ORIGINAL RESEARCH

Effects of Photobiomodulation Therapy Combined with Static Magnetic Field in Severe COVID-19 Patients Requiring Intubation: A Pragmatic Randomized Placebo-Controlled Trial

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Purpose: We aimed to investigate the effects of photobiomodulation therapy combined with static magnetic field (PBMT-sMF) on the length of intensive care unit (ICU) stay and mortality rate of severe COVID-19 patients requiring invasive mechanical ventilation and assess its role in preserving respiratory muscles and modulating inflammatory processes.

Patients and Methods: We conducted a prospectively registered, triple-blinded, randomized, placebo-controlled trial of PBMT-sMF in severe COVID-19 ICU patients requiring invasive mechanical ventilation. Patients were randomly assigned to receive either PBMT-sMF or a placebo daily throughout their ICU stay. The primary outcome was length of ICU stay, defined by either discharge or death. The secondary outcomes were survival rate, diaphragm muscle function, and the changes in blood parameters, ventilatory parameters, and arterial blood gases.

Results: Thirty patients were included and equally randomized into the two groups. There were no significant differences in the length of ICU stay (mean difference, MD = −6.80; 95% CI = −18.71 to 5.11) between the groups. Among the secondary outcomes, significant differences were observed in diaphragm thickness, fraction of inspired oxygen, partial pressure of oxygen/fraction of inspired oxygen ratio, C-reactive protein levels, lymphocyte count, and hemoglobin (p < 0.05).

Conclusion: Among severe COVID-19 patients requiring invasive mechanical ventilation, the length of ICU stay was not significantly different between the PBMT-sMF and placebo groups. In contrast, PBMT-sMF was significantly associated with reduced diaphragm atrophy, improved ventilatory parameters and lymphocyte count, and decreased C-reactive protein levels and hemoglobin count.

Trial Registration Number (Clinical Trials.gov): NCT04386694.

Keywords: COVID-19, photobiomodulation, intensive care unit, respiratory muscles, ventilatory parameters, mechanical ventilation

Introduction

The novel coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] SARS-CoV-2 infection triggers hematological abnormalities such as leucopenia, lymphopenia, increased prothrombin time, increased D-dimer and C-reactive protein (CRP) levels, and increased cytokine levels such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α.[2–4] The clinical features observed in COVID-19 patients mainly include fever, dry cough, and fatigue or myalgia[2,5] and can range from no symptoms to severe pneumonia and even death.[1] Severe cases usually

progress to complications such as dyspnea, hypoxia, acute hypoxemic respiratory insufficiency, arrhythmia, acute cardiac injury, and shock,[2,5,6] which often require critical care in the intensive care unit (ICU).[2,5,7]

To date, there has been no effective treatment for SARS-CoV-2 infection. Therefore, several therapeutic agents such as chloroquine, hydroxychloroquine,[8] lopinavir-ritonavir,[9] remdesivir,[10] and dexamethasone[11] have been tested and used in the attempt to treat COVID-19. Reports on the effectiveness of such therapeutic agents have been conflicting,[8–10] and further high-quality randomized controlled trials are necessary to confirm whether the benefits outweigh the harm. However, given the lack of robust evidence regarding the effects of available therapeutic agents and the lack of effective treatment available to combat SARS-CoV-2, it is necessary to base management strategies on the signs and symptoms of COVID-19 patients, especially in the most severe cases. Respiratory management of severe COVID-19 ICU patients can be achieved through oxygen therapy, non-invasive ventilation, and intubation.[12] Respiratory failure due to hypoxemia is one of the most prominent complications in these patients,[13] and usually requires mechanical ventilation via an endotracheal tube.[14] Acute respiratory failure reduces lung compliance, increases respiratory work, and affects blood oxygenation, leading to a shallow breathing pattern.[15] The strength of respiratory muscles may also be reduced.[16] The diaphragm is one of the respiratory muscles widely affected by critical illness and mechanical ventilation.[17,18] Prolonged diaphragm inactivity during mechanical ventilation is not only associated with changes in diaphragm thickness[19] but also causes a rapid progression of diaphragm weakness.[17,20] All these characteristics render the weaning of patients from mechanical ventilation difficult and increase the risk of ICU readmission, leading to a poorer clinical trajectory.[21,22]

Photobiomodulation therapy (PBMT) combined with static magnetic field (sMF) has the potential as a non-pharmacological intervention for the respiratory management of severe COVID-19 patients. PBMT is a non-thermal and non-ionizing light therapy applied in the form of light amplification by stimulated emission of radiation (LASER), light-emitting diodes (LEDs), and/or broad-band irradiation in the visible and infrared spectra.[23] PBMT increases cellular metabolism,[24,25] microcirculation,[26] oxygen availability,[27,28] and redox metabolism,[29] as well as modulates inflammatory processes.[30–32] Recently, PBMT has been used in combination with sMF.[31–34] sMF interacts with biological systems[35] by increasing the production of adenosine triphosphate (ATP) and consequently enhancing cell metabolism.[36] In addition, sMF reduces oxidative stress.[37,38] Regardless of the exact mechanism of action, sMF has been shown to exert analgesic effects.[39–41] PBMT and sMF have been found to produce a synergistic effect by generating greater transfer of electrons.[33,34,34–36,36–42,42,43] Robust evidence has shown that PBMT, isolated or in combination with sMF (PBMT-sMF), exerted beneficial effects on skeletal muscles, including increased physical performance, reduced fatigue,[42,43] decreased performance loss and function during the detraining period,[34] and increased oxygen saturation.[27,28] In contrast, there is a lack of evidence on the effects of PBMT or PBMT-sMF on respiratory muscles and the respiratory system in general. To date, only one clinical trial has evaluated the effects of PBMT on respiratory muscles and has shown its effectiveness in improving the functional capacity of chronic obstructive pulmonary disease patients.[44] Furthermore, experimental studies on the respiratory system of animals have shown that PBMT can modulate pulmonary inflammation[45–47] and relieve bronchial hyperresponsiveness.[47]

The previously reported beneficial effects coupled with the lack of known adverse effects suggest that PBMT-sMF may be a safer alternative to the pharmacological treatments of severe COVID-19 patients. However, it is unknown whether the effects of PBMT (or PBMT-sMF) on respiratory muscles are similar to those previously observed on skeletal muscles and whether PBMT-sMF can modulate inflammatory processes and contribute to the clinical improvement of these patients. Therefore, our study aimed to investigate the effects of PBMT-sMF on the length of ICU stay and mortality rate of severe COVID-19 patients requiring invasive mechanical ventilation, and assess its role in preventing respiratory muscle atrophy, increasing oxygen availability, modulating inflammatory process, and improving the general clinical outcomes of such patients.

Patients and Methods
Trial Design
A prospectively registered (NCT04386694), pragmatic, two-arm, parallel randomized, triple-blinded (patients, therapists, and outcome assessors), placebo-controlled trial was conducted. There were two deviations from the registered protocol. The first involved our estimation that the endpoint would be up to 20 days after randomization, with patients either discharged or dead from any cause within this period; however, as the study period directly

depended on the length of ICU stay, it eventually extended beyond 20 days. The second deviation was regarding the failure to assess three secondary outcomes: immunoglobulin G (IgG), immunoglobulin M (IgM), and D-dimer levels, as the laboratory in charge was unable to implement the necessary routines before beginning this trial. This study adhered to the CONSORT guidelines.

Ethics

This study was submitted and approved by the Research Ethics Committee of Associação Dr. Bartholomeu Tacchini/Hospital Tacchini/RS (protocol number: 3,985,226) and by the National Research Ethics Commission of the Brazilian Ministry of Health (protocol number: 4,021,485). This trial was performed in accordance with the Declaration of Helsinki and the Research Involving Human Subjects guidelines. All eligible patients or their legal representatives (if the patients were too unwell to provide consent) were informed of the study objectives and provided written informed consent before enrollment. All appropriate and customary steps were taken to ensure data security and patient confidentiality.

Participants and Recruitment

The participants were patients with laboratory-confirmed COVID-19 via the reverse transcription-polymerase chain reaction (RT-PCR) test, who were admitted to the adult ICU of the Hospital Tacchini, Bento Gonçalves, Brazil, between May 2020 and July 2020. The inclusion criteria included age ≥ 15 years and the need for invasive mechanical ventilation through orotracheal intubation due to respiratory failure. The exclusion criteria included negative COVID-19 results, duration of prone position > 24 hours, cancer patients, and pregnant women.

Randomization and Blinding

Prior to treatment initiation, the patients were randomized into two intervention groups: the active and placebo PBMT-sMF groups. Randomization was generated by a website (http://randomization.com/) and was performed by an independent researcher not involved in the recruitment, assessment, or treatment of the patients. The same researcher programmed the PBMT-sMF device to either the active or placebo mode according to the group allocations and was instructed not to disclose the programmed interventions to anyone involved in the study until its completion. The assessors, patients, and therapists were hence blinded throughout the treatment. Concealed allocation was achieved by the use of sequentially numbered, sealed, and opaque envelopes.

Interventions

All patients received standard ICU care in addition to the allocated PBMT-sMF treatment. Standard ICU care involved the administration of antibiotics, antivirals, corticoids, and sedatives. In addition, physical therapies such as bronchial hygiene techniques, passive mobilization exercises of the lower and upper limbs, pressure ulcer management (positional changes), bronchial secretion aspiration with a closed system, positive end-expiratory pressure (PEEP) titration, and passive sitting were implemented. The clinical evolution of the patients determined the progression of care. Active and placebo PBMT-sMF were performed using the same devices, with the same irradiated sites. To ensure blinding of the therapists, the devices emitted the same sounds and displayed the same information regardless of the programmed mode. Furthermore, since the amount of heat produced by the devices is undiscernible,[33] blinding was not compromised by this aspect.

All patients underwent the allocated PBMT-sMF treatment once a day for seven days per week throughout their ICU stay (after study enrollment) until discharge or death, whichever came first. The intervention specifications were as follows:

1) Active PBMT-sMF: PBMT-sMF was performed using a cordless, portable MR5 Activ Pro LaserShower™ device (PhotOxyl™ prototype, Multi Radiance Medical™, Solon, OH, USA) (Figure 1). A cluster probe with 20 diodes was used, which included 4 diodes of 905 nm (peak power: 50W, average mean optical output: 1.25 mW, power density: 3.91 mW/cm^2, spot size: 0.32 cm^2, and operating mode: superpulsed); 8 diodes of 633 nm (average optical output: 25 mW, power density: 29.41 mW/cm^2, spot size: 0.85 cm^2, and operating mode: pulsed); and 8 diodes of 850 nm (average optical output: 40 mW, power density: 71.23 mW/cm^2, spot size: 0.56 cm^2, and operating mode: pulsed). PBMT-sMF was applied to six sites (33 cm^2 each site) of the lower thoracic/upper abdominal region and two sites (33 cm^2 each site) of the neck area (sternocleidomastoid muscle), as illustrated in Figure 1. The exposure time was 60 s per site, yielding a total treatment time of 480 s (8 min). The energy delivered per site was 31.50 J, yielding a total delivered energy of 189 J and 63 J in the lower thoracic and neck regions, respectively. A total area of 264 cm^2
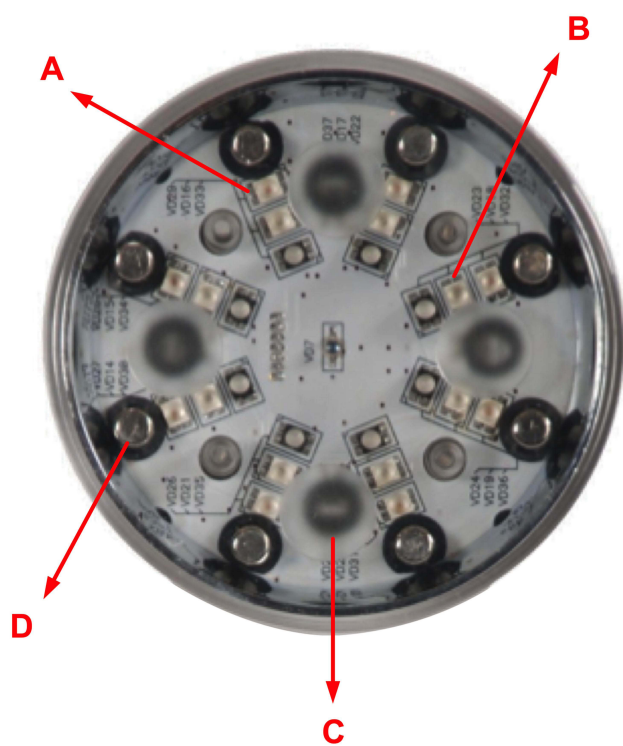
Figure 1 PBMT-sMF device. Figure 1 shows the device used to applied the PBMT-sMF and placebo. A cluster probe with 20 diodes containing 4 diodes of 905 nm, 8 diodes of 633 nm and 8 diodes of 850 nm was used: A - Red LEDs; B - Infrared LEDs; C - Super-pulsed laser; D - Magnetic field.

was irradiated with a dose of 0.95 J/cm^2. The energy delivered per site and the irradiation sites were established based on the previous study that applied PBMT to respiratory muscles.[44] PBMT-sMF was applied using the direct contact method, with slight pressure on the skin. A full description of the intervention is presented in Figure 2.

2) Placebo PBMT-sMF: The same device was used without any emission of the therapeutic dose. In the placebo mode, the infrared laser diodes, infrared LED diodes, and sMF were deactivated. In addition, the mean power of each LED diode was decreased to 0.5 mW to maintain the visual aspect of the red light, while maintaining the energy delivered (0.24 J per site) and dose (0.007 J/cm^2) below therapeutic level. According to currently available evidence, the therapeutic windows of PBMT on small and large muscle groups are 20–60 J and 60–300 J, respectively.[42,43] The irradiated sites and exposure time were kept the same as those in active PBMT-sMF treatment.

Outcomes

Demographic and clinical characteristics such as age, gender, comorbidities, disease severity, and health status

before hospital admission measured using the Simplified Acute Physiology Score 3 (SAPS 3)[48] were collected from the electronic medical records. The primary and secondary outcomes were as follows:
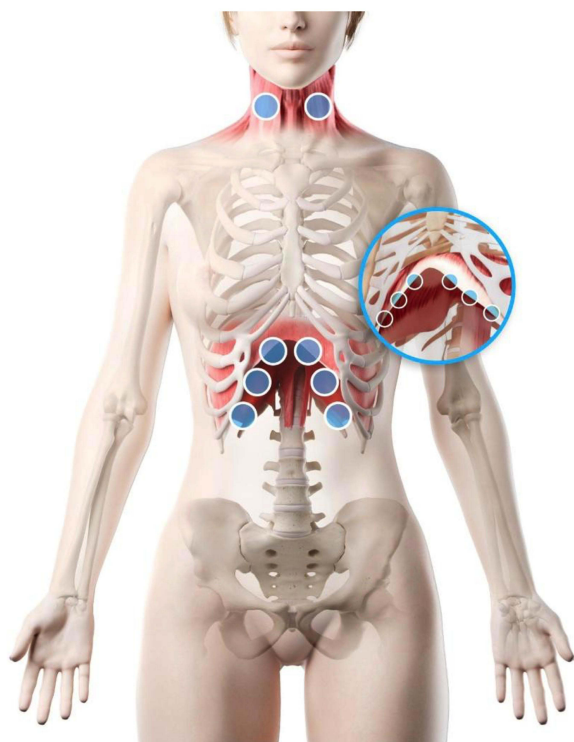
Primary Outcome

- Length of ICU stay: The length of ICU stay was measured by the number of days of ICU admission from randomization to discharge or death from any cause.

Secondary Outcomes

- Survival rate: The survival rate was measured as the rate of those who survived and were discharged from the ICU against those who died during the study period.
- Diaphragm thickness: The diaphragm thickness was measured using ultrasound.[48,49] The LOGIQe device (GE Healthcare, Chicago, USA) and a linear transducer (ML6-15 5–15 MHz, and 9L D – 8 MHz) were used. The measurements were performed with the patient in the supine position. The transducer was positioned in the zone of apposition between the anterior and midaxillary lines at the level of the ninth or tenth intercostal space.[50] End-expiratory diaphragm thickness was measured in two consecutive breaths on two separate images. Measurements were repeated at least once until they were consistently within 10%. The mean of all four measurements was used for analysis.[50] Measurements were obtained at baseline (up to 24 hours after the initiation of invasive mechanical ventilation), 10 days after randomization (day 10), and within 24 hours of discharge or death from any cause (the endpoint).
- Blood parameters: C-reactive protein (CRP), TNF-α, and vitamin D levels, and erythrocyte, hemoglobin, hematocrit, leucocyte, segmented neutrophil, eosinophil, basophil, lymphocyte, monocyte, and platelet counts were assessed at baseline (ICU admission), day 10, and the endpoint. All measured parameters were part of the daily routine blood tests performed at the hospital and were hence collected directly from the electronic medical records. The data were collected by two assessors who were blinded to the group allocations.
- Mechanical ventilation parameters: Positive end-expiratory pressure levels (PEEP) and fraction of inspired oxygen (FiO$_2$) were measured using a mechanical ventilator. The data were collected at baseline, day 10, and the endpoint.

A

B

	Diaphragm	Neck area
Number of lasers	4	4
Wavelength (nm)	905	905
Frequency (Hz)	250	250
Peak power (W) - each	50	50
Average mean optical output (mW) - each	1.25	1.25
Power density (mW/cm^2) - each	3.91	3.91
Energy density (J/cm^2) - each	0.234	0.234
Energy delivered (J) - each	0.075	0.075
Spot size of laser (cm^2) - each	0.32	0.32
Number of red LEDs	8	8
Wavelength of red LEDs (nm)	633	633
Frequency (Hz)	2	2
Average optical output (mW) - each	25	25
Power density (mW/cm^2) - each	29.41	29.41
Energy density (J/cm^2) - each	1.765	1.765
Energy delivered (J) - each	1.50	1.50
Spot size of red LED (cm^2) - each	0.85	0.85
Number of infrared LEDs	8	8
Wavelength of infrared LEDs (nm)	850	850
Frequency (Hz)	250	250
Average optical output (mW) - each	40	40
Power density (mW/cm^2) - each	71.23	71.23
Energy density (J/cm^2) - each	4.286	4.286
Energy delivered (J) - each	2.40	2.40
Spot size of infrared LED (cm^2) - each	0.56	0.56
Magnetic field (mT)	110	110
Irradiation time per site (sec)	60	60
Total energy delivered per site (J)	31.50	31.50
Number of irradiated sites	6	1 (bilaterally)
Total energy delivered to the muscle group (J)	189.00	31.50 (bilaterally)
Aperture of device (cm^2)	33	33
Dose (J/cm^2)	0.95	0.95
Application mode	Cluster probe held stationary in skin contact with a 90-degree angle and slight pressure	Cluster probe held stationary in skin contact with a 90-degree angle and slight pressure

Figure 2 Irradiation of interventions. (**A**) shows the sites where PBMT-sMF and placebo were irradiated. The interventions were irradiated at six sites in the lower thorax/upper abdominal cavity and two sites in the neck area. (**B**) shows the full description of the parameters of PBMT-sMF applied in the treatment. This figure is owned by the authors.

- Arterial blood gases: The arterial partial pressure of oxygen (PO_2) and the PO_2/FiO_2 ratio were assessed at baseline, day 10, and the endpoint. Both parameters were part of the routine blood tests performed by the hospital and were hence collected directly from the electronic medical records. The data were collected by two assessors who were blinded to the group allocation groups.

Statistical Analysis

To date, there are no published studies on the effects of PBMT-sMF in severe COVID-19 patients; thus, a convenience sample was used for this trial. To estimate our sample size, we considered the number of patients admitted to the ICU in the month before the start of our trial (April 2020) who met our inclusion and exclusion criteria (n = 10). By further estimating a 3-month enrollment period (after the randomization of the first patient), the convenience sample size was expected to reach 30 patients.

Statistical analysis was conducted following the intention-to-treat principle.[51,52] Data normality was tested using the Kolmogorov–Smirnov test. Since the data were normally distributed, inter-group differences (treatment effects) were analyzed using the unpaired, two-tailed t test (hospitalization data), and the two-way repeated measures analysis of variance (time vs experimental group) with post-hoc Bonferroni correction (ventilatory parameters, biochemical markers, and hemogram parameters). The association between categorical variables was analyzed using the Chi-square test. Data are expressed as mean, standard deviation (SD), mean difference (MD), and 95% confidence interval (CI). Data are expressed as frequency (%). The significance level was defined as p < 0.05. To evaluate practical significance, the magnitude of differences (Cohen's d) between groups was calculated using the means and SDs of the placebo and PBMT-sMF groups (using G*Power 3.1). The following criteria of Cohen were adopted: small, 0.2; moderate, 0.50; and

Results

Patients

Among the 62 patients who were assessed for eligibility, 30 were included and equally randomized into the placebo and PBMT-sMF groups (n = 15 each). All patients received the allocated treatment (Figure 3). The mean age was 66.06 years, the mean height was 166.53 cm, the mean body mass was 75.18 kg, and 53.33% were male. The baseline demographic and clinical characteristics were similar between the groups (p > 0.05), as shown in Table 1. No adverse effects were observed in either group.

Primary Outcome

The mean lengths of ICU stay of the placebo and PBMT-sMF groups were 23.06 days and 16.26 days, respectively, with no significant differences observed (MD = −6.80; 95% CI = −18.71 to 5.11) (Table 2).

Secondary Outcomes

There were no differences in diaphragm thickness at baseline (p = 0.99; MD = 4.27; 95% CI = −7.41 to 15.95); however, significantly increased diaphragm thickness was shown in the PBMT-sMF group at day 10 (p = 0.0117; MD = 14.19; 95% CI = 2.50 to 25.87) and the endpoint (p = 0.0001; MD = 20.46; 95% CI = 8.77 to –32.14) (Table 3). There were no differences in PEEP at baseline (p = 0.99; MD = −0.13; 95% CI = −2.69 to 2.43), day 10 (p = 0.5540; MD = −1.40; 95% CI = −3.96 to 1.16), and the endpoint (p = 0.3931; MD = −1.60; 95% CI = −4.16 to 0.96) (Table 3). In addition, there were no differences in PO_2 at baseline (p = 0.99; MD = 16.76; 95% CI = −8.64 to 42.16), day 10 (p = 0.99; MD = 6.72; 95% CI = −18.68 to 32.12), and the endpoint (p = 0.34; MD = 16.55; 95% CI = −8.85 to 41.95) (Table 3). There were also no significant differences in FiO_2 at baseline (p = 0.33; MD = −7.33; 95% CI = −30.92 to 16.26), and at day 10 (p = 0.6590; MD = −11.94; 95% CI = −35.53 to 11.65); however, the PBMT-sMF group demonstrated significantly lower FiO_2 at the endpoint (p = 0.0456; MD = −23.93; 95% CI = −47.52 to −0.34) (Table 3). Finally, there were no difference in the PO_2/FiO_2 ratio at baseline (p = 0.67; MD = 52.94; 95% CI = −53.63 to 158.50) and day 10 (p = 0.2246; MD = 77.95; 95% CI = −27.63 to 183.50), but the PBMT-sMF group showed significantly higher PO_2/FiO_2 ratio at the endpoint (p = 0.0233; MD = 117.90; 95% CI = 12.31 to 223.50) (Table 3).

There were no differences in CRP levels at baseline (p = 0.41; MD = −49.01; 95% CI = −131.60 to 33.58) and day 10 (p = 0.99; MD = −20.09; 95% CI = −102.70 to 62.50); however, the PBMT-sMF group showed significantly lower CRP levels (p = 0.045; MD = −83.87; 95% CI = −166.50 to −1.28) at the endpoint (Table 4). There were no differences in TNF-α levels at baseline (p = 0.99; MD = 2.48; 95% CI = −7.71 to 12.68), day 10 (p = 0.44; MD = 6.11; 95% CI = −4.08 to 16.32), and the endpoint (p = 0.99; MD = −2.87; 95% CI = −13.07 to 7.33) (Table 4). There were no differences in vitamin D levels at baseline (p = 0.43; MD = 4.15; 95% CI = −2.75 to 11.07), day 10 (p = 0.99; MD = −0.42; 95% CI = −7.34 to 6.48), and the endpoint (p = 0.0502; MD = 6.90; 95% CI = −0.004 to 13.82) (Table 4). There was no differences in erythrocyte count at baseline (p = 0.19; MD = −0.45; 95% CI = −1.02 to 0.13), day 10 (p = 0.065; MD = −0.55; 95% CI = −1.13 to 0.02), and the endpoint (p = 0.389; MD = −0.36; 95% CI = −0.94 to 0.21) (Table 4). There were no differences in hemoglobin count at baseline (p = 0.29; MD = −1.23; 95% CI = −3.03 to 0.56) and at the endpoint (p = 0.6173; MD = −0.94; 95% CI = −2.74 to 0.86), but the PBMT-sMF group showed significantly lower hemoglobin count (p = 0.026; MD = −1.97; 95% CI = −3.77 to −0.17) at day (Table 4). There were no differences in hematocrit at baseline (p = 0.29; MD = −3.25; 95% CI = −7.98 to 1.48), at day 10 (p = 0.194; MD = −3.62; 95% CI = −8.36 to 1.10), and the endpoint (p = 0.459; MD = −2.79; 95% CI = −7.52 to 1.94) (Table 4). There were no differences in leukocytes count at baseline (p = 0.99; MD = 709.30; 95% CI = −15,147 to 16,566), at day 10 (p = 0.476; MD = 9227; 95% CI = −6629 to 25,083), and the endpoint (p = 0.99; MD = 5444; 95% CI = −10,412 to 21,300) (Table 4). There were no differences in segmented neutrophil count at baseline (p = 0.99; MD = −56.02; 95% CI = −6836 to 6724), day 10 (p = 0.91; MD = −2856; 95% CI = −9636 to 3924), and the endpoint (p = 0.99; MD = 1362; 95% CI = −5418 to 8142) (Table 4). There were no differences in eosinophil count at baseline (p = 0.99; MD = 2.30; 95% CI = −1032 to 1037), day 10 (p = 0.99; MD = 95.70; 95% CI = −939 to 1130), and the endpoint (p = 0.146; MD = −846.40; 95% CI = −1881 to 188.3) (Table 4). There were no differences in basophil count at baseline (p = 0.99; MD = 1.73; 95% CI = −15.79 to 19.26), day 10 (p = 0.99; MD = 2.93; 95% CI = −14.59 to 20.46), and the
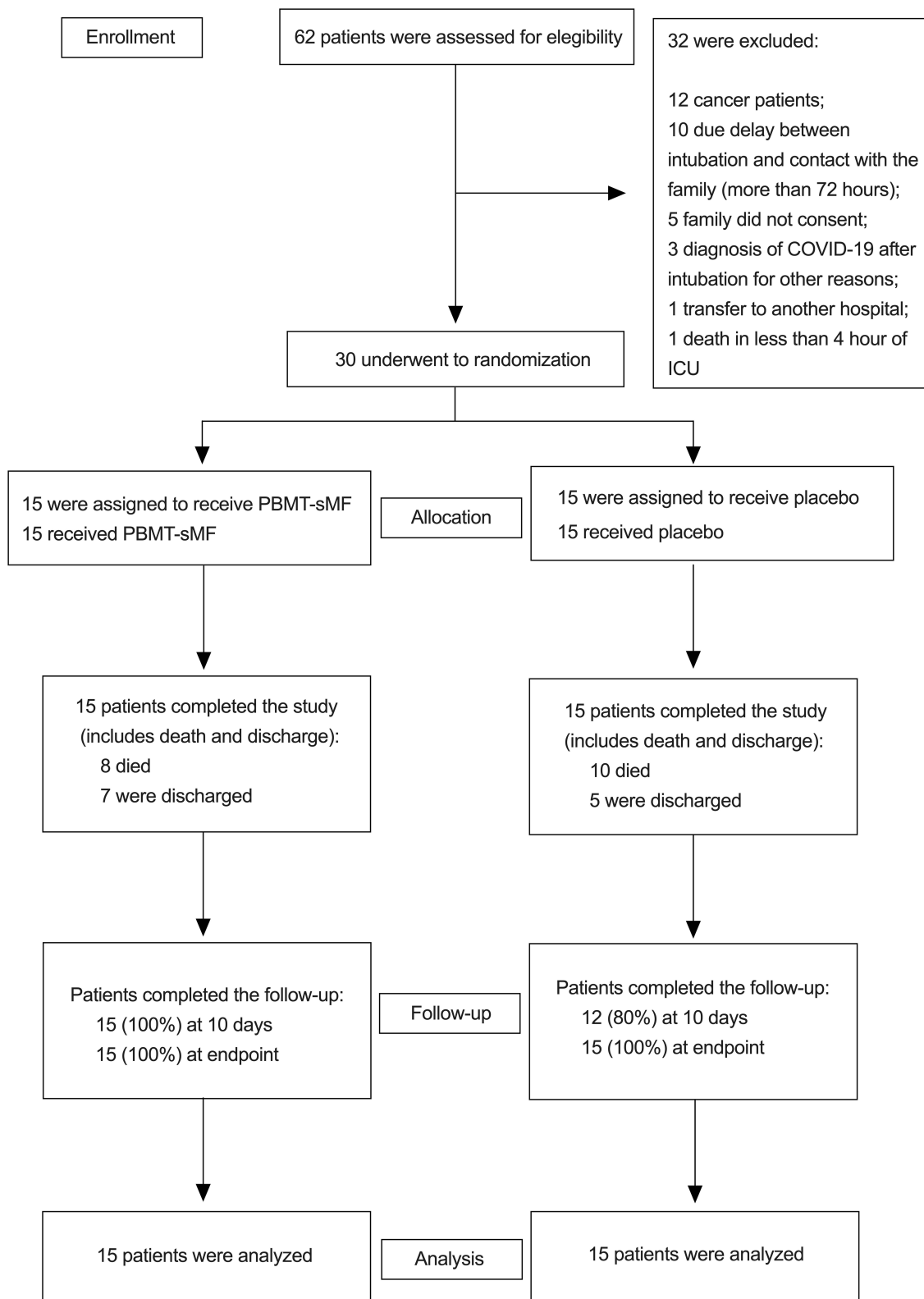
Figure 3 CONSORT flow diagram of the study. Figure 3 shows the flow diagram of the study including enrollment, randomization, intervention allocation, follow-up and data analysis of the two groups.

Table 1 Demographic and Clinical Characteristics of the Patients at Baseline

	Placebo (n = 15)	PBMT-sMF (n = 15)	P value
Age (years)	67.93 (11.68)	64.2 (18.67)	0.51
Gender (%) Female Male	8 6	6 8	
Body mass (Kg)	77.96 (18.49)	72.40 (16.94)	0.39
Height (cm)	165.06 (11.57)	168 (7.98)	0.42
SPAS 3	63.40 (13.43)	59.73 (13.24)	0.46
Comorbidities (%) Hypertension Diabetes Obesity Dementia Depression	9 (60.0) 8 (53.3) 4 (26.7) 3 (20.0) 2 (13.3)	5 (33.3) 4 (26.7) 3 (20.0) 2 (13.3) 0 (0.00)	

Notes: Categorical variables are expressed as number (%). Continuous variables are expressed as mean (SD).
Abbreviations: PBMT-sMF, photobiomodulation therapy combined with static magnetic field; SAPS 3, Simplified Acute Physiology Score 3.

endpoint (p = 0.99; MD = 4.33; 95% CI = −13.19 to 21.86) (Table 4). There were no differences in lymphocyte count at baseline (p = 0.87; MD = 418.9; 95% CI = −543 to 1381) and day 10 (p = 0.99; MD = 170.8; 95% CI = −791.1 to 1133), while the PBMT-sMF group showed

significantly higher lymphocyte count at the endpoint (p = 0.0125; MD = 1160.00; 95% CI = 197.90 to 2122.00) (Table 4). There were no differences in monocytes count at baseline (p = 0.99; MD = 104.2; 95% CI = −431.3 to 639.6), day 10 (p = 0.576; MD = 288.2; 95% CI = −247.2 to 823.6), and the endpoint (p = 0.571; MD = 289.2; 95% CI = −246.2 to 824.6) (Table 4). There were no differences in platelet count at baseline (p = 0.99; MD = 20,228; 95% CI = −83,748 to 124,204), day 10 (p = 0.99; MD = 23,967; 95% CI = −80,009 to 127,943), and the endpoint (p = 0.54; MD = 57,533; 95% CI = −46,443 to 161,509) (Table 4).

Sample Size Calculation for Future Randomized Controlled Trials

Based on the results of our primary outcome, we were able to determine the sample size required for future studies investigating the effects of PBMT-sMF in COVID-19 ICU patients requiring invasive mechanical ventilation.

The lengths of ICU stay of the placebo and PBMT-sMF groups were 23.06 ± 20.37 days and 16.26 ± 9.61 days, respectively. By considering a β-value of 20% and an α-value of 5%, the required sample size was 68 patients per group. This was calculated using the SPH™ Analytics website (https://www.sphanalytics.com/sample-size-calculator-using-average-values/).

Table 2 Hospitalization Outcomes

	Placebo	PBMT-sMF	Between Groups Comparisons	Mean Difference [95% CI]	Effect Size Cohen d
Length of stay in ICU (Days)	Total (n=15): 23.06 (± 20.37) Non-survivors (n=10): 20.4 (± 20.58) Survivors (n=5): 28.4 (± 21.12)	Total (n=15): 16.26 (± 9.61) Non-survivors (n=8): 18.65 (± 9.88) Survivors (n=7): 13.57 (± 9.25)	Total: p=0.25 Non-survivors: p=0.82 Survivors: p=0.12	Total: - 6.80 [−18.71 to 5.11] Non-survivors: −1.75 [−18.6 to 15.1] Survivors: −14.83 [−34.61 to 4.94]	Total: 0.4 (small) Non-survivors: 0.1 (small) Survivors: 0.9 (large)
Hospitalization length (Days)	Total (n=15): 24.33 (± 20.24) Non-survivors (n=10): 20.40 (± 20.58) Survivors (n=5): 32.20 (± 19.09)	Total (n=15): 18.33 (± 8.95) Non-survivors (n=8): 18.75 (± 10.08) Survivors (n=7): 17.85 (± 8.17)	Total: p=0.30 Non-survivors: p=0.83 Survivors: p=0.10	Total: −6.0 [−17.7 to 5.69] Non-survivors: −1.65 [−18.56 to 15.26] Survivors: −14.35 [−32.13 to 3.43]	Total: 0.4 (small) Non-survivors: 0.1 (small) Survivors: 0.9 (large)
Deaths/Discharges	10/5	8/ 7	p=0.46	———	———

Notes: Continuous variables are expressed as mean (standard deviation). Categorical variables are expressed as number.
Abbreviations: PBMT-sMF, photobiomodulation therapy combined with static magnetic field; ICU, intensive care unit; CI, confidence interval.

Table 3 Ventilatory Parameters

	Placebo (n = 15)			PBMT-sMF (n = 15)			Placebo vs PBMT-sMF		
	Baseline	Day 10	Endpoint	Baseline	Day 10	Endpoint	Between Groups Comparisons	Mean Difference [95% CI]	Effect Size Cohen d
Thickness fraction diaphragm (%)	25.28 (± 12.85)	22.90 (± 7.58)	23.76 (± 13.15)	29.55 (± 13.56)	37.09 (± 17.02)	44.22 (± 12.59)**	Baseline: p=0.99 Day 10: p=0.0117 Endpoint: p=0.0001	Baseline: 4.27 [−7.41 to 15.95] Day 10: 14.19 [2.50 to 25.87] Endpoint: 20.46 [8.77 to 32.14]	Day 10: 1.1 (large) Endpoint: 1.6 (large)
PEEP (cmH$_2$O)	9.13 (± 2.64)	8.93 (± 2.46)	8.20 (± 4.09)	9.00 (±1.96)	7.53 (± 2.82)	6.60 (± 2.82)	Baseline: p=0.99 Day 10: p=0.5540 Endpoint: p=0.3931	Baseline: −0.13 [−2.69 to 2.43] Day 10: −1.40 [−3.96 to 1.16] Endpoint: −1.60 [− 4.16 to 0.96]	Day 10: 0.5 (moderate) Endpoint: 0.5 (moderate)
PO$_2$ (mmHg)	91.46 (± 20.78)	99.74 (± 29.93)	92.74 (± 28.00)	108.22 (± 29.68)	106.46 (± 33.98)	109.29 (± 26.16)	Baseline: p=0.99 Day 10: p=0.99 Endpoint: p=0.34	Baseline: 16.76 [−8.64 to 42.16] Day 10: 6.72 [−18.68 to 32.12] Endpoint: 16.55 [−8.85 to 41.95]	Day 10: 0.2 (small) Endpoint: 0.6 (moderate)
FiO$_2$ (%)	69.66 (± 21.91)	63.20 (± 26.72)	75.46 (± 31.59)	62.33 (± 21.03)	51.26 (± 26.07)	51.53 (± 29.68)	Baseline: p= 0.33 Day 10: p=0.6590 Endpoint: p=0.0456	Baseline: −7.33 [−30.92 to 16.26] Day 10: −11.94 [−35.53 to 11.65] Endpoint:− 23.93 [−47.52 to −0.34]	Day 10: 0.5 (moderate) Endpoint: 0.8 (large)
PO$_2$/FiO$_2$ (mmHg)	146.71 (± 61.85)	183.32 (± 81.85)	158.26 (± 102.37)	199.65 (± 109.82)	261.28 (± 156.21)	276.15 (± 163.07)	Baseline: p=0.67 Day 10: p=0.2246 Endpoint: p=0.0233	Baseline: 52.94 [−53.63 to 158.50] Day 10: 77.95 [−27.63 to 183.50] Endpoint: 117.90 [12.31 to 223.50]	Day 10: 0.6 (moderate) Endpoint: 0.9 (large)

Notes: **Intragroup difference (PBMT-sMF) compared to the baseline (p<0.01). Continuous variables are expressed as mean (SD).
Abbreviations: PBMT-sMF, photobiomodulation therapy combined with static magnetic field; PEEP, positive end-expiratory pressure levels; PO$_2$, arterial partial pressure of oxygen; FiO$_2$, fraction of inspired oxygen; PO$_2$/FiO$_2$, ratio of arterial partial pressure of oxygen and fraction of inspired oxygen.

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Table 4 Biochemical Markers and Hemogram Parameters

	Placebo (n = 15)			PBMT-sMF (n = 15)			Between Groups Comparisons	Mean Difference [95% CI]	Effect Size Cohen d
	Baseline	**Day 10**	**Endpoint**	**Baseline**	**Day 10**	**Endpoint**			
CRP (mg/dL)	201.30 (± 65.39)	118.79 (± 71.00)	156.53 (± 124.49)	152.29 (± 108.88)	98.70 (± 95.24)	72.66 (± 75.40)	Baseline: p=0.41 Day 10: p=0.99 Endpoint: p=0.045	Baseline: -49.01 [−131.60 to 33.58] Day 10: −20.09 [−102.70 to 62.50] Endpoint: −83.87 [−166.50 to −1.28]	Day 10: 0.2 (small) Endpoint: 0.8 (large)
TNF-alpha (pg/mL)	17.23 (± 13.46)	12.28 (± 5.42)	17.05 (± 7.54)	19.71 (± 17.51)	18.40 (± 13.43)	14.19 (± 5.44)	Baseline: p=0.99 Day 10: p=0.44 Endpoint: p=0.99	Baseline: 2.48 [−7.71 to 12.68] Day 10: 6.11 [−4.08 to 16.32] Endpoint: -2.87 [−13.07 to 7.33]	Day 10: 0.5 (moderate) Endpoint:0.4 (small)
Vitamin D (ng/mL)	14.88 (± 5.13)	18.28 (± 9.95)	14.16 (± 4.80)	19.04 (± 10.83)	17.85 (± 6.67)	21.07 (± 7.07)	Baseline: p=0.43 Day 10: p=0.99 Endpoint: p=0.0502	Baseline: 4.15 [−2.75 to 11.07] Day 10: −0.42 [−7.34 to 6.48] Endpoint: 6.90 [−0.004 to 13.82]	Day 10: 0.1 (small) Endpoint:1.1 (large)
Erythrocytes (millions/mm^3)	4.20 (± 0.58)	3.73 (± 0.55)	3.40 (± 0.54)$^{###}$	3.75 (±0.64)	3.18 (± 0.59)	3.04 (± 0.90)**	Baseline: p=0.19 Day 10: p=0.065 Endpoint: p=0.389	Baseline: −0.45 [−1.02 to 0.13] Day 10: −0.55 [−1.13 to 0.02] Endpoint: −0.36 [−0.94 to 0.21]	Day 10: 0.9 (large) Endpoint: 0.5 (moderate)

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Table 4 (Continued).

	Placebo (n = 15)			PBMT-sMF (n = 15)			Between Groups Comparisons	Mean Difference [95% CI]	Effect Size Cohen d
	Baseline	**Day 10**	**Endpoint**	**Baseline**	**Day 10**	**Endpoint**			
Haemoglobin (g/dL)	12.57 (± 1.91)	11.47 (± 1.78)	9.97 (± 1.73)##	11.34 (± 2.14)	9.50 (± 1.55)*	9.03 (± 2.75)**	Baseline: p=0.29 Day 10: p=0.026 Endpoint: p=0.6173	Baseline: −1.23 [−3.03 to 0.56] Day 10: −1.97 [−3.77 to −0.17] Endpoint: −0.94 [−2.74 to 0.86]	Day 10: 1.2 (large) Endpoint: 0.4 (small)
Hematocrit (%)	36.92 (± 4.94)	32.66 (± 5.46)	30.57 (± 4.58)##	33.67 (± 5.04)	29.03 (± 4.04)	27.78 (± 7.20)**	Baseline: p=0.29 Day 10: p=0.194 Endpoint: p=0.459	Baseline: −3.25 [−7.98 to 1.48] Day 10: −3.62 [−8.36 to 1.10] Endpoint: −2.79 [−7.52 to 1.94]	Day 10: 0.8 (large) Endpoint: 0.5 (moderate)
Leukocytes (mm^3)	11,466.00 (± 6677.06)	14,221.92 (± 5231.44)	17,742.66 (± 11,630.20)	12,175.33 (± 9330.37)	23,449.13 (± 33,682.68)	23,186.66 (± 21,612.65)	Baseline: p=0.99 Day 10: p=0.476 Endpoint: p=0.99	Baseline: 709.30 [−15,147 to 16,566] Day 10: 9227 [−6629 to 25,083] Endpoint: 5444 [−10,412 to 21,300]	Day 10: 0.4 (small) Endpoint: 0.3 (small)
Segmented Neutrals (mm^3)	9890.16 (± 5904.32)	13,010.82 (± 3191.26)	13,655.49 (± 10,037.05)	9834.14 (± 7425.83)	10,154.81 (± 3450.12)	15,017.58 (± 11,566.73)	Baseline: p=0.99 Day 10: p=0.91 Endpoint: p=0.99	Baseline: −56.02 [−6836 to 6724] Day 10: −2856 [−9636 to 3924] Endpoint: 1362 [−5418 to 8142]	Day 10: 0.9 (large) Endpoint: 0.1 (small)

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Table 4 (Continued).

	Placebo (n = 15)			PBMT-sMF (n = 15)			Between Groups Comparisons	Mean Difference [95% CI]	Effect Size Cohen d
	Baseline	**Day 10**	**Endpoint**	**Baseline**	**Day 10**	**Endpoint**			
Eosinophiles (mm³)	47.43 (± 90.35)	19.41 (± 29.58)	1070.21 (± 2825.97)#	49.73 (± 108.99)	115.11 (± 114.62)	223.86 (± 229.56)	Baseline: p=0.99 Day 10: p=0.99 Endpoint: p=0.146	Baseline: 2.30 [−1032 to 1037] Day 10: 95.70 [−939 to 1130] Endpoint: - 846.40 [−1881 to 188.3]	Day 10: 1.1 (large) Endpoint: 0.4 (small)
Basophils (mm³)	5.84 (± 6.07)	7.83 (± 11.16)	18.54 (± 27.53)	7.57 (± 11.23)	10.77 (± 18.75)	22.88 (± 30.30)	Baseline: p=0.99 Day 10: p=0.99 Endpoint: p=0.99	Baseline: 1.73 [−15.79 to 19.26] Day 10: 2.93 [−14.59 to 20.46] Endpoint: 4.33 [−13.19 to 21.86]	Day 10: 0.2 (small) Endpoint: 0.2 (small)
Lymphocyte (mm³)	764.31 (± 415.39)	1138.70 (± 484.81)	1554.00 (± 1072.52)	1183.26 (±1224.40)	1309.53 (± 560.02)	2713.83 (± 1899.26)**, $*	Baseline: p=0.87 Day 10: p=0.99 Endpoint: p=0.0125	Baseline: 418.9 [−543 to 1381] Day 10: 170.8 [−791.1 to 1133] Endpoint: 1160.00 [197.90 to 2122.00]	Day 10: 0.3 (small) Endpoint: 0.8 (large)
Monocytes (mm³)	557.06 (± 459.19)	936.71 (± 499.14)	1057.01 (± 646.43)	661.23 (± 542.04)	1224.89 (± 496.07)*	1346.20 (± 862.56)**	Baseline: p=0.99 Day 10: p=0.576 Endpoint: p=0.571	Baseline: 104.2 [−431.3 to 639.6] Day 10: 288.2 [−247.2 to 823.6] Endpoint: 289.2 [−246.2 to 824.6]	Day 10: 0.6 (moderate) Endpoint: 0.4 (small)

(*Continued*)

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Table 4 (Continued).

	Placebo (n = 15)			PBMT-sMF (n = 15)			Between Groups Comparisons	Mean Difference [95% CI]	Effect Size Cohen d
	Baseline	Day 10	Endpoint	Baseline	Day 10	Endpoint			
Platelets (mm³)	189,800.00 (± 92,499.57)	237,890.86 (± 71,588.08)	231,266.66 (± 104,779.67)	210,028.26 (± 90,254.29)	261,858.03 (± 145,159.59)	288,800.00 (± 166,270.09)	Baseline: p=0.99 Day 10: p=0.99 Endpoint: p=0.54	Baseline: 20,228 [−83,748 to 124,204] Day 10: 23,967 [−80,009 to 127,943] Endpoint: 57,533 [−46,443 to 161,509]	Day 10: 0.2 (small) Endpoint: 0.4 (small)

Notes: *Intragroup difference (PBMT-sMF) compared to the baseline (p<0.05); $^{\$}$*Intragroup difference (PBMT-sMF) compared to the Day 10 (p<0.01); **Intragroup difference (PBMT-sMF) compared to the baseline (p<0.01); #Intragroup difference (Placebo) compared to the baseline (p<0.05); ##Intragroup difference (Placebo) compared to baseline (p<0.01). Continuous variables are expressed as mean (standard deviation).
Abbreviations: PBMT-sMF, photobiomodulation therapy combined with static magnetic field; CI, confidence interval; CRP, C-reactive protein; TNF-alpha, tumor necrosis factor.

Discussion

This is the first randomized, triple-blinded, placebo-controlled trial evaluating the effects of PBMT-sMF in severe COVID-19 patients requiring mechanical ventilation. The length of ICU stay was observed to be shorter in the PBMT-sMF group than in the placebo group, but with no significant difference. In addition, the PBMT-sMF group demonstrated increased diaphragm thickness and the improvement in several ventilatory parameters and biochemical markers.

Although there is speculation about the possible benefits of PBMT (isolated or combined with sMF) in COVID-19 patients,[53] our study is the only randomized controlled trial on this topic to date. Several clinical studies with low levels of evidence have reported on the use of PBMT in COVID-19 patients. Two case reports described the reduction of inflammatory markers such as IL-6, ferritin, and CRP with the use of PBMT in severe COVID-19 patients. Moreover, improvement in radiological findings, reduction in lung involvement, and increase in oxygen saturation have been observed.[54,55] A small open label study (n = 10) demonstrated that patients treated with PBMT showed improvement in pulmonary indices such as the SMART-COP score for pneumonia severity, the Brescia-COVID respiratory severity scale (BCRSS) score, the radiographic assessment of lung edema (RALE) scale score, and the Community-Acquired Pneumonia (CAP)

score, in addition to a rapid recovery from COVID-19.[56] Although the aforementioned studies were carried out in severe COVID-19 patients, the profile of patients treated in our study was different, as we included only severely ill COVID-19 ICU patients requiring orotracheal intubation due to respiratory failure. In addition, the measured outcomes, irradiation protocols, and PBMT parameters involved in previous studies were different to those applied in our randomized controlled trial. Finally, isolated PBMT, instead of PBMT-sMF, were employed in previous studies. Heterogeneity in these characteristics, therefore, limited the direct comparison among the studies.

In our study, we observed that the use of PBMT-sMF not only increased muscle thickness and muscle strength,[57–59] but also prevented atrophy due to disuse,[34] as previously observed in skeletal muscles. In addition, there is evidence that PBMT modulates inflammatory processes by decreasing the CRP levels in skeletal muscles,[60,61] which was also observed in our study. With regards to treatments other than PBMT, it has been shown that the time taken for patients treated with lopinavir-ritonavir to achieve clinical improvement was similar to that for patients treated with standard care alone.[9] In addition, the time to achieve clinical improvement within 28 days following treatment with combined convalescent plasma therapy and standard treatment was similar to those following standard treatment alone.[62] In

contrast, patients treated with remdesivir showed shorter recovery times than those treated with placebo.[10] In our trial, patients treated with PBMT-sMF also had shorter recovery times, but with no significant difference compared to the placebo group, probably due to the small sample size. It is important to highlight that we included only ICU patients requiring invasive mechanical ventilation, which is distinct from the aforementioned studies.[9,10,62] Finally, we did not observe any adverse effects with the use of PBMT-sMF, while treatment involving convalescent plasma therapy, lopinavir-ritonavir, and remdesivir have been associated with adverse effects such as gastrointestinal events, chills, rashes, acute kidney injury, and even severe respiratory failure.[9,10,62]

PBMT-sMF associated with improvements in the ventilatory parameters, inflammatory and infectious processes, and immune responses of severe COVID-19 patients requiring mechanical ventilation. It was previously observed that such patients presented with elevated ventilation-perfusion mismatch due to high dead space fraction.[63] In our study, higher ventilation/perfusion ratio with preservation and improvement of diaphragm thickness were observed in patients treated with PBMT-sMF. These improvements possibly led to decreased inflammation and infection (as reflected by CRP level), which enhanced the immune response (as reflected by lymphocyte count). These findings suggest that the preservation of the main respiratory muscle may trigger a cascade of positive effects and improve the clinical condition of patients. Furthermore, patients in the PBMT-sMF group also demonstrated shorter lengths of ICU stay, suggesting that PBMT-sMF may reduce the burdens of both the hospital and the healthcare system and minimize the use of scarce healthcare resources during the pandemic. In addition, PBMT-sMF has proven to be safe, with no adverse effects observed. In addition, the mortality rate of the PBMT-sMF group was lower than that of the placebo group, although with no statistical significance.

This trial was prospectively registered. True randomization, concealment allocation, and blinding of the therapists, outcome assessors, and patients were employed. Statistical analysis was conducted following the intention-to-treat principle and was performed by a researcher blinded to the treatment allocations. Moreover, a placebo group was used to control for confounders such as the placebo effect, regression to mean, and natural recovery. Although attempts were made to minimize bias, this study had several limitations. There were two deviations from the registered protocol. First, we estimated that the study endpoint would be up to 20 days

after randomization. However, as the endpoint directly depended on the length of ICU stay, the study period of some cases extended beyond 20 days. The second deviation involved the lack of evaluations on IgG, IgM, and D-dimer levels due to failures at the laboratory in charge in implementing the necessary routines before the beginning of our trial. In addition, the lack of post-discharge follow-ups and the small sample size were other limitations of our study.

This trial is the first to assess the effects of PBMT-sMF in severe COVID-19 patients; therefore, the required sample size for precise comparison of the primary outcome was unknown a priori. However, this study presents as a guide for the estimation of appropriate sample sizes for future studies with length of ICU stay as a primary outcome. Based on our results, we estimated that 68 patients per group would be required in future studies to detect a significant difference. Therefore, rigorous methodological quality assessments and adequate sample sizes are warranted to investigate whether PBMT-sMF decreases the length of ICU stay in severe COVID-19 ICU patients requiring invasive mechanical ventilation. In addition, post-ICU discharge follow-ups should be considered in future randomized controlled trials to observe the medium- and long-term effects, as well as the adverse effects, of PBMT-sMF in severe COVID-19 patients. Moreover, future studies should investigate whether modifying the power output of the ultrasound device while measuring for diaphragm thickness could increase the effect size of PBMT-sMF. Finally, different treatment protocols should be trialed to achieve better results with the use of PBMT-sMF in severe COVID-19 patients.

Conclusion

PBMT-sMF failed to show superiority to the placebo in decreasing the length of ICU stay in severe COVID-19 patients requiring invasive mechanical ventilation, although our sample size may have been underpowered to detect a significant difference. However, PBMT-sMF is associated with increased diaphragm thickness, PO_2/FiO_2 ratio, and lymphocyte count and decreased FiO_2, CRP levels, and hemoglobin count.

Abbreviations

COVID-19, Coronavirus disease; CRP, C-reactive protein; IL, Interleukin; TNF, Tumor necrosis factor; ICU, Intensive care unit; PBMT, Photobiomodulation therapy; sMF, Static magnetic field; LASER, Light amplification by stimulated emission of radiation; LEDs, light-emitting diodes; ATP, Adenosine triphosphate; PBMT-sMF, Photobiomodulation

therapy combined with static magnetic field; IgG, Immunoglobulin G; IgM, Immunoglobulin M; RT-PCR, Reverse transcription-polymerase chain reaction; PEEP, Positive end-expiratory pressure; SAPS 3, Simplified Acute Physiology Score 3; FiO$_2$, Fraction of inspired oxygen; PO$_2$, Arterial partial pressure of oxygen; PO$_2$/FiO$_2$ ratio, Ratio of arterial partial pressure of oxygen and fraction of inspired oxygen; SD, Standard deviation; MD, Mean difference; CI, Confidence interval; BCRSS, Brescia-COVID respiratory severity scale; RALE, Radiographic assessment of lung edema; CAP, Community-Acquired Pneumonia.

Data Sharing Statement
The datasets used and/or analyzed in the study are available from the corresponding author on reasonable request.

Ethics Approval and Informed Consent
This study was submitted and approved by the Research Ethics Committee of Associação Dr. Bartholomeu Tacchini/Hospital Tacchini/RS (protocol number: 3,985,226), and by the National Research Ethics Commission of the Brazilian Ministry of Health (protocol number: 4,021,485). All eligible patients or their legal representatives (if the patients were too unwell to provide consent) were informed of the study objectives and provided written informed consent before enrollment.

Disclosure
Professor Ernesto Cesar Pinto Leal-Junior receives research support from Multi Radiance Medical (Solon, OH, USA), a laser device manufacturer. Professor Ernesto Cesar Pinto Leal-Junior also has a patent Laser therapy for patients requiring mechanical ventilation pending to Multi Radiance Medical. Shaiane Silva Tomazoni has a personal relationship with Ernesto Cesar Pinto Leal-Junior. Multi Radiance Medical had no role in the planning, conducting, and analysis of the data. The remaining authors declare that they have no support from any organizations for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the past three years; and no other relationships or activities that could appear to have influenced the submitted work.

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