart disease	14 (22.6 %)	9 (21.4 %)	5 (25.0 %)	1.000
Symptomatic treatment, n (%)				
Cough suppressants	23 (37.1 %)	15 (35.7 %)	8 (40.0 %)	0.744
Expectorants	18 (29.0 %)	11 (26.2 %)	7 (35.0 %)	0.475
Anti-inflammatory drugs	16 (25.8 %)	10 (23.8 %)	6 (30.0 %)	0.603
Immunomodulatory drugs	12 (19.4 %)	8 (19.0 %)	4 (20.0 %)	0.929

Data not normally distributed were expressed as the median (interquartile range).
Categorical variables were expressed as percentages.

Statistical analysis

Normally distributed data were expressed as the mean (standard deviation), whereas data not normally distributed were expressed as the median (interquartile range). The independent sample test was used for the comparison between groups, and the paired sample test was used for the comparison within the group before and after treatment. Categorical variables were expressed as percentages. A χ^2 test or Fisher's exact test was used to compare categorical data. A p-value <0.05 was considered statistically significant. Statistical analysis was carried out using SPSS software, version 26.

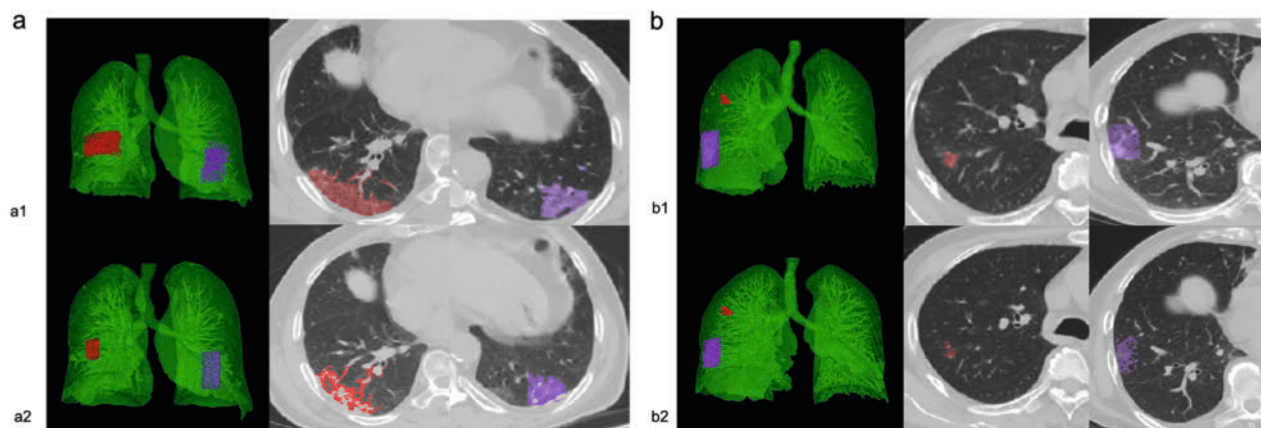
Results

62 (82.7 %) of 75 patients enrolled in the LIPUS therapy trial were eligible for random assignment (► **Fig. 1**). 42 patients were randomly allocated to the treatment group and 20 were randomly allocated to the external control group. In the treatment group, the inflammation site of each patient was randomly divided into the

LIPUS treatment area and the internal control area. All patients were Asian, the median age was 68.5 years (IQR, 60.8–75.3); sex distribution was 26 (61.9 %) men versus 16 (38.1 %) women in the treatment group and 12 (60.0 %) versus 8 (40.0 %) in the external control group. At baseline, there was no statistically significant difference between the two groups (► **Table 1**).

After an average of 7.2 days of treatment, the area and volume of lung inflammation in the three groups were reduced ($p < 0.05$) (► **Fig. 2**), but the AAR and VAR in Group 1 were higher than those in Group 2 (AAR 0.25 vs. 0.12, $p = 0.013$; VAR 0.35 vs. 0.11, $p = 0.005$) and in Group 3 (AAR 0.25 vs. 0.11, $p = 0.047$; VAR 0.35 vs. 0.19, $p = 0.042$) (► **Table 2**, ► **Fig. 3**), and so was the volume absorption difference (► **Table 2**, ► **Fig. 4**). However, there was no statistically difference between Group 2 and Group 3 (AAR, $p = 0.957$; VRR, $p = 0.392$).

Before and after treatment, some laboratory results, clinical symptoms, and signs of patients in Group 1 and Group 3 were improved, but the effectiveness in Group 1 was more obvious (► **Table 3**). After LIPUS treatment, CRP, IL-6, leukocyte and neutrocyte were decreased compared with before treatment (CRP 19.1 vs.



► **Fig. 2** The target lung inflammation area and volume of the CT image were marked and 3D reconstructed using pair software. (Red represents the area with LIPUS treatment, and purple represents the area without LIPUS treatment). **a** The LIPUS treatment area and the self-control area in the treatment group were located in different lungs. (a1 represents before treatment, a2 represents after treatment). The inflammation area with LIPUS treatment (red) was reduced 42.7 % (543.01/311.14mm²). The inflammation volume with LIPUS treatment (red) was reduced 66.2 % (32.17/10.87 mm³). The inflammation area without LIPUS treatment (purple) was reduced 13.9 % (411.68/354.45mm²). The inflammation volume without LIPUS treatment (purple) was reduced 8.4 % (38.24/35.03 mm³). **b** The LIPUS treatment area and the self-control area in the treatment group were located in the same lungs. (b1 represents before treatment, b2 represents after treatment.) The inflammation area with LIPUS treatment (red) was reduced 40.2 % (9.14/5.46mm²). The inflammation volume with LIPUS treatment (red) was reduced 56.0 % (0.84/0.37 mm³). The inflammation area without LIPUS treatment (purple) was reduced 33.4 % (384.47/256.05mm²). The inflammation volume without LIPUS treatment (purple) was reduced 41.4 % (31.20/18.28 mm³).

► **Table 2** Comparisons of changes in the area and volume of pneumonia on CT images before and after treatment in the LIPUS treatment area, self-control area, and external control group.

Variable		LIPUS treatment area (n = 42)	Self-control area (n = 42)	External control group (n = 20)	p ^a value	p ^b value	p ^c value
Area of lung inflammation, median (IQR), mm ²	Before treatment	543.4 (302.6–1643.1)	500.7 (290.2–1766.1)	927.0 (308.6–1643.1)	0.837	0.436	0.330
	After treatment	344.8 (210.0–1103.7)	372.8 (221.7–1374.4)	631.3 (288.8–1489.6)	0.570	0.159	0.290
Volume of lung inflammation, median (IQR), mm ³	Before treatment	37.6 (17.1–58.6)	38.4 (12.0–59.5)	29.3 (8.6–49.2)	1.000	0.233	0.286
	After treatment	20.3 (5.4–42.4)	29.9 (8.3–50.0)	20.8 (6.9–46.3)	0.168	0.708	0.418
Area absorption difference, median (IQR), mm ²		132.0 (54.3–340.7)	62.1 (22.1–233.4)	129.2 (17.0–288.4)	0.027	0.354	0.625
Area absorption rate, median (range)		0.25 (0.10–0.49)	0.12 (0.06–0.26)	0.11 (0.04–0.26)	0.013	0.047	0.957
Volume absorption difference, median (IQR), mm ³		10.0 (3.0–29.2)	3.3 (1.3–10.4)	3.1 (1.9–9.1)	0.039	0.038	0.646
Volume absorption rate, median (range)		0.35 (0.13–0.71)	0.11 (0.06–0.37)	0.19 (0.11–0.28)	0.005	0.042	0.392

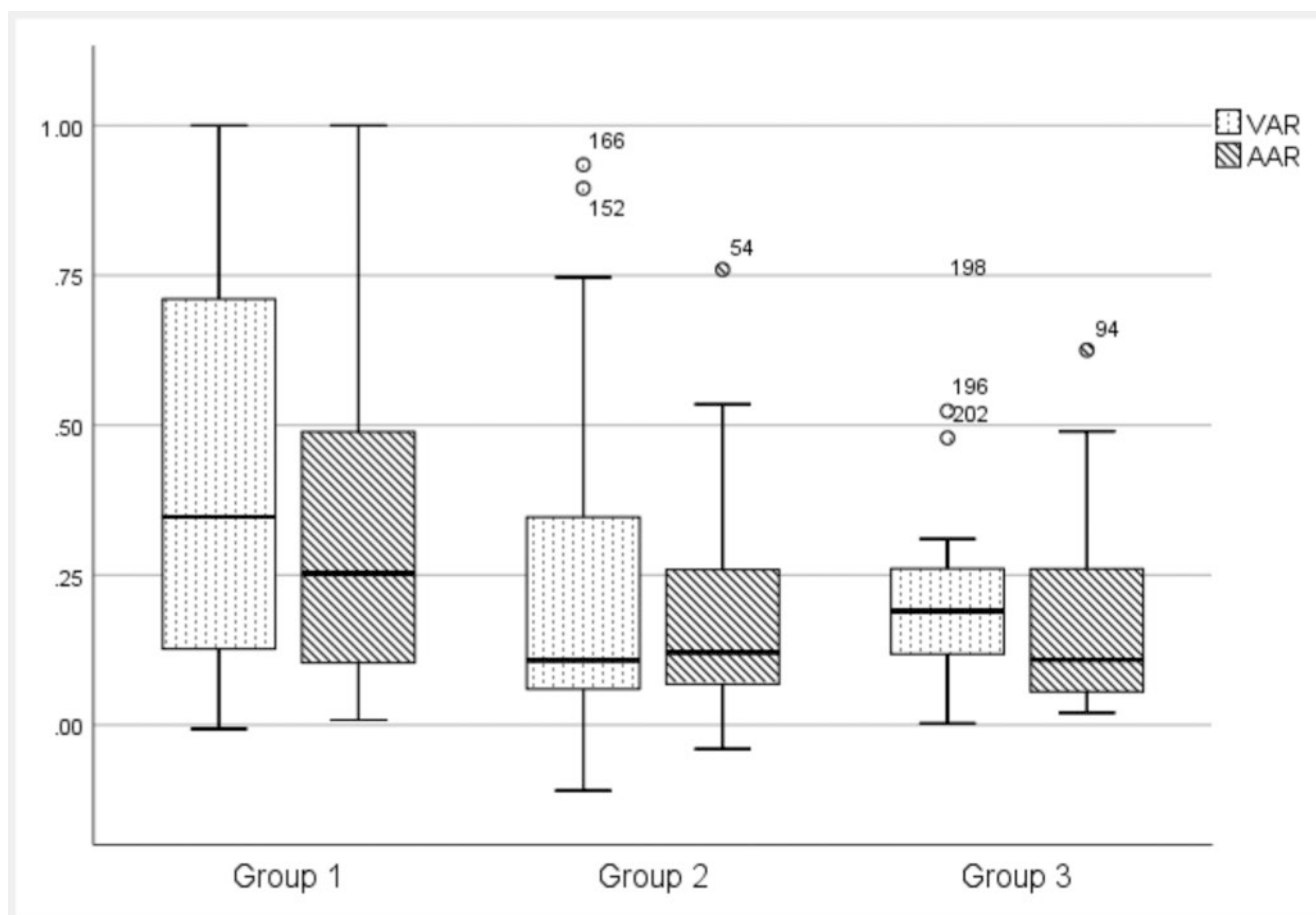
Data not normally distributed were expressed as the median (interquartile range).

Categorical variables were expressed as percentages.

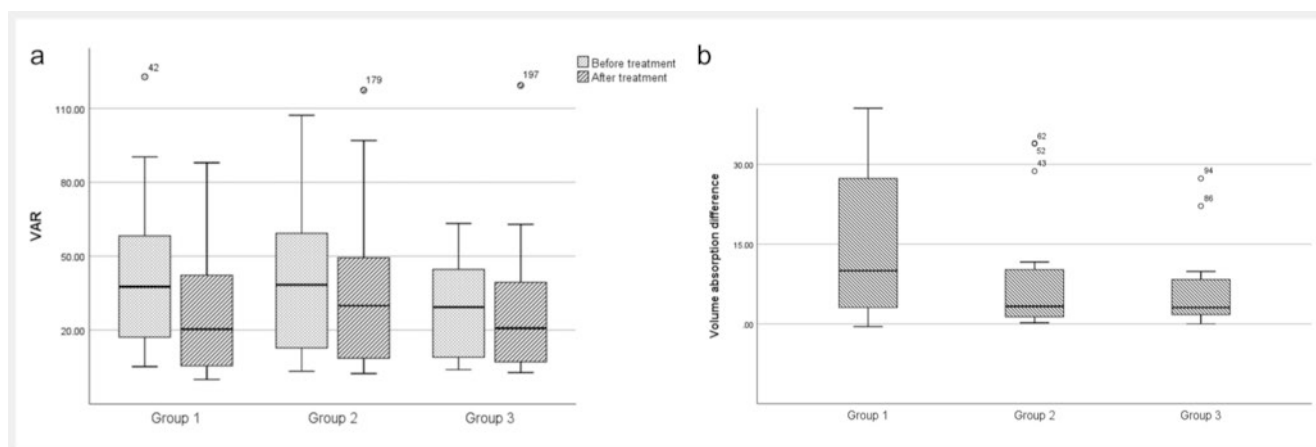
The p^a value represents the statistical significance of the difference between the LIPUS treatment area and the self-control area.

The p^b value represents the statistical significance of the difference between the LIPUS treatment area and the external control group.

The p^c value represents the statistical significance of the difference between the internal control area and the external control group.



► **Fig. 3** Comparison of VAR and AAR in LIPUS area (Group 1), self-control area (Group 2), and external control group (Group 3). There was a statistically significant difference in VAR between Group 1 and Group 2 (0.35 vs. 0.11, $p = 0.005$), and also between Group 1 and Group 3 (0.35 vs. 0.19, $p = 0.042$), but not between Group 2 and Group 3. AAR was also statistically different between Group 1 and Group 2 (0.25 vs. 0.12, $p = 0.013$), and also between Group 1 and Group 3 (0.25 vs. 0.11, $p = 0.047$), but not between Group 2 and Group 3. VAR = the volume reduction rate, AAR = the area reduction rate.



► **Fig. 4** Comparison of the volume of lung inflammation and the volume absorption difference in the LIPUS area (Group 1), self-control area (Group 2), and external control group (Group 3) before and after treatment. **a** There was a statistically significant difference in the volume of lung inflammation before and after treatment in Group 1 (37.6 vs. 20.3, $p < 0.001$), Group 2 (38.4 vs. 29.9, $p < 0.001$), and Group 3 (29.3 vs. 20.8, $p < 0.001$). **b** There was a statistically significant difference in the volume absorption difference before and after treatment between Group 1 and Group 2 (10.0 vs. 3.3, $p = 0.039$), and between Group 1 and Group 3 (10.0 vs. 3.1, $p = 0.038$), but not between Group 2 and Group 3 (3.3 vs. 3.1, $p = 0.646$).

► **Table 3** Comparison of laboratory results and symptoms before and after treatment between the LIPUS treatment group and the external control group.

Variable	LIPUS treatment area (n = 42)			External control group (n = 20)		
	Before treatment	After treatment	p-value	Before treatment	After treatment	p-value
Leukocyte count× 10 ⁹ , mean (SD), /L	7.6 (3.9)	6.6 (3.2)	0.026	7.8 (3.4)	7.5 (2.5)	0.746
Lymphocyte count× 10 ⁹ , median (IQR), /L	1.1 (0.7–1.9)	1.0 (0.7–1.6)	0.324	0.9 (0.7)	1.0 (0.6)	0.546
Monocyte count× 10 ⁹ , mean (SD), /L	0.5 (0.2)	0.7 (1.6)	0.499	0.8 (0.3)	0.6 (0.3)	0.055
CRP, median (IQR), mg/L	19.1 (3.9–67.8)	3.7 (1.5–9.6)	0.000	28.4 (2.1–58.1)	11.2 (2.1–21.7)	0.099
Neutrophil count× 10 ⁹ , median (IQR), /L	5.0 (2.9–7.5)	4.3 (2.6–6.0)	0.047	5.5 (4.1–6.5)	5.2 (3.5–8.7)	0.094
IL-6, median (IQR), pg/mL	9.7 (2.6–37.7)	2.8 (1.3–12.2)	0.000	32.9 (10.7–80.6)	20.4 (11.6–39.0)	0.472
Serum lactate, median (IQR), mmol/L	1.5 (1.0–2.5)	1.4 (0.9–2.9)	0.603	1.4 (1.1–1.9)	1.4 (1.2–2.4)	0.075
PaCO ₂ , mean (SD), mmHg	36.9 (5.4)	38.3 (6.2)	0.253	36.0 (6.24)	40.2 (6.0)	0.024
PaO ₂ , mean (SD), mmHg	94.2 (22.1)	95.5 (31.6)	0.868	106.4 (38.1)	132.1 (58.7)	0.090
Fingertip SaO ₂ , median (IQR), %	96.0 (93.8–98.8)	99.0 (98.0–99.4)	0.000	98.0 (95.7–98.9)	99.0 (97.6–99.5)	0.026
Body temperature, median (IQR), °C	36.9 (36.5–38.4)	36.5 (36.4–37.0)	0.000	36.6 (36.3–36.9)	36.6 (36.4–37.1)	0.796
Fever, n (%)	20 (47.6 %)	3 (7.1 %)	0.000	3 (15.0 %)	1 (5.0 %)	0.605
Cough, n (%)	20 (47.6 %)	10 (23.8 %)	0.023	12 (60.0 %)	2 (10.0 %)	0.002
Expectoration, n (%)	24 (57.1 %)	8 (19.0 %)	0.000	11 (55.0 %)	2 (10.0 %)	0.006
Sore throat, n (%)	3 (7.3 %)	1 (2.4 %)	0.360	0	0	–
Headache, n (%)	3 (7.3 %)	1 (2.4 %)	0.360	0	0	–
Muscular ache, n (%)	17 (40.5 %)	1 (2.4 %)	0.000	0	0	–
Number of days since Hospitalization, mean (SD), days	11.4 (5.0)			17.7 (7.7)		

Normally distributed data were expressed as the mean (standard deviation).

Data not normally distributed were expressed as the median (interquartile range).

Categorical variables were expressed as percentages.

CRP = C-reactive protein, IL-6 = interleukin-6, PaCO₂ = arterial partial pressure of carbon dioxide, PaO₂ = arterial partial pressure of oxygen; SaO₂ = arterial oxygen saturation

3.7 mg/L, $p = 0.000$; IL-6 9.7 vs. 2.8 pg/mL, $p = 0.000$; leukocyte 7.6 vs. 6.6 mg/L, $p = 0.026$; neutrocyte 5.0 vs. 4.3 mg/L, $p = 0.047$), but there was no significant difference in Group 3. The fingertip SaO₂ increased (96.0 % vs. 99.0 %, $p = 0.000$) and

body temperature decreased (36.9 vs. 36.5°C, $p = 0.000$) in Group 1, while in the external control group, only the fingertip SaO₂ (98.0 vs. 99.0 %, $p = 0.026$) increased.

During LIPUS treatment, no adverse events such as local swelling, spotting bleeding, increased local pain response, and hemoptysis occurred.

Discussion

The results of this randomized trial demonstrate that LIPUS is an effective therapy for hospitalized patients with COVID-19 pneumonia. Following the treatment of soft tissue and musculoskeletal diseases with LIPUS, our study is the first clinical trial to use LIPUS to conduct an exploratory intervention in COVID-19 pneumonia, and no safety problems and side effects occurred during the treatment.

We found that after LIPUS treatment, the volume and area of local pneumonia in COVID-19 patients were reduced, showing a positive effect compared with those without LIPUS treatment. At the same time, the serum inflammatory factors CRP and IL-6 of the patients were decreased compared with those before treatment, suggesting that the anti-inflammatory biological effects of LIPUS are also applicable to patients with pulmonary inflammation. For CRP in particular, early clinical studies in COVID-19 pneumonia reported its correlation with severity and prognosis [21, 22].

After an average of 7.2 days of treatment, the patient's pulmonary symptoms were improved, and fingertip SaO_2 increased, but a similar result was also seen in the external control group, so it is difficult to explain the independent effect of LIPUS, and large-scale blank control clinical studies still need to be carried out. In addition, the working principle and optimal working mode of LIPUS in clinical treatment have not been fully elucidated. Prada et al. have proposed the idea of applying LIPUS to the clinical treatment of COVID-19 [23], but no relevant clinical trials have been reported and the selection of the optimal working mode and treatment parameters is a difficult point. Based mainly on previous research on LIPUS in mitigating soft tissue inflammation, we chose this working parameter. However, the optimal parameters of LIPUS for lung treatment need to be further explored and verified.

Chest CT is currently considered the main reference standard for the imaging diagnosis of pneumonia. Therefore, we quantitatively calculated the area and volume of lung inflammation to evaluate the effect of LIPUS treatment. In recent years, the clinical value of lung ultrasound (LUS), which is favored for its convenience, real-time, reliability, and lack of radiation, has gradually been confirmed. Nazerian et al. [24] and Soldati et al. [25] compared the value of chest CT and LUS in the diagnosis of lung lesions qualitatively and semi-quantitatively and found that the two had a good consistency and were significantly better than chest radiographs. We conducted LUS follow-up in a small number of COVID-19 pneumonia patients and found that the LUS score decreased significantly after LIPUS treatment (median: 12 vs. 2, $p < 0.001$) (**Supplementary Figure 3**). Lugarà et al. [26] performed LUS scores and high-resolution CT scores on 99 patients with COVID-19 pneumonia, and the results also showed a significant correlation and synergy between the two in the diagnosis and the assessment of disease severity. It can be seen that LUS combined with chest CT for pneumonia diagnosis and localization and guidance of LIPUS treatment will further improve the curative effect.

However, there are differences in the distribution of lung lesions in the population [27], and its accessibility should be evaluated before ultrasound localization and treatment, so as to select patients suitable for LIPUS therapy. Due to the existence of sound attenuation, whether there is a difference in the efficacy of LIPUS in the treatment of deep and superficial lung lesions requires more clinical research.

No side effects such as pulmonary hemorrhage and hemoptysis were found during the treatment. The ultrasound intensity of LIPUS is far lower than that used in most clinical diagnoses and treatments, and its reliability has been demonstrated by many researchers in both animal and clinical studies [14, 15, 16, 19, 20, 21]. However, various frequencies and intensities of LIPUS application to the lungs need to be established and validated. The underlying cellular and molecular mechanisms underlying the biological effects of LIPUS on inflammation remain to be further explored.

Our study had several limitations. First, this was a single-center and small-sample exploratory clinical study. Due to the limited number of wearable LIPUS devices and control of the local epidemic, the study was terminated, and the data were analyzed. Secondly, all participants enrolled in our study were infected with Omicron, and the effect of LIPUS in reducing pulmonary inflammation may currently only be applicable to this type of virus infection. Whether LIPUS has a role in other types of viral infections or bacterial pneumonia needs further study. Finally, the study did not compare whether there were differences in treatment effects between different diseased lobes. Due to the limited number of patients included, we randomly assigned lung lobes with different lesions, and there was no statistical difference between the LIPUS treatment area and the self-control area before treatment. Subsequent multi-center large-sample studies will further analyze whether there are differences in the efficacy of inflammation in different locations.

Conclusion

This clinical trial has shown for the first time that LIPUS is effective in mitigating inflammation in COVID-19 pneumonia. In addition, combined with the therapeutic mechanism, it is speculated that LIPUS may be more extensive than limited to COVID-19 pneumonia, and may play a certain role in the treatment of other viral and even bacterial types of pneumonia. However, large-scale clinical trials are still needed for further verification.

Funding

Shanghai Science and Technology Commission (21Y11910900) | National Key R&D Program (2021YFC2009100) | Shanghai Sixth People's Hospital COVID-19 Pneumonia Epidemic Emergency Special Project (ynxg202209) | Shanghai Sixth People's Hospital Surface Cultivation Project (ynms202110)

Clinical Trial

Registration number (trial ID): ChiCTR2200059550 | Trial registry: Chinese Clinical Trial Registry (<http://www.chictr.org/>) | Type of Study: Randomized

Acknowledgement

We thank the patients who participated in this study. We would also like to thank the many doctors, nurses, other allied health personnel and research administrators in the isolation hospital for their support. Thanks to the core financial support provided by Shanghai Jiao Tong University Affiliated Sixth People's Hospital.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

- [1] da Costa CHS, de Freitas CAB, Alves CN et al. Assessment of mutations on RBD in the Spike protein of SARS-CoV-2 Alpha, Delta and Omicron variants. *Sci Rep* 2022; 12: 8540. doi:10.1038/s41598-022-12479-9
- [2] Jassat W, Abdool Karim SS, Mudara C et al. Clinical severity of COVID-19 in patients admitted to hospital during the omicron wave in South Africa: a retrospective observational study. *Lancet Glob Health* 2022; 10: e961–e969
- [3] Costela-Ruiz VJ, Illescas-Montes R, Puerta-Puerta JM et al. SARS-CoV-2 infection: The role of cytokines in COVID-19 disease. *Cytokine Growth Factor Rev* 2020; 54: 62–75. doi:10.1016/j.cytogfr.2020.06.001
- [4] Yi Y, Lagniton PNP, Ye S et al. COVID-19: what has been learned and to be learned about the novel coronavirus disease. *Int J Biol Sci* 2020; 16: 1753–1766
- [5] Hu G, Christman JW. Editorial: Alveolar Macrophages in Lung Inflammation and Resolution. *Front Immunol* 2019; 10: 2275. doi:10.3389/fimmu.2019.02275
- [6] Rahman S, Montero MTV, Rowe K et al. Epidemiology, pathogenesis, clinical presentations, diagnosis and treatment of COVID-19: a review of current evidence. *Expert Rev Clin Pharmacol* 2021; 14: 601–621. doi:10.1080/17512433.2021.1902303
- [7] Gonzales JN, Lucas R, Verin AD. The Acute Respiratory Distress Syndrome: Mechanisms and Perspective Therapeutic Approaches. *Austin J Vasc Med* 2015; 2: 1009
- [8] Huang C, Wang Y, Li X et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China [published correction appears in *Lancet*]. *Lancet* 2020; 395: 497–506
- [9] Wang D, Hu B, Hu C et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China [published correction appears in *JAMA*. 2021 Mar 16;325(11):1113]. *JAMA* 2020; 323: 1061–1069
- [10] Best TM, Wilk KE, Moorman CT et al. Low Intensity Ultrasound for Promoting Soft Tissue Healing: A Systematic Review of the Literature and Medical Technology. *Intern Med Rev (Wash D C)* 2016; 2: 271. doi:10.18103/imr.v2i11.271
- [11] Jiang X, Savchenko O, Li Y et al. A Review of Low-Intensity Pulsed Ultrasound for Therapeutic Applications. *IEEE Trans Biomed Eng* 2019; 66 (10): 2704–2718. doi:10.1109/TBME.2018.2889669
- [12] Yang Q, Nanayakkara GK, Drummer C et al. Low-intensity ultrasound-induced anti-inflammatory effects are mediated by several new mechanisms including gene induction, immunosuppressor cell promotion, and enhancement of exosome biogenesis and docking. *Front Physiol* 2017; 8: 818
- [13] Zheng C, Wu SM, Lian H et al. Low-intensity pulsed ultrasound attenuates cardiac inflammation of CVB3-induced viral myocarditis via regulation of caveolin-1 and MAPK pathways. *J Cell Mol Med* 2019; 23 (3): 1963–1975. doi:10.1111/jcmm.14098
- [14] Zhang B, Chen H, Ouyang J et al. SQSTM1-dependent autophagic degradation of PKM2 inhibits the production of mature IL1B/IL-1 β and contributes to LIPUS-mediated antiinflammatory effect. *Autophagy* 2020; 16: 1262–1278. doi:10.1080/15548627.2019.1664705
- [15] Song J, Li N, Xia Y et al. Arctigenin Confers Neuroprotection Against Mechanical Trauma Injury in Human Neuroblastoma SH-SY5Y Cells by Regulating miRNA-16 and miRNA-199a Expression to Alleviate Inflammation. *J Mol Neurosci* 2016; 60: 115–129. doi:10.1007/s12031-016-0784-x
- [16] da Silva Junior EM, Mesquita-Ferrari RA, Cristiane Miranda França C et al. Modulating effect of low intensity pulsed ultrasound on the phenotype of inflammatory cells. *Biomed Pharmacother* 2017; 96: 1147–1153
- [17] Zhang ZC, Yang YL, Li B et al. Low-intensity pulsed ultrasound promotes spinal fusion by regulating macrophage polarization. *Biomed Pharmacother* 2019; 120: 109499. doi:10.1016/j.biopha.2019.109499
- [18] Tsukada M, Takiuchi T, Watanabe K. Low-Intensity Pulsed Ultrasound for Early-Stage Lumbar Spondylolysis in Young Athletes. *Clin J Sport Med* 2019; 29 (4): 262–266. doi:10.1097/JSM.0000000000000531
- [19] Bajpai A, Nadkarni S, Neidrauer M et al. Effects of Non-thermal, Non-cavitational Ultrasound Exposure on Human Diabetic Ulcer Healing and Inflammatory Gene Expression in a Pilot Study. *Ultrasound Med Biol* 2018; 44 (9): 2043–2049. doi:10.1016/j.ultrasmedbio.2018.05.011
- [20] Yadava M, Le DE, Dykan IV et al. Therapeutic Ultrasound Improves Myocardial Blood Flow and Reduces Infarct Size in a Canine Model of Coronary Microthromboembolism. *J Am Soc Echocardiogr* 2020; 33 (2): 234–246. doi:10.1016/j.echo.2019.09.011
- [21] Guan WJ, Zhong NS. Clinical Characteristics of COVID-19 in China. *Reply*. *N Engl J Med* 2020; 382: 1861–1862. doi:10.1056/NEJMc2005203
- [22] Qin C, Zhou L, Hu Z et al. Dysregulation of Immune Response in Patients With Coronavirus 2019 (COVID-19) in Wuhan, China. *Clin Infect Dis* 2020; 71: 762–768
- [23] Prada F, Cogliati C, Wu MA et al. Can Low-Intensity Pulsed Ultrasound Treat Discrete Pulmonary Lesions in Patients With COVID-19? *J Ultrasound Med* 2021; 40 (7): 1445–1450
- [24] Nazerian P, Volpicelli G, Vanni S et al. Accuracy of lung ultrasound for the diagnosis of consolidations when compared to chest computed tomography. *Am J Emerg Med* 2015; 33 (5): 620–625
- [25] Soldati G, Smargiassi A, Inchingolo R et al. Proposal for International Standardization of the Use of Lung Ultrasound for Patients With COVID-19: A Simple, Quantitative, Reproducible Method. *J Ultrasound Med* 2020; 39 (7): 1413–1419
- [26] Lugarà M, Tamburrini S, Coppola MG et al. The Role of Lung Ultrasound in SARS-CoV-19 Pneumonia Management. *Diagnostics (Basel)* 2022; 12 (8): 1856. doi:10.3390/diagnostics12081856
- [27] Kovács A, Palásti P, Veréb D et al. The sensitivity and specificity of chest CT in the diagnosis of COVID-19. *Eur Radiol* 2021; 31: 2819–2824. doi:10.1007/s00330-020-07347-x