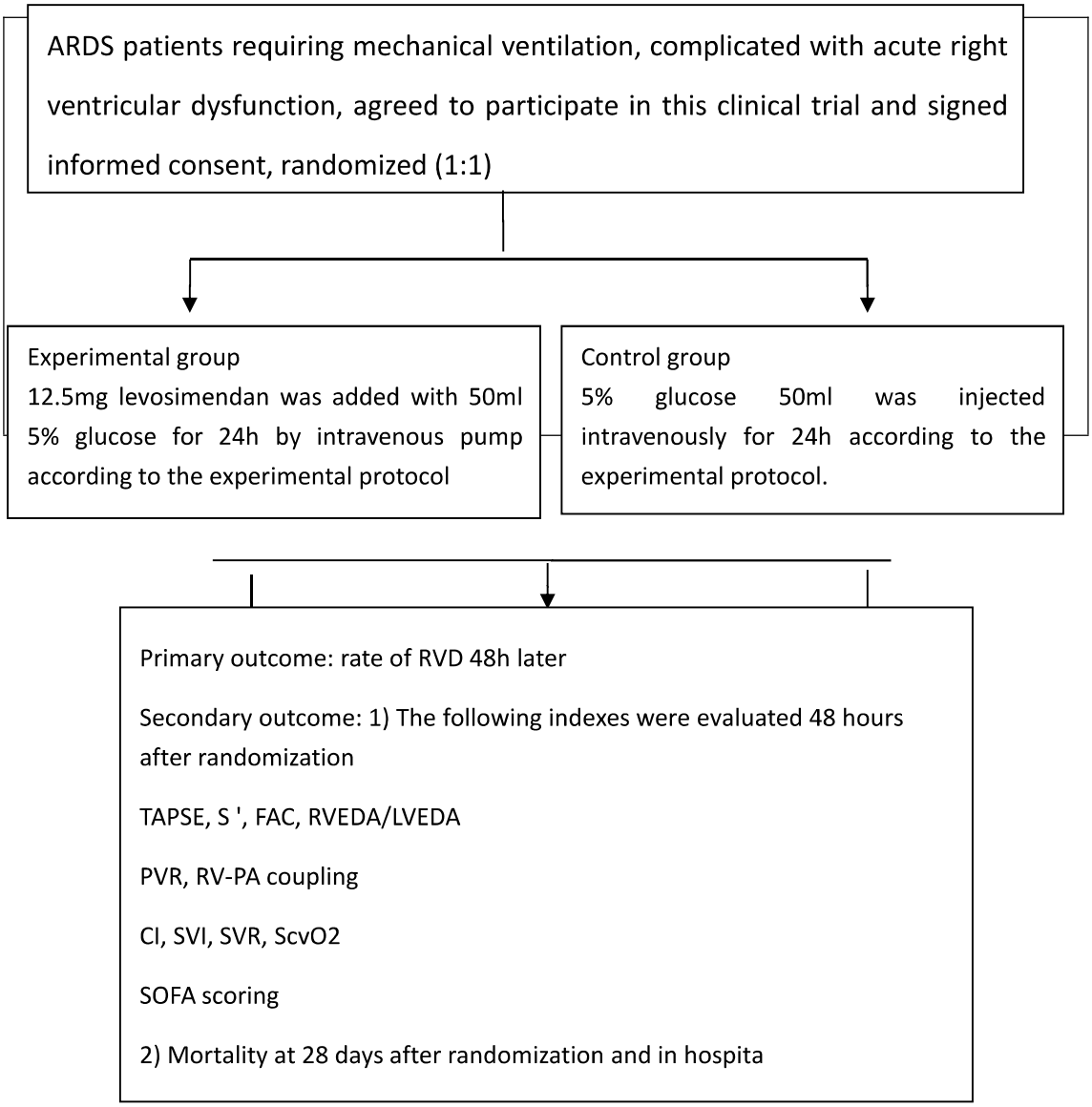
project title	Using transesophageal echocardiography to evaluate the effect of levosimendan on patients with acute respiratory distress syndrome associated with right ventricular dysfunction during mechanical ventilation
numbering scheme	ARVD20220810
version number	V1
Version date	20220810
major investigators	Xiaojing zou
Estimated starting and ending date	2022.12.01-2025.12.01

Project abstract	
Research title	Using transesophageal echocardiography to evaluate the effect of levosimendan on patients with acute respiratory distress syndrome associated with right ventricular dysfunction during mechanical ventilation
Introduction	Acute respiratory distress syndrome (ARDS) is often complicated by right ventricular dysfunction (RVD),the incidence can be as high as 64%, Acute cor pulmonale is the most serious form of ARDS complicated with RVD. The prognosis of ARDS patients with RVD is worse and the risk of death would be significantly increased.Currently, there are very limited clinical drug treatment options for RVD.Levosimendan is indicated for short-term treatment of acute decompensated heart failure that is not responding well to conventional therapy and requires increased myocardial contractile force.In 2016, the European Society of Cardiology issued recommendations for the management of acute right heart failure, stating that levosimendan can improve right ventriculo-pulmonary artery coupling by both increasing right heart contractility and reducing pulmonary vascular resistance. Levosimendan is therefore recommended to be given priority over dobutamine in the treatment of acute right heart failure.At present, there are also relevant clinical studies reporting that levosimendan can be used in acute right heart failure caused by different causes, including a single center randomized controlled study reporting that levosimendan can do work in the right ventricle of patients

	with sepsis ARDS, and has beneficial hemodynamic effects.However, the clinical application of levosimendan in the treatment of ARDS right heart dysfunction is insufficient.Therefore, this study intends to use transesophageal ultrasound to evaluate right ventricular function, reduce the limitation of poor right ventricular window in transthoracic echocardiography, and conduct a multi-center randomized controlled study to further explore the effects of levosimendan on right ventricular function in ARDS patients, such as tricuspid ring systolic displacement (TAPSE) and tricuspid ring systolic displacement velocity (S '). Effects of right ventricular area change fraction (RV FAC), right ventricular end-diastolic area/left ventricular end-diastolic area (RVEDA/LVEDA), pulmonary circulation resistance (PVR), RV-PA coupling, hemodynamics and mortality.
Study objective	To investigate the effects of levosimendan on the coupling of RVD, PVR, RV-PA, hemodynamics and mortality of ARDS patients with mechanical ventilation.
Study design	Prospective, multicenter, randomized controlled, clinical study.
Total number of patients enrolled	A total of 58 subjects are expected to be enrolled
Number of study groups Number of control groups	There were 29 cases in the experimental group and 29 cases in the control group.
diagnostic criteria	The diagnosis of ARDS is based on the 2012 Berlin definition

Inclusion criteria	1) Patients who agree to participate in this clinical trial and sign informed consent; 2) Age 18-80 years old, gender unlimited; 3) Patients with ARDS requiring mechanical ventilation; 4)Acute right ventricular dysfunction due to ARDS；
Exclusion criteria	1) Pregnant or lactating women; (2) Chronic cardiac dysfunction, pulmonary hypertension and/or right ventricular enlargement caused by chronic cardiopulmonary disease, cardiogenic shock or after major cardiac surgery before ARDS; 3) right ventricular myocardial infarction; 4) Uncorrected hypotension, hypoxemia and hypercapnia, or acid-base balance disturbance 5) Mechanical ventilation driving pressure >=18cmH2O before randomization 6) Rapid arrhythmia; 7) pericardial tamponade; 8) Pulmonary embolism; 9) severe renal insufficiency; 10) severe liver insufficiency; 11) Failure to sign informed consent; 12) known allergy to the test drug; 13) Patients who have participated in other clinical trials within 30 days
Intervention	Patients will be randomized to treatment group A: intravenously injected levosimendan 12.5mg with 5% glucose injection 50ml configuration at 2ml/h for 24h, or treatment group B: control group with 5% glucose injection 2ml/h for 24h.

Evaluation criteria: Primary endpoint: Secondary end point: Safety evaluation	Primary endpoint: The rate of RVD occurrence within 48 hours after randomization. secondary end points: 1) Changes in the following indexes were assessed 48h after random administration including : TAPSE, S ', RV FAC, RVEDA/LVEDA PVR, RV-PA coupling, CI, SVI, SVR, ScvO2 SOFA scoring 2) Mortality at 28 days after randomization and in hospital
Statistical Methods：	Sample size: 23 cases in the experimental group and 23 cases in the control group. Considering the 20% shedding rate, the statistical sample size was 29 cases in the experimental group and 29 cases in the control group, and a total of 58 subjects were enrolled. Primary endpoints: incidence of RVD 48h after randomization Safety endpoints: Safety endpoints are those where subjects have severe adverse reactions related to the drug that are persistent and cannot be corrected according to the protocol
Study duration	2023.02-2025.12
Subject attendance time	From the time subjects were recruited to the time each subject completed all follow-up visits
Research unit/location	Wuhan Union Medical College Hospital, the First Affiliated Hospital of Zhengzhou University, Henan Provincial People's Hospital
Name, qualifications and contact information of the principal investigator	
	Zou Xiaojing, 249126734@qq.com, has obtained the GCP Certificate



The list of abbreviations	
abbreviations	Benelux
ARDS	acute respiratory distress syndrome
RVD	Right ventricular dysfunction
TEE	Transesophageal ultrasound
FAC	Right ventricular area change fraction
PVR	Pulmonary circulation resistance
RV-PA	Right ventriculo-pulmonary artery coupling

1. Introduction

1.1 Background

1.1.1 General Overview

Acute Respiratory Distress Syndrome (ARDS) is often complicated with Right Ventricular Dysfunction, Acute pulmonary heart disease is the most serious form of ARDS complicated with RVD. The pathogenesis includes pulmonary vascular dysfunction and right ventricular systolic dysfunction. Excessive inflammation, hypoxic pulmonary vasoconstriction, hypercapnia, pulmonary hyperventilation or collapse leading to increased pulmonary vascular resistance, pulmonary capillary microthrombosis and pulmonary vascular remodeling are important factors leading to ARDS right heart dysfunction. Meta-analysis has shown that patients with ARDS combined with RVD have worse prognosis and significantly increased risk of death, and RVD is not an independent risk factor for death in patients with COVID-19 ARDS [1-3]. RVD also occurs frequently in patients with COVID-19 related ARDS, and its incidence is even higher than the incidence of left heart dysfunction, and the fatality rate of patients with RVD increases significantly from 30.6% to 56.3%[4,5]. Despite the implementation of right cardiac protective mechanical ventilation, the incidence of RVD in ARDS patients is still as high as 21%[6]. It can be seen that the adjustment of mechanical ventilation strategy cannot completely solve RVD in ARDS patients. RVD can often lead to low cardiac displacement and hemodynamic failure in patients, resulting in increased mortality [7].

Currently, there are very limited clinical drug treatment options for RVD. The clinical indication for the use of levosimendan is for short-term treatment of acute decompensated heart failure that does not respond well to conventional therapy and requires increased myocardial contractile force. In 2016, the European Association of Cardiology issued recommendations on the management of acute right heart failure, indicating that levosimendan can improve right ventriculo-pulmonary artery coupling by increasing the right heart contractile force and reducing pulmonary vascular resistance, so it is recommended that levosimendan should be given priority over

dobutamine in acute right heart failure [8]. At present, relevant clinical studies have reported that levosimendan can be used in acute right heart failure caused by different causes, including a single-center randomized controlled study that reported that levosimendan can do work in the right ventricle of patients with septic ARDS, and has beneficial hemodynamic effects [9,10]. However, there is limited clinical experience in the treatment of right-heart dysfunction in patients with ARDS on mechanical ventilation and lack of high-quality clinical evidence. Therefore, multi-center randomized controlled studies are needed to obtain more and higher level of clinical evidence support, so it is necessary to carry out this clinical study.

In addition, the ratio of right ventricular end-diastolic area to left ventricular end-diastolic area (RVEDA/LVEDA) was used to evaluate ARDS RVD in the past, but this index cannot fully reflect right ventricular dysfunction. Right Ventricular Fractional Area Change (RV FAC) is significantly correlated with right ventricular end-diastolic area, and is more reflective of right ventricular systolic function [11]. In addition, lateral systolic displacement (TAPSE) of the tricuspid ring is also an important indicator of right ventricular systolic function. Therefore, in this study, RV expansion (RVEDA/LVEDA ＞ 0.6), TAPSE ＜ 16mm, tricuspid ring systolic S 'velocity (TS ＜'10cm/s) or RV FAC＜;35% represented RVD, and the incidence of RVD was used as the primary outcome indicator. Due to the poor right voice window in patients with mechanical ventilation, the evaluation of right heart function is limited. Therefore, from the perspective of practicality and operability in critical care medicine, this study uses transesophageal ultrasound to minimize the influence of lung gas on the right ventricular window, evaluates the right ventricular function in patients with mechanical ventilation ARDS, and discusses the influence of levosimendan on ARDS RVD and its relationship with prognosis, hoping to provide evidence support and useful reference for drug treatment of ARDS associated RVD.

1.2 Research Types

Prospective, multicenter, randomized controlled study.

1.3 Research Basis

Clinical studies have shown that inhaled pulmonary vasodilators such as NO and prostaglandins can reduce pulmonary circulatory resistance, improve pulmonary ventilation/perfusion ratio, and improve right cardiac function in ARDS patients [12]. Levosimendan is a calcium-sensitizer that relaxes blood vessels by increasing myocardial contractility while opening up adenosine triphosphate (ATP) -sensitive potassium channels in smooth muscle cells. Therefore, theoretically, levosimendan can improve the function of the right heart by increasing the systolic force of the right heart and reducing the Pulmonary Vascular Resistance (PVR) at the same time, and levosimendan has no significant effect on the ratio of pulmonary/systemic resistance [13]. Levosimendan has been widely used in acute decompensated left heart failure. Levosimendan has been shown to reduce right ventricular afterload and improve right cardiac function in animal models of right heart failure caused by acute pulmonary hypertension such as hypoxic pulmonary constriction, pulmonary artery ligation, and pulmonary embolism. In addition, levosimendan can also increase coronary blood flow, and has anti-proliferative and anti-inflammatory effects in animal models of chronic pulmonary hypertension, which can reduce pulmonary vascular remodeling [9,10]. Therefore, we speculate that levosimendan may be a beneficial clinical choice whether RVD occurs in the early or late stage of ARDS, and whether RVD in ARDS is due to impaired right heart function or elevated pulmonary circulation resistance.

At present, only one small sample single-center randomized controlled study has reported that levosimentan can relax the pulmonary vessels in patients with sepsis ARDS, improve the right ventricular - pulmonary artery (RV-PA) coupling in patients with pulmonary hypertension, improve the right ventricular work, and have beneficial hemodynamic effects [14]. However, the limitation of this study is single-center, small sample size, and MRI detection of right heart function may lead to some severe ARDS patients undergoing mechanical ventilation therapy unable to go out for cardiac MRI examination, resulting in selection bias. Therefore, this study intends to use transesophageal ultrasound to evaluate right heart function, reduce the limitation of poor right voice window in transthoracic echocardiography, and conduct a multicenter randomized controlled study to further explore the effects of

levosimendan on RVD, PVR[15], RV-PA coupling, hemodynamics and mortality in ARDS patients [16].

1.4 Risk/Benefit assessment

1.4.1 Known potential risks

Levosimentan is a marketed drug, which has been widely used in clinical treatment of acute and chronic heart failure, as well as some types of acute right heart failure in severe patients [10]. The purpose of this study was to observe the effects of levosimentan on right heart function in patients with ARDS on mechanical ventilation, except for possible adverse reactions in the drug instructions, and the drug itself does not pose risks to patients. The contents of the study were all within the scope of routine diagnosis and treatment for patients with mechanical ventilation, and blood drawing examination was also a routine examination item for patients with severe illness, so the frequency of examination would not be increased. In this study, sampling and transesophageal ultrasound were conducted to monitor right heart function, which was completed during the sedation and analgesia of patients with mechanical ventilation, without causing additional psychological or physical pain to patients. Transesophageal ultrasound was performed by clinicians participating in the study, so there was no additional cost.

1.4.2 Probability of injury

the possibility of subject harm may involve adverse effects associated with levosimendan. Clinically possible adverse effects are increased heart rate or temporary and slight decrease in blood pressure, which can be treated with dose reduction or withdrawal and symptomatic management.

1.4.3 Degree of injury

An increase in heart rate or a temporary slight drop in blood pressure is considered mild injury and can be treated appropriately.

1.4.4 Known potential benefits

The occurrence of ARDS related right heart failure is not uncommon, and the

combination of acute right heart failure will lead to hemodynamic failure and significantly increase mortality in mechanical ventilation ARDS patients. At present, there is no clear and effective drug for ARDS right heart failure in clinical practice, and there is also a lack of relevant clinical evidence. The results of this study will provide certain clinical evidence for drug treatment of right heart failure in patients with mechanical ventilation ARDS, and may even help to reduce the mortality of ARDS patients and improve the prognosis.

Of course, the study design for this subject will minimize the need for subjects to be exposed to risk. First, avoid all contraindications of levosimondan use in the drug label and exclude patients who already have rapid arrhythmia or hypotension. The management of possible risks has been elaborated in the medication procedure. Vital signs of patients will be monitored in real time throughout the experiment, and adverse reactions will be handled according to the experimental procedure to minimize risks.

1.4.6 Discussion

ARDS is often complicated by right ventricular dysfunction (RVD), the incidence of which can be as high as 64%. The pathogenesis includes elevated pulmonary vascular resistance and right ventricular systolic dysfunction [1-3]. The fatality rate of ARDS patients with RVD increased significantly from 30.6% to 56.3%[4,5]. For ARDS patients with right heart failure, currently available drugs include dobutamine and Milrinone, but dobutamine has a high risk of rapid malignant arrhythmia, increases myocardial oxygen consumption, and large doses will increase the proportion of body and lung circulation, leading to systemic hypotension, which is not conducive to the treatment of ARDS patients with right heart failure [14]. Milrinone also has the effect of increasing myocardial contractility, but its application is limited due to systemic hypotension [14]. While levosimendan increases myocardial contractility, it does not increase myocardial oxygen consumption and does not lead to malignant arrhythmias. Many animal experiments have shown that levosimendan can reduce pulmonary circulation resistance, protect myocardium

during myocardial ischemia, improve right ventricular/pulmonary artery coupling, and improve myocardial energy metabolism. Compared with dobutamine and Milrinone commonly used in clinical practice, levosimendan has more advantages and has fewer side effects on patients [14]. Therefore, the purpose of this study is to provide a more powerful research basis for the use of levosimendan in ARDS patients with right heart failure. Because the drug itself has few side effects of arrhythmia and hypotension, and the protocol has fully considered the management of adverse reactions, the risk to subjects will be minimized even if there is no clinical benefit.

2. Study objective/end point

The main objective is to (confirm) about whether or not the leosimendan improve mechanical ventilation in patients with ARDS right heart function.

secondary objective is to exploratory whether or not levosimendan reduces mechanical ventilation in patients with ARDS mortality

Primary endpoint: the incidence of RVD after 48h ending of randomization

Secondary endpoint: (1): TAPSE, S ', FAC, RVEDA/LVEDA PVR, RV-PA coupled CI, SVI, SVR, ScvO2 SOFA score after 48h ending of randomization. (2) 28-day mortality rate, in-hospital mortality rate.

3. Research design

3.1 Overall Design

Prospective, multicenter, randomized controlled study. According to the random allocation method, the subjects were divided into experimental group and control group; The control group was injected with 50ml 5% glucose for 24h, and the experimental group was injected with 12.5mg levosimendan for 24h according to the experimental protocol. Right ventricular function was compared between the two groups before and 48h after medication: Right ventricular area change fraction (RV FAC), tricuspid annulus systolic displacement (TAPSE), tricuspid annulus systolic S 'velocity (S'), right ventricular end-diastolic area/left ventricular end-diastolic area (RVEDA/LVEDA), pulmonary circulation resistance (PVR), right ventricular - pulmonary artery coupling (RV-PA), heart index (CI), Per Bo index (SVI), systemic

resistance (SVR), central venous oxygen saturation (ScvO2). SOFA score of patients with mechanical ventilation ARDS treated with levosimendan 48h after medication was evaluated, mortality rate at random 28 days, and all-cause in-hospital mortality.

3.2 Sample size

After discussion by clinical experts and combined with the literature results, it is expected that the mean difference and standard deviation between the experimental group and the control group in this study are 20% and 20% respectively. α=0.05 (bilateral), power=0.9, the proportion of experimental group and control group was 1:1, the number of cases meeting the statistical requirements of the experimental group and control group were 23 cases each. Considering the 20% shedding rate, the statistical sample size was 29 cases in the experimental group and 29 cases in the control group, and a total of 58 subjects were enrolled.

4. Study population

4.1 Diagnostic Criteria

According to the diagnostic criteria of ARDS in Berlin 2012

4.2 Inclusion Criteria

Each subject must meet all inclusion criteria to be eligible to participate in the study.

(1) Patients who have agreed to participate in the clinical trial and signed informed consent;

(2) Age 18-80 years old, gender unlimited;

(3) Patients with ARDS requiring mechanical ventilation;

(4) Acute right ventricular dysfunction due to ARDS;

4.3 Exclusion Criteria

All baseline subjects meeting any of the exclusion criteria will be excluded from the study.

(1) Pregnant or lactating women;

(2) Chronic cardiac insufficiency, pulmonary hypertension and/or right ventricular

enlargement caused by chronic cardiopulmonary disease, cardiogenic shock or after major cardiac surgery before ARDS;

(3) right ventricular myocardial infarction;

(4) Uncorrected hypotension, hypoxemia and hypercapnia, or acid-base balance disturbance;

(5) pre-randomized mechanical ventilation drive pressure ⩾18cmH2O;

(6) rapid arrhythmia;

(7) pericardial tamponade;

(8) Pulmonary embolism;

(9) Severe renal insufficiency;

(10) Severe liver insufficiency;

(11) Failure to sign informed consent;

(12) known allergy to the test drug and control drug;

(13) Patients who have participated in other clinical trials within 30 days;

4.4 Grouping method of subjects

Patients will be randomized to treatment group A: intravenously injected levosimendan 12.5mg with 5% glucose injection 50ml configuration at 2ml/h for 24h, or treatment group B: control group with 5% glucose injection 2ml/h for 24h.

The randomization method of the experiment was block randomization and stratification according to the center. The statistical specialty used SAS 9.4 software to generate the blind base of the treatment group randomized according to the 1:1 allocation ratio between the experimental group and the control group. According to the enrollment sequence of patients, random numbers were issued from small to large and groups were assigned. The random number is reproducible, and the set parameters such as the number of centers, section length and seed number are recorded in the blind base.

4.5 Criteria for subject withdrawal

Early withdrawal All patients who fill in the informed consent form and are screened for qualified randomized entry into the trial are referred to as shedding cases

(shedding and excluding cases should be controlled below 20% of the total number of cases), no matter when they quit for any reason, as long as they do not complete the observation period specified in the scheme. The reasons are as follows: (1) The investigator considered it most beneficial for the patient to terminate treatment due to safety concerns (such as adverse events); (2) Serious protocol violations (any protocol violations will be assessed by the investigator, who will consult the principal investigator of the group leader unit to determine whether they are serious enough to warrant withdrawal from the study); (3) Patients voluntarily terminate the study. Patients are free to terminate the study under any circumstances and will not be discriminated against in their subsequent treatment.

5. Research interventions

5.1 Description of the study intervention

Patients will be randomized to treatment group A: intravenously injected levosimendan 12.5mg with 5% glucose injection 50ml configuration at 2ml/h for 24h, or treatment group B: control group with 5% glucose injection 2ml/h for 24h.

Only when the clinician is satisfied that adequate fluid resuscitation has been achieved can the drug be investigated for pumping. Both groups were injected at a constant rate of 2ml/h starting at 8am after randomization. If there was no significant hypotension or tachycardia, the injection was completed at 24h. Any incipient hypotension can be treated with fluid replacement or/and pressors depending on the actual condition. During the entire infusion period, systolic blood pressure ＜ 80mmHg and heart rate ＞140bpm is sustained for more than 10min or the heart rate increases by more than 25bpm, the pumping rate should be reduced or discontinued until hypotension and tachycardia disappear, and the dose should be halved when intravenous pumping is restarted [15]. Depending on clinical condition, clinician can use fluids and norepinephrine to maintain MAP≥70mmHg, dobutamine was used to maintain CI ＞2.5L/min/m^2.

All patients received right cardiac protective mechanical ventilation, tidal volume (VT) 4-8ml/kg, platform pressure Pplat≤27cmH2O, driving pressure ＜

18cmH2O, the optimal PEEP was titrated by lung compliance method after lung reexpansion; Adjust the respiratory rate (RR) not more than 35 times/min to PaCO2＜48mmHg, FiO2 levels were adjusted to maintain SpO2 88%-95% and PaO2 55-80mmHg. In hypotension (mean arterial pressure ＜60mm Hg) or in the case of pneumothorax, PEEP can be further adjusted according to the patient's needs; If PaO2/FiO2＜150, FiO2＞0.6. Based on the clinician's judgment, PEEP levels can be further titrated by optimal compliance after lung recruited. Check the Pplat and drive pressure at least 4 hours after each change in PEEP or VT. If lung reexpansion and PEEP titration do not maintain oxygenation or hypercapnia occurs, prone position may be performed for more than 16 hours per day; If the prone position does not improve oxygenation and correct hypercapnia, VV-ECMO can be considered if one of the following conditions is met: PaO2/FiO＜50 mmHg for more than 3 h; PaO2/FiO2＞80 mmHg for more than 6 h; Or arterial blood pH＜ 7.25 accompanied by PaCO2＞60 mmHg over 6 h[16].

All patients were titrated with sedative analgesic agents according to sedative analgesic targets. If PaO2/FiO2＜150, then deep sedation, RASS-4 points to reduce respiratory drive; If PaO2/FiO2> 150, light sedation, RASS 0 ~ -2, maintain RR＜30, VT＜10ml/kg. All patients maintained COPT score of 0 for titrated analgesics.

6. Research steps

(1) Patients eligible for inclusion in the study were screened and informed consent was signed.

(2) Patients were randomly assigned to the experimental group or the control group. Vital signs and hemodynamic parameters were recorded 1 hour before administration, and related indexes such as cardiac function and pulmonary circulation were detected by transesophageal ultrasound, as well as arterial blood gas, central vein blood gas, blood routine, liver and kidney function, electrolyte, myocardial enzyme profile and BNP were detected by blood sampling.

(3) According to the study protocol, all subjects in the two groups began administration at 8am, and 8am vital signs, arterial blood gas, central vein blood gas,

blood routine, liver and kidney function, electrolytes, myocardial enzyme profile and BNP during medication period and the first 24 hours after medication completion were recorded. The related indexes of cardiac function, pulmonary circulation and hemodynamics were monitored by transesophageal ultrasound. Study withdrawal/early termination: Family members refuse to continue treatment if: Family members withdraw from the study; In the presence of experimental-related serious adverse reactions.

7 Evaluation

7.1 Primary and Secondary Endpoints/Outcome Evaluation Primary outcome:

Rate of RVD at 48h randomized administration. Secondary outcome: 48h after random administration, the following indicators were assessed. Other indicators of right cardiac function were: TAPSE, S ', FAC, RVEDA/LVEDA PVR RV-PA coupling CI, SVI, SVR, ScvO2 SOFA score after random 28-day mortality, in-hospital mortality

7.2 Safety evaluation

(1)Vital signs, physical examination, laboratory examination indicators.

(2) The incidence of adverse events/adverse reactions was evaluated according to CTCAE4.0 (Annex 4).

(3) Incidence of serious adverse events/serious adverse reactions.

8. Adverse Events and serious adverse events

Adverse Event Reports: Adverse medical events in patients treated with levosimendan that are not necessarily causal to the treatment.

Reports of serious adverse events: Events requiring hospitalization, prolonged hospitalization, disability, inability to work, life threatening or death occurred during levosimendan treatment. During clinical trials, the clinical investigator is obligated to take necessary measures to ensure the safety of the subjects and record them. In the event of a serious adverse event occurring during a clinical trial, the investigator shall

immediately take appropriate treatment measures for the subject and report to the principal investigator, the Ethics committee of the clinical trial unit, the Food and Drug Administration and the State Food and Drug Administration of the province, municipality or autonomous region, and the sponsor within 24 hours by telephone or fax. At the same time, write a written report to the above institutions within 15 days. The occurrence of serious adverse events must be completed in the CRF Serious Adverse Event Report form.

For all serious adverse events, the investigator should follow up until resolution, recovery to baseline, proven unresolveable/permanent, switch to other treatments, or death. Medical documentation of the serious adverse events, including results of laboratory and auxiliary tests, should be recorded in the original documentation and follow-up form. The investigator and other leaders should analyze and determine cause-and-effect relationships from a clinical perspective for serious adverse events in subjects.

9. Statistical analysis and statistical methods

(1) Case enrollment analysis: The total number of enrolled and completed cases in each center was listed, and three analytical data sets (FAS, PPS, SS) were determined. List the cases of shedding and elimination and their causes.

(2) Demographic data and baseline analysis: descriptive demographic data and other baseline characteristic values; The number of cases, mean value, standard deviation, median, minimum value and maximum value of measurement data were calculated. Count and grade data calculation frequency and component ratio.

(3) Effect analysis: CMH-$\chi 2$ test considering central effect was used for comparison between groups. t test or Wilcoxon rank sum test were used to compare the measures between groups. $\chi 2$ test or Fisher's exact probability method were used to compare the classification indexes between groups. Rank data were obtained by Wilcoxon rank sum test or CMH-$\chi 2$ test. Survival data will be described using Kaplan-Meier curves and life tables. A nonparametric confidence interval for the median lifetime is calculated. Linear rank tests (logrank test and Wicoxon test) were used to compare

survival between the treatment group and the control group. The Cox risk model was used to analyze the potential influence of covariables (predictors). Multivariate logistic regression was used to analyze dichotomous outcomes, such as recurrence rates, and this multivariate model would identify the most significant predictors. Logical or proportional advantage models will be used to adjust for prognostic factors. Precise confidence intervals will be calculated for the binary event incidence.

(4) Safety analysis: the incidence of adverse reactions was calculated; The sub-system lists the frequency and frequency of adverse reactions and calculates the percentage; A detailed list of adverse event cases; A detailed list of adverse event cases; The number and rate of "abnormal normal conversion" or "abnormal intensification" of laboratory indicators, electrocardiogram and physical examination after the test; List laboratory indicators, electrocardiograms, abnormal cases of physical examination and clinical explanations.

(5) Statistical software and general requirements use SAS software for analysis. All statistical tests were two-sided, and $P \leq 0.05$ would be considered statistically significant.

10. Medical treatment and protection of subjects

10.1 Risk assessment of subjects in the study and risk disposal measures and plans

(1) Reporting method: Any adverse event, such as the subject's subjective discomfort and abnormal laboratory test, shall be treated seriously, analyzed carefully, and immediately taken measures to protect the safety of the subject.

(2) Processing procedures: record in detail, retest according to the situation, and record its duration, return, disappearance, etc.

(3) Unmitigated adverse events should be followed up, and all adverse events should be followed up until they are properly resolved or stable.

10.2 Medical treatment and protection of subjects during the study

The subjects' rights and welfare will be protected during the study, and it is

stressed that the quality of their medical care will not be affected by their refusal to participate or withdrawal from the study. If a patient withdraws from the study, we will no longer perform tests related to the study. The Investigator will provide insurance for the subjects participating in the study program, and the subjects will receive timely and free treatment if they suffer damage related to the study, and will be compensated or compensated in accordance with laws, regulations and mutual agreements.

10.3 Medical Treatment and Protection of Subjects after the study

The patient will continue to receive routine treatment after the study is completed. Subjects shall not be required to waive their right to free treatment and compensation for research-related damage.

11. Supporting documentation and notes

11.1 Informed Consent Process

Informed consent is given before the subject's relatives agree to participate in the study and continues throughout the study. Informed consent With the consent of the Ethics Committee, relatives of the subject should read the informed consent. The researchers explain the study process and answer questions from the subjects' relatives; The subjects' relatives were informed of possible risks and their rights. Subject relatives may discuss with other family members or guardians before agreeing to participate. Researchers must inform their relatives that participation in the study is voluntary and that they may withdraw from the study at any time. Copies of the informed consent can be provided to relatives of the subjects for preservation. The rights and welfare of relatives of the subjects will be protected, and it is stressed that the quality of their medical care will not be affected by their refusal to participate in the study.

11.2 Privacy Protection

The relevant data of the subjects shall be kept by the project quality controller, who shall store the relevant forms, records and samples. Under the condition that the

confidentiality principle and relevant regulations are not violated, the supervisors, ethics committee and inspectors of the drug regulatory department can access the original medical records, and any research information shall not be disclosed to unauthorized third parties without prior approval.

11.3 Collection and use of specimens and data

The blood samples collected by the institute are only used for routine testing of medical tests during treatment, so no remaining samples will be retained. After the study, the retained images and other data will be used for future research with the consent of the subjects.

11.4 Quality Control and quality Assurance

All physicians participating in this study should be familiar with the diagnosis and treatment of ARDS, right heart failure, and the use of transesophageal ultrasound. The CRF form needs to be checked by two people. The project quality control leader shall conduct weekly quality control on the study data to ensure the safety, accuracy and orderly conduct of the test.

11.5 Data processing and record saving

11.5.1 Data Collection and Management

The required data were collected from the medical records of patients who met the inclusion criteria and filled in a paper case report form (CRF). Data collection will be conducted by clinical investigators under the supervision of the responsible person, who will be responsible for the accuracy, completeness and timeliness of the reported data. All data should be clear to ensure accurate interpretation and traceability. Data entry: After training the data entry clerk, remote data entry is carried out. Double entry method is adopted, which is completed independently by two people. Review of data: Manual comparison of data in the case report form and the database to ensure that the data in the database is consistent with the results in the case report form. Data locking: In addition to the data review as described above, the principal investigator, statistician, data manager and sponsor representative will further discuss and confirm

the main content of the study proposal and the statistical analysis proposal. Conduct an audit to confirm that all data has been entered into the database, all questions have been resolved, and the analysis population has been defined and judged to lock the data. Clinical data will be stored in a database, which should be password protected, and logic proofreading procedures should be set up when the database is established.

11.5.2 Study data retention

The minimum retention time of all data and original documents studied is 1 year, and permission should be obtained before destruction.

11.6 Conflict of Interest Statement

This study has no conflict of interest with any of the physicians involved in the clinical study.

12. References

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