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Levosimendan Versus Dobutamine in Myocardial Injury Patients with Septic Shock: A Randomized Controlled Trial

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Background: We aimed to investigate the effect of levosimendan on biomarkers of myocardial injury and systemic hemodynamics in patients with septic shock.





Material/Methods: After achieving normovolemia and a mean arterial pressure of at least 65 mmHg, 38 septic shock patients with low cardiac output (left ventricular ejective fraction), LVEF \leq 45% were randomly divided into two groups: levosimendan dobutamine. Patients in the levosimendan and dobutamine groups were maintained with intravenous infusion of levosimendan (0.2 μ g/kg/minute) and dobutamine (5 μ g/kg/minute) for 24 hours respectively. During treatment we monitored hemodynamics and LVEF, and measured levels of heart-type fatty acid binding protein (HFABP), troponin I (TNI), and brain natriuretic peptide (BNP). In addition, the length of mechanical ventilation, intensive care unit (ICU) stay, hospital stay, and 28-day mortality were compared between the two groups.

Results: The levosimendan group and the dobutamine group were well matched with respect to age (years, 55.4 \pm 1 7.5 versus 50.2 \pm 13.6) and gender (males, 68.4% versus 57.9%). Levosimendan-treated patients had higher stroke volume index (SVI), cardiac index (CI), LVEF, and left ventricular stroke work index (LVSWI), and lower extravascular lung water index (EVLWI) compared to dobutamine-treated patients (p <0.05). HFABP, TNI, and BNP in the levosimendan group were less than in the dobutamine group (p <0.05). There was no difference in the mechanical ventilation time, length of stay in ICU and hospital, and 28-day mortality between the two groups.

Conclusions: Compared with dobutamine, levosimendan reduces biomarkers of myocardial injury and improves systemic hemodynamics in patients with septic shock. However, it does not reduce the days on mechanical ventilation, length of stay in ICU and hospital, or 28-day mortality.

MeSH Keywords: **Cardiomyopathies • Fatty Acid-Binding Proteins • Hemodynamics • Shock, Septic**

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Background

The latest several decades have witnessed the progress in the treatment of severe sepsis and septic shock, acute organ dysfunction and consequential multiple organ dysfunction [1], however, cardiovascular dysfunction due to severe infection is still a major contributor to sepsis related morbidity and mortality [2].

Cardiovascular dysfunction induced by severe sepsis and septic shock is characterized by signs of distributive shock and septic cardiomyopathy consisting of bi-ventricular myocardial contractility impairment and diastolic dysfunction [3]. The characteristics of septic cardiomyopathy include left ventricular dilatation, depressed ejection fraction and recovery during 7–10 days. In severe sepsis and septic shock, myocardial depression is the manifestation of septic cardiomyopathy and may attribute to the overwhelming production of inflammatory cytokines, mitochondrial dysfunction, and decreased myofibrillar sensitivity to calcium [4,5].

Rivers et al. suggested in 2001 that early goal-directed therapy (EGDT) was effective for severe sepsis management [6], however, several recent studies, including the ProCESS [7] and ARISE [8] trials in 2014 and the ProMiSe trial [9] in 2015 indicated that EGDT did not improve outcomes compared to usual care.

International sepsis guidelines have been adopted worldwide, and it is widely accepted that the standard treatment for sepsis should concentrate on infection control and optimization of hemodynamic parameters by fluid resuscitation and vasopressor therapy including noradrenaline and vasopressin [10]. These standard treatment also apply to septic cardiomyopathy. In addition, using dobutamine to increase the cardiac index is recommended by international sepsis guidelines [10]. However, several studies have demonstrated that the use of dobutamine to increase cardiac output did not improve microcirculation, peripheral perfusion, or the outcome of septic shock patients [11], and even increased the 90-day mortality rate [12].

Another inotropic agent is levosimendan, a Ca^{2+} sensitizer and inodilator, which has been used successfully in the management of acute heart failure. Levosimendan not only has inotropic and vasodilator effects, but also has anti-inflammatory and anti-apoptotic effects [13]. In addition, meta-analysis has shown that levosimendan reduced mortality in critically ill patients and chronic advanced heart failure patients [14,15]. However, levosimendan is not widely used in intensive care units (ICUs).

Therefore, the aim of the present study was to compare the effects of levosimendan and dobutamine on biomarkers of

myocardial injury and systemic hemodynamics in patients with septic shock in the ICU.

Material and Methods

Ethics statement

This pilot study was conducted in the medical-surgical ICU at Tongde Hospital of Zhejiang Province in Hangzhou, China between March 2014 and January 2016. It was conducted in strict accordance with the protocol approved by the Ethics Committee of Tongde Hospital of Zhejiang Province (Hangzhou, China). All participants were recruited by Tongde Hospital of Zhejiang Province and they all (or their guardians) signed informed consents prior to enrollment.

Study population

Patients with low cardiac output (left ventricular ejective fraction (LVEF) $\leq 45\%$) were enrolled within the first 24 hours from the onset of septic shock after having established normovolemia (CVP=12 to 15 mmHg) [10] and mean arterial pressure (MAP) of at least 65 mmHg using norepinephrine, if needed. Red blood cells were transfused when hemoglobin concentrations decreased to below 7 g/dL [10] to elevate systemic oxygen supply.

Inclusion criteria were: (1) all participants were diagnosed with septic shock and established normovolemia, and used norepinephrine to maintain MAP of at least 65 mmHg [10]; (2) all participants had LVEF $\leq 45\%$ after fluid resuscitation and vasopressor therapy. Exclusion criteria included: (1) onset of septic shock >24 hours; (2) patients younger than 18 years of age; (3) LVEF $\leq 45\%$ before fluid resuscitation; (4) patients with pre-existing cardiomyopathy, valvular heart disease, or heart failure; (5) present or suspected acute coronary syndrome within recent two weeks; (6) pregnancy; and (7) ventricular outflow tract obstruction.

Experimental procedure

All patients received mechanical ventilation using a volume-controlled mode with a tidal volume of 6 to 8 mL/kg of predicted body weight, and were sedated with midazolam and fentanyl. According to the international sepsis guidelines [10], for septic shock treatment we used norepinephrine to maintain a mean arterial pressure (MAP) of at least 65 mmHg despite quantitative fluid resuscitation (the variation of CVP from 12 to 15 mmHg) within the first 24 hours from the onset of septic shock. After these goals were achieved, cardiac ultrasound scans were used to measure LVEF immediately. Finally, 38 patients with low cardiac output (LVEF $\leq 45\%$) were enrolled and

randomized (by the use of a table of random numbers) into two groups: a group receiving 0.2 µg/kg/minute (without a loading bolus dose) of levosimendan (levosimendan group, n=19), and a group receiving 5 µg/kg/minute of dobutamine (dobutamine group, n=19). During the 24-hour drug intervention period, all patients also received fluid therapy and norepinephrine to maintain normovolemia and MAP of more than 65 mmHg. After this period, levosimendan and dobutamine were discontinued and the attending ICU physicians decided whether dobutamine should be started based on the hemodynamic status of the patients. In addition, in order to avoid the interference of depurative extracorporeal circulation on biomarkers of myocardial injury, all patients did not receive continuous blood purification (CBP) before or within 48 hour after enrollment. The treating physicians and echocardiographers were not blinded to the experimental procedure or the echocardiographic and laboratory results during the study.

Standard echocardiographic examination

Transthoracic echocardiography was performed before and again after fluid resuscitation and vasopressor therapy, and 24 hours after inclusion. All echocardiograms were performed by an expert echocardiographer not involved in patient care, using a Vivid E9 ultrasound scanner and acquiring LVEF (modified Simpson's rule).

Hemodynamics monitoring and lactate

All patients were monitored using a pulse-indicated continuous cardiac output (PiCCO) system (Pulsion Medical System, Munich, Germany). They were required to have both a left femoral artery catheter and a right central venous catheter. The correct placement of the catheter insertion was further confirmed by chest radiography.

A 5-French thermistor-tipped catheter (PV2013L16, Pulsion Medical System, German) was inserted into the femoral artery and a central venous catheter (CS-277202-E, ARROW, USA) was placed into a central vein (jugular or subclavian vein); both were connected to the PiCCO system. Thermodilution parameters and pulse contour parameter were obtained with the PiCCO monitor, based on triplicate injections of 15 mL of cold isotonic saline 0.9% (<8°C) via the central venous catheter, and were recorded as the average of the three measurements. The corresponding ventilator function and perfusion parameters were observed and kept constant during the 6-hour period preceding the measurements. The patients were kept in a horizontal position.

Blood gas samples, including lactate samples, were obtained from the arterial catheters via 3 mL heparinized syringes (PL67BP; BD Diagnostics, Plymouth, UK) anaerobically and

analyzed on blood gas bedside machines (ABL800: Radiometer, Copenhagen, Denmark).

Outcome measures

The changes in heart rate (HR), CVP, MAP, stroke volume index (SVI), cardiac index (CI), left ventricular stroke work index (LVSWI), systemic vascular resistance index (SVRI), intrathoracic blood volume index (ITBVI), global end diastolic volume index (GEDV), extravascular lung water index (EVLWI), oxygen delivery index (DO₂I), oxygen consumption index (VO₂I), LVEF, lactate, positive end-expiratory pressure (PEEP), and norepinephrine dose were recorded at baseline and 24 hours after randomization.

We collected 3 mL venous blood samples from the two groups of patients at baseline and 24 hours after randomization and sent samples immediately to Department of Biochemistry at Tongde Hospital of Zhejiang Province to measure plasma levels of heart-type fatty acid binding protein (HFABP), troponin I (TNI), and brain natriuretic peptide (BNP).

Statistical analysis

PASS software (version 11; NCSS, LLC) was used to calculate sample size. Sample size was determined by two-sample *t*-test power analysis using preliminary data obtained in our laboratory with the following assumptions: α of 0.05 (two-tailed), power of 80%, differences in the mean of HFABP between patients in the levosimendan and the dobutamine groups of -3.4 ng/mL, and a standard deviation of 1.4 ng/mL. Therefore, we calculated that a sample size of 17 would provide 80% power of detecting a difference at a 0.05 level of significance.

The multivariate linear regression analysis was used to assess the effect of levosimendan on biomarkers of myocardial injury (HFABP, TNI, and BNP) and heart function (LVEF and CI) after controlling for age, gender, APACHEII scores, SOFA scores, and baseline values in the septic shock patients. Statistically significant levels were two-tailed and set at $p < 0.05$.

Data were expressed as mean \pm standard deviation of the mean (SDM) for quantitative variables, and as count and percentages for qualitative values. Distributions of the discrete variables were compared between the two treatment groups with either the chi-square test or Fisher exact tests. Two sample *t*-test was used to compare between the two groups; and paired *t*-test was used for continuous variables before and after treatment. SPSS software (version 16; SPSS Inc., Chicago, IL, USA) was used for statistical analysis; all tests were 2-tailed and $p < 0.05$ was considered to be statistically significant.

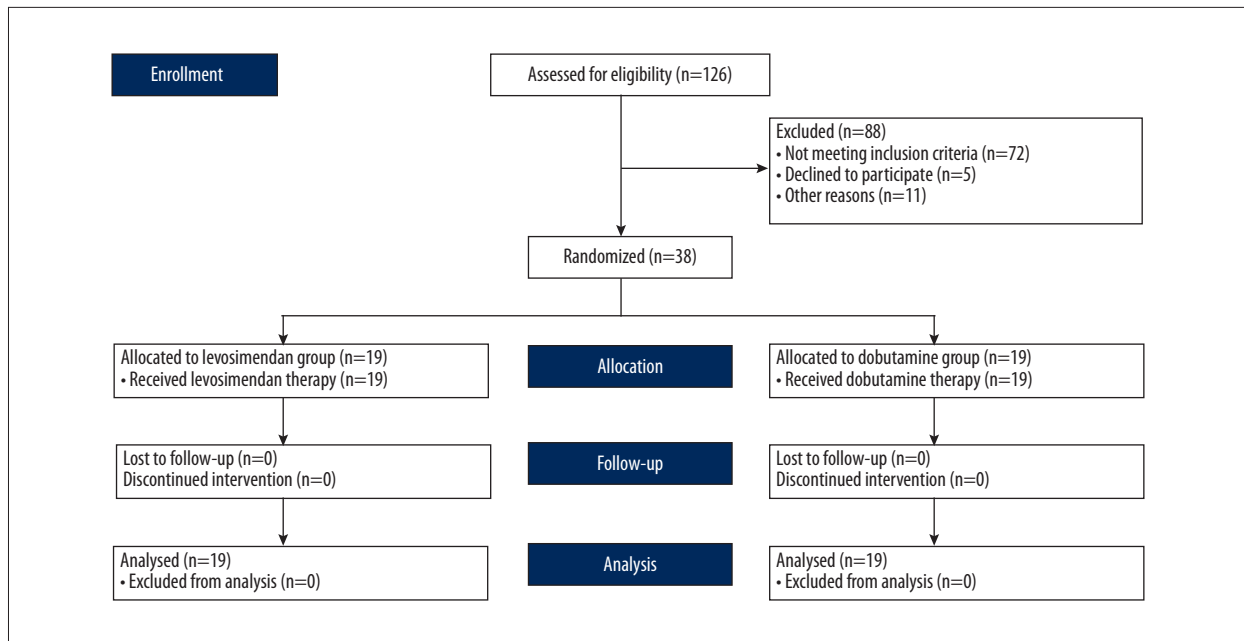


Figure 1. Flow diagram.

Table 1. Demographic variables at baseline in each group.

Characteristics	Levosimendan (n=19)	Dobutamine (n=19)	P-value
Age(years)	55.4±17.5	50.2±13.6	0.313
Male (n,%)	13 (68.4)	11 (57.9)	0.501
APACHEII	18.4±4.5	19.5±4.3	0.446
SOFA	4.2±1.8	4.3±2.6	0.891
Type of infection (n,%)			
Pneumonia	8 (42.1%)	10 (52.6%)	0.516
Peritonitis	5 (26.3%)	4 (21.1%)	0.703
CRBSI	4 (21.1%)	2 (10.5%)	0.374
Urinary tract infection	2 (10.5%)	3 (15.8%)	0.631
Days on MV	6.9±5.5	7.2±5.3	0.865
Length of stay in ICU(days)	12.6±10.1	13.3±10.5	0.835
Length of stay in hospital(days)	20.4±21.5	22.5±23.1	0.773
28-day mortality% (n)	31.6% (6)	36.8% (7)	0.732

yr – years; APACHE II – acute physiology and chronic health evaluation; SOFA – sequential organ failure assessment; CRBSI – catheter related bloodstream infection; MV – mechanical ventilation; ICU – intensive care unit.

Results

Demographic and clinical characteristics of the study population

Of the 126 cases assessed for eligibility, 38 subjects were enrolled. Figure 1 shows a flow diagram that quantifies progress through the trial. Of the 72 patients that did not meet the inclusion criteria, five patients declined to participate, 11

patients were excluded from the study for other reasons (six because of an onset of septic shock >24 hours, three because of LVEF ≤45% before fluid resuscitation, and two due to acute coronary syndrome within the recent two weeks).

The demographic data of patients are summarized in Table 1. Age, gender, APACHE II scores, SOFA scores, and type of infection were balanced between the two groups at admission. There were no difference in the duration of mechanical

Table 2. Hemodynamic data at different points in each group.

		Levosimendan (n=19)	Dobutamine (n=19)	P-value
HR (beats/min)	Baseline	116.1±7.5	113.8±6.9	0.332
	24 hours	111.6±6.8	110.3±6.5	0.551
CVP (mmHg)	Baseline	13.2±1.1	13.6±1.4	0.334
	24 hours	13.1±0.9	13.7±1.2	0.090
MAP (mmHg)	Baseline	67.6±2.0	67.4±2.1	0.765
	24 hours	68.1±1.8	67.9±1.9	0.741
SVI (ml/m ²)	Baseline	33.5±8.9	32.5±10.3	0.750
	24 hours	40.5±9.1	33.6±9.7	0.030
CI (L/min/m ²)	Baseline	3.0±0.2	2.9±0.3	0.235
	24 hours	3.5±0.3	3.1±0.4	0.001
LVSWI (kg/min/m ²)	Baseline	31.5±1.8	32.6±3.2	0.200
	24 hours	36.9±2.7	33.8±2.9	0.002
SVRI (kPa·s·L/m ²)	Baseline	1185±109	1236±121	0.181
	24 hours	1257±117	1198±99	0.102
LVEF (%)	Baseline	36.2±5.1	37.2±7.2	0.624
	24 hours	45.6±7.6	39.1±8.5	0.018
ITBVI (ml/m ²)	Baseline	889.8±124.9	850.3±162.2	0.406
	24 hours	873.6±134.8	860.5±122.9	0.756
GEDI (ml/m ²)	Baseline	709.7±97.6	683.3±130.6	0.484
	24 hours	693.4±101.6	685.0±95.2	0.794
EVLW I(ml/kg)	Baseline	9.5±3.6	9.3±3.8	0.869
	24 hours	6.4±2.8	8.9±3.0	0.012
DO ₂ I (kg·min/·m ²)	Baseline	716.8±56.2	725.5±58.7	0.644
	24 hours	755.0±52.1	719.8±49.6	0.040
VO ₂ I (kg·min/·m ²)	Baseline	123.2±16.9	125.6±13.4	0.631
	24 hours	139.5±18.3	127.4±14.8	0.031
Lactate (mmol/L)	Baseline	5.1±1.2	4.7±1.1	0.291
	24 hours	3.6±0.8	4.3±1.0	0.022
PEEP (cmH ₂ O)	Baseline	5.68±2.05	6.05±2.24	0.633
	24 hours	5.26±1.63	5.47±1.54	0.823
Norepinephrine dose (ug/kg/min)	Baseline	0.42±0.13	0.40±0.11	0.619
	24 hours	0.36±0.11	0.37±0.09	0.761

HR – heart rate; CVP – central venous pressure; MAP – mean arterial pressure; SVI – stroke volume index; CI – cardiac index; LVSWI – left ventricular stroke work index; SVRI – systemic vascular resistance index; ITBVI – intrathoracic blood volume index; GEDI – global end diastolic volume index; EVLWI – extravascular lung water index; DO₂I – oxygen delivery index; VO₂I – oxygen consumption index; LVEF – left ventriculus ejective fraction; PEEP – positive end-expiratory pressure.

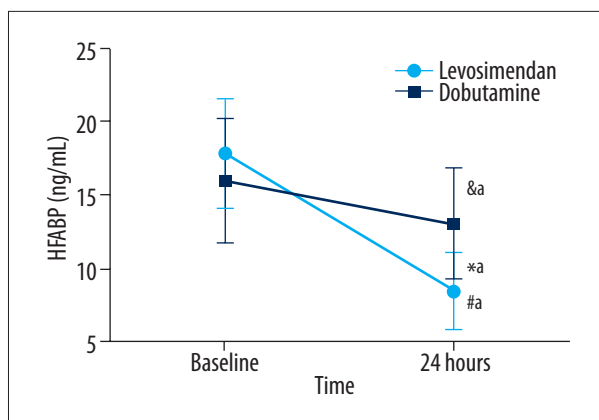


Figure 2. The change of HFABP in the different groups. No significant differences were observed at baseline. It was decreased after 24 hours of treatment compared to baseline values in the dobutamine group (^{&a} $p=0.032$), and significantly reduced after 24 hours of treatment compared to baseline values in levosimendan group (^{#a} $p<0.001$). HFABP was lower in the levosimendan group than in the dobutamine group (^{*a} $p<0.001$). HFABP – heart-type fatty acid binding.

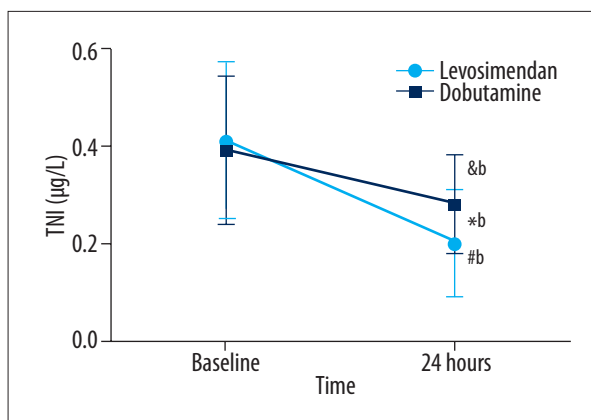


Figure 3. The change of TNI in the different groups. No significant differences were observed at baseline. It was decreased after 24 hours of treatment compared to baseline values in the dobutamine group (^{&b} $p=0.011$), and significantly reduced after 24 hours of treatment compared to baseline values in the levosimendan group (^{#b} $p<0.001$). TNI was lower in the levosimendan group than in the dobutamine group (^{*b} $p=0.025$). TNI – troponin I.

ventilation, length of stay in ICU and hospital, or 28-day mortality between the two groups (all $p>0.05$).

The changes of hemodynamics and lactate in the different groups

As shown in Table 2, no significant differences were observed in HR, CVP, MAP, SVRI, ITBVI, GEDI, PEEP, and norepinephrine dose (all $p>0.05$). Compared to dobutamine, levosimendan increased CI ($p=0.001$), SVI ($p=0.030$), LVSWI ($p=0.002$), and LVEF ($p=0.018$), and decreased EVLWI and lactate ($p=0.012$ and $p=0.022$, respectively) after 24 hours of intervention. In addition, levosimendan improved DO_2 and VO_2 of systemic tissues ($p=0.040$ and $p=0.031$, respectively).

Levosimendan decreased the plasm levels of biomarkers of cardiac injury induced by septic shock

Compared with the dobutamine group, concentrations of HFABP, TNI, and BNP were significantly decreased at 24 hours after intervention in the levosimendan group ($p<0.001$, $p=0.025$, and $p<0.001$, respectively, see Figures 2–4.)

Multivariate regression analysis showed that compared to the dobutamine group, after adjusting for the patients' age, gender, APACHE II, SOFA, and baseline of HFABP, TNI, BNP, LVEF, and CI, the levosimendan group had significantly decreased levels of HFABP, TNI, and BNP ($p<0.001$, $p=0.03$ and $p<0.001$, respectively, Tables 3–5), and increased LVEF and CI ($p=0.03$ and $p<0.001$, respectively, see Tables 6, 7)

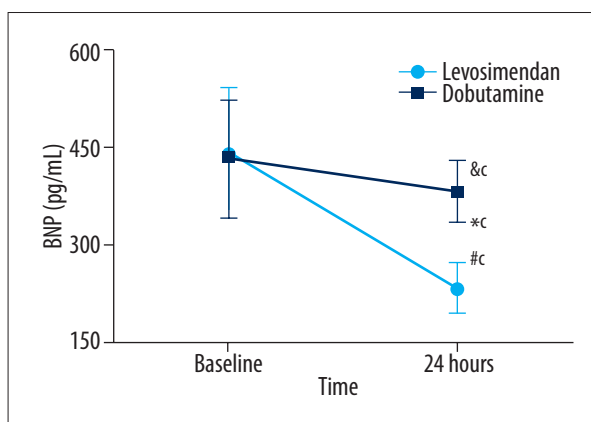


Figure 4. The change of BNP in the different groups. No significant differences were observed at baseline. It was decreased after 24 hours of treatment compared to baseline values in the dobutamine group (^{&c} $p=0.037$), and significantly reduced after 24 hours of treatment compared to baseline values in the levosimendan group (^{#c} $p<0.001$). BNP was lower in the levosimendan group than in the dobutamine group (^{*c} $p<0.001$). BNP – brain natriuretic peptide.

Discussion

Septic cardiomyopathy, a kind of cardiovascular dysfunction induced by severe sepsis and septic shock, is manifested by low cardiac output and is closely related to higher mortality in septic shock patients [16–18]. Inotropic agents including dobutamine and levosimendan may show benefits in the treatment of cardiovascular dysfunction induced by sepsis [10,19]. The major findings of the present study were that levosimendan

Table 3. Multivariate regression analysis for the effect of levosimendan on the HFABP.

Variables	Parameter estimate	Standardized estimate	Standard error	t-value	p-value
Intercept	1.53	0.00	5.81	0.26	0.79
Age	-0.01	-0.03	0.04	-0.22	0.83
Gender	-1.73	-0.21	1.26	-1.37	0.18
APACHEII	0.01	0.02	0.15	0.09	0.93
SOFA	0.21	0.12	0.27	0.78	0.44
Baseline HFABP	0.19	0.20	0.17	4.40	0.25
Levosimendan	-5.05	-0.64	1.15	1.16	<0.001

APACHEII – acute physiology and chronic health evaluation; SOFA – sequential organ failure assessment; HFABP – heart-type fatty acid binding protein.

Table 4. Multivariate regression analysis for the effect of levosimendan on the TNI.

Variables	Parameter estimate	Standardized estimate	Standard error	t-value	p-value
Intercept	0.23	0.00	0.12	1.94	0.06
Age	-0.0002	-0.04	0.001	-0.26	0.80
Gender	-0.06	-0.26	0.04	-1.57	0.13
APACHEII	0.001	0.04	0.01	0.26	0.79
SOFA	0.01	0.20	0.01	1.19	0.24
Baseline TNI	-0.18	-0.27	0.11	-1.62	0.12
Levosimendan	-0.08	-0.37	0.03	-2.34	0.03

APACHEII – acute physiology and chronic health evaluation; SOFA – sequential organ failure assessment; TNI – troponin I.

Table 5. Multivariate regression analysis for the effect of levosimendan on the BNP.

Variables	Parameter estimate	Standardized estimate	Standard error	t-value	p-value
Intercept	189.81	0.00	59.86	3.17	0.003
Age	-0.73	-0.13	0.44	-1.66	0.11
Gender	34.67	0.19	14.48	2.39	0.02
APACHEII	-1.41	-0.07	1.62	-0.87	0.39
SOFA	-1.35	-0.03	3.23	-0.42	0.68
Baseline BNP	-0.16	-0.18	0.08	-2.14	0.04
Levosimendan	-142.05	-0.83	13.46	-10.55	<0.001

APACHEI – acute physiology and chronic health evaluation; SOFA – sequential organ failure assessment; BNP – brain natriuretic peptide.

increased LVSWI and LVEF, decreased EVLWI and lactate, and improved tissue perfusion, hemodynamics, and cardiac function. However, levosimendan did not reduce the days on mechanical ventilation, lengths of stay in ICU and hospital, or 28-day mortality.

Elevated concentrations of TNI and BNP are frequently observed in patients with severe sepsis and septic shock even in the absence of an acute coronary syndrome (ACS) [20,21]. Kandil et al. demonstrated the relationship between elevated BNP level and severity of sepsis regardless of congestive heart failure; their findings also supported the utility of BNP level as

Table 6. Multivariate regression analysis for the effect of levosimendan on the LVEF.

Variables	Parameter estimate	Standardized estimate	Standard error	t-value	p-value
Intercept	43.20	0.00	11.55	3.74	<0.001
Age	0.08	0.14	0.09	0.88	0.38
Gender	3.90	0.22	2.98	1.31	0.20
APACHEII	-0.003	-0.002	0.33	-0.01	0.99
SOFA	-0.016	-0.004	0.64	-0.02	0.98
Baseline LVEF	-0.016	-0.01	0.22	-0.07	0.94
Levosimendan	6.41	0.38	2.78	2.31	0.03

APACHEII – acute physiology and chronic health evaluation; SOFA – sequential organ failure assessment; LVEF – left ventricular ejective fraction.

Table 7. Multivariate regression analysis for the effect of levosimendan on the CI.

Variables	Parameter estimate	Standardized estimate	Standard error	t-value	p-value
Intercept	5.13	0.00	0.82	6.29	<0.001
Age	-0.003	-0.13	0.004	-0.87	0.39
Gender	-0.05	-0.07	0.12	-0.44	0.66
APACHEII	-0.01	-0.05	0.01	-0.34	0.74
SOFA	0.04	0.21	0.03	1.46	0.15
Baseline CI	-0.35	-0.23	0.23	-1.53	0.14
Levosimendan	0.43	0.55	0.11	3.81	<0.001

APACHEII – acute physiology and chronic health evaluation; SOFA – sequential organ failure assessment; CI – cardiac index.

a marker for mortality in septic shock [22]. Studies have shown that TNI was sensitive and superior to creatinine kinase-MB (CK-MB) for the detection of myocardial injury in septic shock and after coronary angioplasty with or without stenting [20,23]. Hence, BNP and TNI have been identified as cardiac biomarkers and predictors of cardiac dysfunction and death in septic patients and successfully used in ICUs. However, it is difficult to distinguish septic patients with or without cardiac dysfunction related to septic shock via utilization of BNP alone [24]. Furthermore, Tiruvoipati et al. suggested TNI was related to lower ejection fraction and higher need for inotropic/vasopressor support, but did not independently predict mortality in critically ill patients with severe sepsis [25].

HFABP is a more sensitive and specific biomarker of myocardial injury than TNI and BNP, which make it superior to TNI and BNP for the assessment of recurrent or persistent myocardial injury [26,27]. Zhang et al. found that serum HFABP was frequently elevated among patients with severe sepsis and was associated with sepsis-related myocardial dysfunction, and elevated HFABP independently predicted 28-day mortality in severe sepsis [28]. Therefore, HFABP, TNI, and BNP were utilized

as biomarkers of myocardial injury for septic shock patients in the present study.

This study demonstrated that both dobutamine and levosimendan reduced the concentrations of biomarkers of myocardial injury in critically ill patients with septic shock. But HFABP, TNI, and BNP were lower in the levosimendan group than in the dobutamine group, which suggested that levosimendan could play a more important role in attenuating septic myocardial injury. Wu et al. [29] conducted a study in patients with acute myocardial infarction (AMI) who received emergency percutaneous coronary intervention (PCI) and found that the TNI concentration decreased much more in the levosimendan group than in the placebo group, suggesting that the myocardium was suffering less damage. In myocardium injury and stunning, the activation of the Ca²⁺-dependent protease results in degradation of TNI partially and selectively during reperfusion [30]. Because levosimendan has the ability to increase responsiveness to calcium while keeping the cytosolic Ca²⁺ concentration unchanged, levosimendan prevented the degradation of TNI, which could prevent the contractile dysfunction of myocardium stunning and damage [29].

Studies found that BNP levels were elevated in severe sepsis and septic shock patients and elevated BNP were related to myocardial dysfunction, global tissue hypoxia, and mortality [22,31]. Several mechanisms probably account for the elevated BNP levels in sepsis, including neuro-hormonal activation, volume resuscitation, and sepsis-induced biventricular dilation [32], as well as stimulation of lipopolysaccharide or pro-inflammatory cytokines, acute lung injury, and acute respiratory distress syndrome induced sepsis [33]. Levosimendan was found to have significantly decreased BNP levels at Day 1 and Day 3 in patients with decompensated heart failure (DHF) and renal dysfunction [34]. Feola et al. demonstrated that BNP concentrations decreased more significantly in a levosimendan group than a furosemide group [35]. Kyrzopoulos et al. suggested that the decreasing effect of levosimendan treatment on BNP was associated with its decreasing effect on end-diastolic cardiac wall tension [36]. Immunomodulatory and anti-inflammatory properties of levosimendan might also contribute to its effect on decreased BNP [37,38]. These probably mechanisms of decreased TNI and BNP concentrations could explain the changes of TNI and BNP in our study.

HFABP, a stable low molecular weight protein found in the cytoplasm of myocardial cells, is a sensitive and specific biomarker of myocardial injury. It is frequently elevated in patients with severe sepsis and septic shock and is associated with sepsis-related myocardial dysfunction. Elevated HFABP independently predicts 28-day mortality in severe sepsis and septic shock cases [28,39]. The probably mechanisms leading to elevated HFABP concentrations in sepsis and septic shock cases may be related to sepsis-induced myocardial dysfunction and elevated levels of free fatty acids due to an increased catabolism of glycogen and lipids during multiple organ dysfunction syndrome (MODS) associated inflammatory responses [40]. In addition, decreased renal function also contributes to the elevation of HFABP [41].

In this study, compared to dobutamine, levosimendan significantly decreased HFABP levels in patients with cardiomyopathy associated with severe sepsis and septic shock.

To date, however, the mechanisms of decreased HFABP concentrations remains unclear. There are several probably mechanisms. First, levosimendan improves inotropic function due to binding to the Ca^{2+} saturated troponin C of myocardial thin filament and diastolic function because it does not promote calcium flux into the cell [42]. The improvement in systolic and diastolic function and myocardial oxygen supply [43,44] attributed to levosimendan may decrease HFABP concentration via preventing myocardial dysfunction associated sepsis. Second, the anti-inflammatory and anti-apoptotic effects [13,45] enable levosimendan to reduce catabolism of glycogen and lipids via mediating inflammatory responses and decreasing the level

of HFABP during sepsis and septic shock. Finally, levosimendan decreases HFABP through reducing renal injury and improving renal function [34] and because levosimendan leads to augmentation of renal perfusion [46], increases in renal blood flow [47] and increases glomerular capillary surface area and glomerular filtration rate (GFR) [48].

The present study demonstrated that levosimendan had no adverse effects on hemodynamics, on the contrary, it elevated LVEF, SVI, and CI and did not increase the dose of norepinephrine needed. The absence of adverse effects on hemodynamics is in harmony with previous studies [49,50]. In addition, decreased EVLWI and lactate, and increased DO_2I and VO_2I in patients with myocardial dysfunction suggests that levosimendan improved pulmonary vascular permeability and tissue perfusion via elevated cardiac output, and the driving pressure of blood flow entering into microcirculation [51]. In our study, however, although there were no significant difference in mechanical ventilation time, ICU length of stay, hospital length of stay, or 28-day mortality between the two groups, all of these were still shorter in the levosimendan group than the dobutamine group.

Limitations of the study

There are several limitations in our study. First, we chose change in HFABP as the primary endpoint of this study. The number of septic shock patients investigated in our study was small and the study period was relative brief, therefore, the risk of positive results in a study with numerous secondary variables has to be taken into account.

Second, our study did not show a statistically significant difference in 28-day mortality between the two groups and the difference was 5.2%, suggesting that the effect size was much lower than that assumed in the sample size calculation. Post hoc sample size calculations showed that $\geq 1,305$ patients per group would be required to show a statistically significant difference in 28-day mortality between the two groups. It is impossible to achieve a study like this in a single center; a multi-centers investigation would be required in the future.

Finally, it is also indispensable to make clear the molecular biologic mechanisms of decreased HFABP in septic shock patients treated by levosimendan.

Conclusions

Compared with dobutamine, this study demonstrated levosimendan reduced the level of biomarkers of myocardial injury including HFABP, TNI, and BNP, increased LVEF, strengthened systolic function, and improved systemic hemodynamics

significantly in patients with septic shock; but there were no significant difference in duration of mechanical ventilation, length of stay in ICU, or 28-day mortality between the two treatment groups.

In conclusion, although levosimendan improved the biomarkers of myocardial injury and hemodynamic parameters, our study failed to show any improvement in clinically relevant outcomes like length of ICU stay, duration of mechanical ventilation, and 28-day mortality.

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Therefore, further investigations are required to elucidate the fundamental mechanisms underlying these processes.

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