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The effect of levosimendan on mortality in severe sepsis and septic shock: a meta-analysis of randomized trials

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ABSTRACT

Objective We aim to synthesize the up-to-date randomized trials to investigate the effects of levosimendan on mortality and clinical outcomes in severe sepsis and septic shock.

Methods A collection of databases including PubMed, EMBASE, Cochrane Central register and Web of Science were searched updated to August, 2017. Randomized trials were included when pertaining the use of levosimendan in severe sepsis or septic shock compared with any category of inotropes, or as an adjunct to standard therapy with mortalities reported. The primary outcome was the mortality at the longest follow-up and the secondary outcomes were clinical performances including serum lactate, cardiac function, vasopressor requirements, fluid infusion and length of intensive care unit (ICU) stay.

Results A final of 10 studies with 1036 patients were included in this meta-analysis. The results revealed that levosimendan could not reduce mortality significantly in septic shock, with a favouring direction towards levosimendan compared with control group (odds ratio 0.88, 95% CI 0.67-1.16, $P = 0.36$). Levosimendan could reduce serum lactate more effectively, enhance cardiac contractibility with increased cardiac index and left ventricular ejection fraction. However, it could also increase fluid infusion, and no differences in norepinephrine requirement and length of ICU stay were noted. No significant benefit in mortality could be observed of levosimendan vs. dobutamine use, or in patients with definite cardiac dysfunction. Patients younger than 65-year-old and more severe patients (mortality >=50%) were more likely to benefit from levosimendan use, though with no statistical significance.

Conclusions Current evidence is not sufficient to support levosimendan as superior to dobutamine or as an optimal adjunct in severe sepsis and septic shock. More large-scale randomized trials were

necessary for validation of the levosimendan use in sepsis.

Key words sepsis; septic shock; levosimendan; dobutamine; septic cardiomyopathy

Strengths and Limitations of this Study

1. This article synthesized the up-to-date random trials for comprehensive analysis of the effect of levosimendan on mortality in severe sepsis and septic shock.

2. Furthermore, a serious of sub-group analyses were conducted for investigation of the sub-population of patients who were likely to benefit most in levosimendan use.

3. Heterogeneity and biases were appraised between each study, and the optimal of sample size was also calculated.

4. However, the trials included were of limited sample size and quality, and were potentially high biased.

BACKGROUND

Sepsis is still a great challenge to the public health and its mortality increases tremendously when severe sepsis and septic shock occurs [1].The incidence of cardiac dysfunction in severe sepsis and septic shock remains as high as 40%-60%[2] resulted from infectious process, cytokine storm[3], decreased myocardial perfusion and pulmonary injuries[4], and is associated with patient outcomes[5, 6].

Surviving Sepsis Campaign International Guidelines (2016) recommended the usage of dobutamine infusion in patients with persistent hypo-perfusion despite adequate fluid loading and the use of vasopressor agents (weak recommendation, low quality of evidence)[7]. However, its effect on mortality in sepsis is still under debate[8], and its adverse effects including increased myocardial oxygen consumption and risks of dysrhythmia could not be neglected.

Levosimendan, a calcium sensitizer with vasodilatory properties which could improve myocardial contractibility in the absence of increased oxygen consumption, is regarded as a promising adjunct in the treatment of both cardiac systolic and diastolic dysfunctions[9] and was demonstrated to have a beneficial effect on mortality in various clinical settings[10, 11].

Levosimendan was demonstrated superior to dobutamine and milrinone in restoring cardiac function in septic animal model[12]. It could also alleviate inflammatory response by NF-κB-dependent transcription down-regulation[13] and decreased inducible NO synthetase (iNOS) promoter activity and NO expression in vitro[14].

Several meta-analyses were conducted to investigate the effects of levosimendan on mortality in sepsis which revealed a beneficial effect on survival, however with limited sample size[15]. In this study, we aim to make an up-to-date meta-analysis to investigate the effects of levosimendan on

mortality in severe sepsis and septic shock.

METHODS

The manuscript was prepared according to the preferred reporting items for systematic review and meta-analysis (PRISMA) statement[16, 17].

Eligibility Criteria

We aimed to include all the randomised control trials (RCT) studying levosimendan use versus any categories of inotropes or as an adjunct to standard management in severe sepsis and septic shock. The articles would be included in our study if fulfilling the following criteria: (1) study population of severe sepsis or septic shock in adults, (2) randomized allocation of treatment, (3) comparison of levosimendan with any category of inotropic agents or placebo, with no restrictions on dosage or time limits of levosimendan infusion, (4) data on mortality reported; and exclusion criteria were as follows: (1) duplicates, (2) pediatric subjects, (3) animal experiments or *in vitro* studies, (4) no sepsis population and (5) lack of data on mortality.

Information Sources

Two investigators searched a collection of data-bases including PubMed, EMBASE, Cochrane Central register and Web of Science updated to August, 2017 separately with no language restrictions. When relevant systemic reviews or meta-analyses were found, we ran a backward snowballing to obtain further studies.

Search

Following key words were used as search terms: "levosimendan", "simendan", "Simadax", "dextrosimendan", "sepsis", "severe sepsis", "septicemia" and "septic shock". (Complete search strategy see Supplementary File 1)

Study Selection

Abstracts and titles of the articles were initially viewed separately by two investigators, if potentially relevant, the complete articles were retrieved. Articles were assessed and selected separately by two investigators with disagreements solved by consensus.

Data Items

Information was extracted from each of the included trials on: (1) characteristics of the participants (including gender, age and diagnosis); (2) interventions (including the duration and dosage of the levosimendan or other inotropes); (3) outcome measurements with primary outcome determined as the mortality at the longest follow-up, and secondary outcomes as clinical outcomes including serum lactate level, cardiac function, fluid infusion, vasopressor requirement and length of ICU stay (LOS).

Assessment of Risk of Bias

Internal validity and risks of bias were evaluated by two investigators separately following Cochrane Collaboration Methods protocols[18]. Risks of bias were assessed by scrutinizing the articles and rated as "Yes", "No" or "Unclear" according to the procedures taken in the articles.

Summary Measures

Dichotomous outcomes were measured as proportions and odds ratio (OR) were calculated. Continuous outcomes were described as mean ± standard deviation (SD) and calculated by mean difference (MD) or standard mean difference (SMD). The end-point and change range were both compared if the continuous variables were measured at baseline and after treatment. Missing data were imputed from other information whenever possible[19].

Statistical Analysis

The data retrieved from the pertinent articles were computerized and analyzed by Review Manager 5.3 (The Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen). We used Mantel-Haenszel statistic method for dichotomous variable measurements and inverse variance for continuous variables. Random-effects model was used for better accommodation of heterogeneity. Cochrane I^2 statistic was used for heterogeneity assessment between the studies, with a range of 0% to 30% representing no or mild heterogeneity, 30%-60% moderate heterogeneity, whereas > 60% as high heterogeneity. Publication bias was tested by visual inspection of funnel plots. As for sensitivity analysis, the dataset was analyzed in both fixed and randomized-effects models and the favoring directions were inspected, and each study was removed sequentially and remaining data-set re-analyzed to assess the robustness of the results.

Trial sequential analysis was performed to estimate the optimal sample size for the plausible effects of levosimendan in sepsis[20]. Statistical significance was set at 2-lateral 0.05 level as hypothesis establishment.

RESULTS

Study Selection

A total of 566 abstracts were yielded from the search strategy, with 121 duplicates were excluded and 192 excluded due to no eligible abstracts. Complete manuscripts of 253 abstracts were retrieved for further assessment, within which 65 were reviews, 106 animal studies, 27 commentaries, 1 study design, 7 non-RCTs, 13 case reports, 2 pediatric patients, 9 non-septic patients, 10 mortality not reported and 3 in vitro studies. A final of 10 studies were included in this meta-analysis[21-30], within which two were conference abstracts[21, 22], and one was written in Chinese[26] **[Fig 1]**.

Study Characteristics

Within the 10 studies enrolling 1036 patients, no differences were present in age and APACHE II scores between the treatment and control group at the baseline. Patients diagnosed as septic shock or severe sepsis after adequate fluid resuscitation were included in each study, and four studies set explicit criteria of cardiac dysfunctions during patient recruitment[21, 26, 27, 30]. Norepinephrine was used as necessary to achieve the target MAP ranging from 65 to 80mmHg during inotropic therapy depending on the study design. Seven studies used dobutamine (dose ranges from 5μg/kg per min to 20μg/kg per min) as a comparator[21-24, 26, 27, 30] and three used levosimendan as an adjunct to standard therapy[25, 28, 29]. Levosimendan was administered as continuous infusion (dose ranges from 0.05μg/kg per min to 2.0μg/kg per min) over 24 hours with no bolus. Parameters reflecting cellular metabolism, microcirculation, hemodynamics, cardiac function and target organ perfusion were measured in individual studies **[Tab 1].**

Syntheses of Results

Mortality data were randomized and calculated from the ten studies, and the final result in mortality at the longest follow-up day revealed no statistical difference, with a favoring towards levosimendan infusion (total events 198/522 vs. 207/514 in levosimendan and control group respectively, OR 0.88, 95% CI 0.67-1.16, $P = 0.36$), with no evidence of heterogeneity ($I^2 = 3\%$, $P = 0.41$) **[Fig 2]**.

We conducted a serious of sub-group analyses according to the patients' characteristics. No statistical significance could be observed dividing the patients with definite clinical cardiac dysfunction[21, 26, 27, 30] (OR 0.96, 95% CI 0.39-1.50, $P = 0.43$) or those with homogenous cardiac functions[22-25, 28, 29] (OR 0.73, 95% CI 0.44-1.19, $P = 0.21$). Patients were also divided according

to the average age (< 65yr vs. ≥ 65yr) and mortality (< 50% vs. ≥ 50%), although no statistical significance could be noted between each sub-group, more severe patients with mortality ≥ 50%[21-23, 25, 30] (OR 0.55, 95% 0.30-1.03, *P* = 0.06 vs. OR 0.97, 95% 0.71-1.33, *P* = 0.85) and patients with average age < 65yr[24, 26, 27, 30] (OR 0.67, 95% CI 0.32-1.40, *P* = 0.29 vs. OR 0.81, 95% CI 0.50-1.32, *P* = 0.40) were more likely to benefit from levosimendan infusion, however, the huge disparities of sample size between each sub-group could not be neglected.

We also compared the effects of levosimendan vs. dobutamine on mortality in sepsis and find no statistical difference in mortality between levosimendan and dobutamine group (OR 0.65, 95% CI 0.39-1.10, *P* = 0.11) [21-24, 26, 27, 30], neither of levosimendan in comparison of standard therapy[25, 28, 29] (OR 0.80, 95% CI 0.40-1.58, *P* = 0.52) **[Fig 3]**.

We also extracted and compared the data of lactate reduction[22, 23, 26, 30], cardiac function including heart rate (HR)[23, 25-27, 30], cardiac index (CI)[23, 25-27, 30], left ventricular ejection fraction (LVEF)[21, 26, 27, 30] and left ventricular stroke work index (LVSWI)[23, 26, 27, 30], fluid infusion[23, 26, 30], norepinephrine dosage[23, 25-27, 30] and length of ICU stay (LOS)[23, 24, 27-29]. The results revealed that lactate was reduced more effectively, and cardiac function significantly improved (with increased CI, LVEF and LVSWI) in levosimendan group, while the heart rate was decreased though with no significance. Norepinephrine dose was reduced slightly, however total fluid infusion over 24 hours was tremendously increased in levosimendan group. LOS in levosimendan group was slightly shortened (*P* = 0.29) **[Tab 2]**.

Risk of Bias and Sensitivity Analyses

The funnel plot was drawn for testing the bias, and visual inspection of the funnel plot revealed potential asymmetry **[Supplementary Fig 1]**.

The data-set was analyzed both in the fixed and random-effects model for sensitivity analysis and the result revealed no shift of favouring directions **[Supplementary Fig 2]**. Each trial was removed and remaining dataset re-analyzed subsequently, and the result indicated that the statistical significance became obliviated only when the trial by Gordon AC et al. [28], was put into analysis **[Supplementary Fig 3]**.

Trial Sequential Analysis

A trial sequential analysis (TSA) was performed to determine the optimal information size. We estimated a 26% mortality based on the recent epidemiologic data of severe sepsis[31], and an assumed an average of 20% relative risk reduction in reference to the effect of levosimendan on overall mortality reduction in hospitalized patients[32] with 80% power and $\alpha = 0.05$ two-sided. The calculation indicated the optimal information size of 2082 patients for detection of the plausible treatment effect of levosimendan in sepsis. The Lan DeMets sequential monitoring boundary constructed by the optimal information size was not crossed, indicating that the cumulative evidence was not conclusive and reliable **[Fig 4]**.

DISSCUSSION

The main finding of this study was that levosimendan could not reduce the mortality in severe sepsis and septic shock patients significantly, although a favoring towards levosimendan could be observed. Furthermore, levosimendan could reduce serum lactate level, improve cardiac function. However, no change in norepinephrine dose but profound increase in fluid infusion, and no difference in LOS has been noted.

We noticed that, albeit improved cardiac function more fluid was infused after levosimendan use for maintenance of the target MAP probably due to its vasodilatory effect, which could exacerbate

pulmonary and peripheral edema and potentially impeding oxygen uptake and exchange. The use of levosimendan was also suggested to be accompanied with higher incidence of life-threatening arrhythmias like supraventricular tachyarrhythmia, which could bring hemodynamic instability and risks to the patients[28].

The previous study by Zangrillo et al. enrolling a series of small RCTs with limited sample sizes yielded a significantly reduced mortality in levosimendan group in septic shock[15]. However, it should be concerned that, in our study, statistical significance was obliviated after a large, multi-center RCT with a sample size of 514 patients by Gordon AC et al.[28] were included, implying that type I error (false positive) due to limited sample size in previous studies should be suspected, necessitating further large-scale randomised studies for the validation of the efficacy of levosimendan use in sepsis. Trial sequential analysis also indicated an optimal sample size of 2082 patients for detection of the plausible effect of levosimendan in sepsis, with current sample size of 1036 patients, suggesting that more trials are needed.

Although no statistical significance could be observed dividing the patients with definite cardiac dysfunction and heterogenous cardiac functions, we thought that further trials separating the participants with cardiac function should be considered, patients with low cardiac output may benefit more from inotropic therapy, and increase the cardiac output to supranormal level does not improve outcomes[7].

Interestingly, sub-group analysis revealed that patients less than 65-year and with high mortality (>50%) were more likely to benefit from levosimendan use. In spite of the huge disparities in sample size between each group and statistical insignificance, we thought that this benefit gain favouring towards younger and more severe patients may due to the more cardiac reserve and

more severe depression of cardiac function, which were to be elucidated in further investigations.

Limitations

Our study had several limitations. The randomized trials included in this meta-analysis were of limited sample size and potentially high bias. The heterogeneity in study design, characteristics and procedure including ethnic and cardiac function of the patients, dosage of levosimendan infusion, target MAP, supportive therapeutic strategy and fluid infusion decision etc. could potentially cause biases between each trial.

CONCLUSION

Although levosimendan could improve clinical outcomes including cardiac output and tissue perfusion compared with dobutamine or standard therapy, it also increases fluid infusion and has no significance on vasopressor requirements, still, it failed to bring significant benefits to mortality in sepsis. More RCTs are necessary for further elucidation of the effects of levosimendan in sepsis, particularly in those with cardiac dysfunctions.

LIST OF ABBREVIATIONS

APACHE Acute Physiology and Chronic Health Evaluation;

CI cardiac index;

HR heart rate;

ICU intensive care unit;

iNOS inducible NO synthetase;

IQR inter-quartile range;

LOS length of ICU stay;

LV left ventricle;

LVEF left ventricle ejection fraction;

LVSWI left ventricular stroke work index;

MAP mean arterial pressure;

MD mean difference;

NE norepinephrine;

OR odds ratio;

RCT randomized control trial;

ROS reactive oxygen species;

SD standard deviation;

SMD standard mean difference;

TSA trial sequential analysis.

DECLARATIONS

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

WC carried out the analysis and interpretation of data and participated in drafting, editing and submitting the manuscript. The articles were reviewed by two reviewers (WC and JFX) independently in accordance with the inclusion criteria. Disagreements were resolved and by consensus and discussion including a third reviewer (JYX). The quality of the articles was assessed by WC and JFX independently, with disagreements resolved by consulting a third reviewer (JYX). YY was responsible for conception, design and coordination of the study, and revising the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Not applicable.

Fig 1 Flow diagram of search process and study selection

Fig 2 The effect of levosimendan on mortality in severe sepsis and septic patients

Fig 3 Sub-group analysis. (A) Levosimendan in patients with definite cardiac dysfunction vs. patients with heterogeneous cardiac function [OR (95% CI) 0.76 (0.35-1.50), *P* = 0.43 vs. 0.73 (0.44-1.19), *P* = 0.21]; (B) Levosimendan in patients with age >= 65-year-old vs. age < 65-year-old [OR (95% CI) 0.81(0.50-1.32), *P* = 0.40 vs. 0.67(0.32-1.40), *P* = 0.29]; (C) Levosimendan in patients with mortality >=50% vs. morality < 50% [OR (95% CI) 0.55 (0.30-1.03), *P* = 0.06 vs. 0.97 (0.71-1.33), *P* = 0.85]; (D) Levosimendan vs. dobutamine [OR (95% CI) 0.65 (0.39-1.10), *P* = 0.11] or standard therapy [OR (95%CI) 0.80 (0.40-1.58), *P* = 0.52].

Fig 4 Trial sequential analysis. The optimal information size of 2082 patients for detection of the plausible treatment effect of levosimendan in sepsis, and the Lan DeMets sequential monitoring boundary constructed by the optimal information size was not crossed

Tab 1 Characteristics of the included trials. MAP mean artery pressure; LVEF left ventricular ejection fraction; PAOP pulmonary artery occlusion pressure; CI cardiac index; NR not reported; SOFA Sequential Organ Failure Assessment. † A total of 256 patients were finally included for 28-day mortality analysis; ‡ Two patients in control group failed to complete the study and were excluded

Tab 2 Clinical outcomes after randomization. Subscript TRT stands for variables after treatment; Δ stands for change range of variables (value after treatment subscribes value at baseline); CI: cardiac index; HR: heart rate; LVSWI: left ventricular stroke work index; LVEF: left ventricular ejection fraction; NE: Norepinephrine; LOS: length of ICU stay; † Standard mean difference is used in this case due to large difference in means [MD (95% CI) 1464.35 (1182.13-1746.58)].

Supplementary Fig 1 Funnel plot for inspection of bias

Supplementary Fig 2 Sensitivity analysis with data-set analyzed in fixed and random-effects models

Supplementary Fig 3 Sensitivity analysis with single study omitted sequentially

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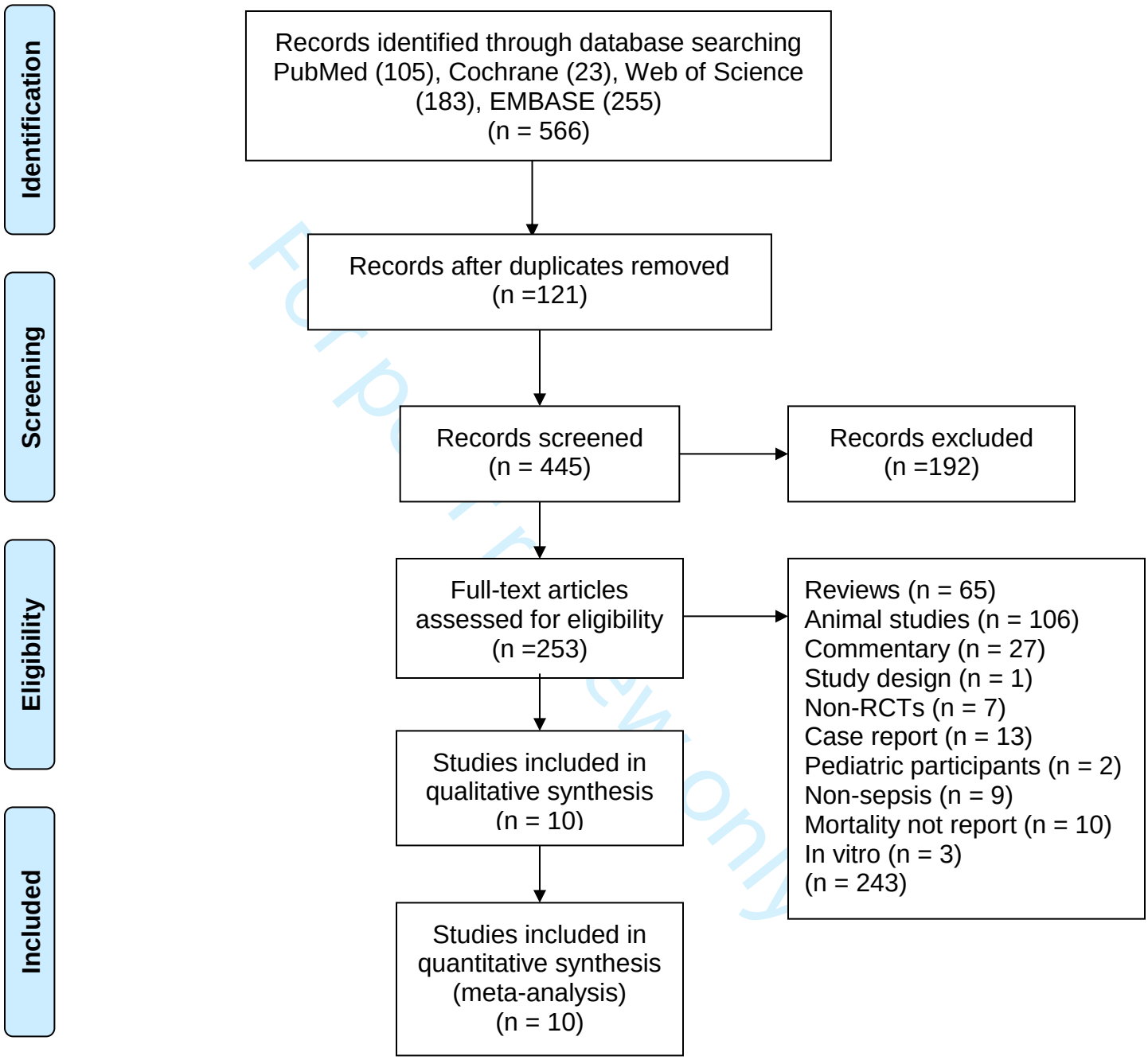
First author	Year	Subjects No	Levosimendan group	Control group	Inclusion criteria	Cardiovascular criteria	Levosimendan therapy	Control therapy	Target MAP (mmHg)	Longest follow-up (day)	Primary outcome
Alhashemi JA [22]	2009	42	21	21	Severe sepsis/septic shock	NR	0.05 to 2μg/kg per min, 24hr	Dobutamine 5 to 20μg/kg per min, 7 days	⩾65	ICU stay	ScvO2 and serum lactate
Fang M [26]	2014	36	18	18	Septic shock	LVEF⩽45%	Dobutamine 0.5μg/kg per min for 24hr; levosimendan 0.2μg/kg per min 24hr subsequently	Dobutamine 5μg/kg per min, 48hr	NR	28	Hemodynamics and cardiac function
Gordon AC [28]	2016	515	258	257*	Septic shock	MAP 60 to 70mmHg	0.05 to 0.2μg/kg per min, 24hr	Standard therapy	65 to 70	Hospital discharge	Daily SOFA score
Memis D [24]	2012	30	15	15	Septic shock	MAP⩽65mmHg	0.1μg/kg per min, 24hr	Dobutamine 10μg/kg per min, 24hr	>65	NR	Liver function
Meng J [27]	2016	38	19	19	Septic shock	MAP⩾65mmHg and LVEF ⩽45%	0.2μg/kg per min, 24hr	Dobutamine 5μg/kg per min, 24hr	⩾65	28	Hemodynamics and myocardial injury biomarkers
Morelli A [30]	2005	28	15	13**	Septic shock	MAP 70 to 80mmHg, PAOP ⩾12mmHg and LVEF<45%	0.2μg/kg per min, 24hr	Dobutamine 5μg/kg per min, 24hr	70 to 80	30	Hemodynamics and cardiac function
Morelli A [23]	2010	40	20	20	Septic shock	MAP⩾65mmHg	0.2μg/kg per min, 24hr	Dobutamine 5μg/kg per min, 24hr	70 ± 5	ICU stay	Systemic and microvascular hemodynamics
Torraco A [25]	2014	26	13	13	Septic shock	MAP⩾65mmHg	0.2μg/kg per min, 24hr	Standard therapy	65 to 75	28	Mitochondrial function
Vaitsis J [21]	2009	42	23	19	Sepsis	CI<2.2, LVEF<35%	0.1μg/kg per min, 24hr	Dobutamine 5 to 10μg/kg per min, 24hr	>65	30	Mortality at 7 and 30 days
Wang X [29]	2017	240	120	120	Septic shock	MAP≥65mmHg	0.1-0.2 μg/kg per min, 24 hours	Standard care	≥65	Hospital discharge	Mortality at 28 days, ICU discharge and hospital discharge

Note: MAP: mean artery pressure; LVEF: left ventricular ejection fraction; PAOP: pulmonary artery occlusion pressure; CI: cardiac index; NR: not reported; SOFA: Sequential Organ Failure Assessment; * A total of 256 patients were finally included for 28-day mortality analysis; ** Two patients in control group failed to complete the study and were excluded

Outcomes	References	No. of subjects	MD [95% CI]	*P* for overall effect	*P* for heterogeneity	I^2 (%)
$Lactate_{TRT}$	[22], [23], [26], [27], [30]	184	-1.04 [-1.69, -0.38]	0.02	0.001	77
ΔLactate	[23], [26], [27], [30]	142	-0.99 [-1.64, -0.35]	0.003	0.02	71
CI_{TRT}	[23], [26], [27], [30]	142	0.44 [0.17, 0.70]	0.001	0.03	67
ΔCI	[21], [23], [26], [27], [30]	184	0.46 [0.28, 0.65]	< 0.00001	0.003	72
HR_{TRT}	[23], [25-27], [30]	168	-0.71 [-3.70, 2.28]	0.64	0.41	0
ΔHR	[23], [25-27], [30]	168	-3.48 [-8.19, 1.22]	0.15	0.13	45
$LVSWI_{TRT}$	[26], [27], [30]	102	3.73 [0.49, 6.98]	0.02	0.0009	86
ΔLVSWI	[23], [26], [27], [30]	142	5.00 [3.95, 6.06]	< 0.00001	0.83	0
$LVEF_{TRT}$	[26], [27], [30]	102	6.76 [3.53, 10.00]	< 0.0001	0.75	0
ΔLVEF	[21], [26], [27], [30]	144	4.98 [0.75, 9.21]	0.02	0.001	81
Norepinephrine $dose_{TRT}$	[23], [26], [27], [30]	142	-0.08 [-0.21, 0.06]	0.26	< 0.00001	95
ΔNE dose	[23], [25], [27], [30]	132	-0.04 [-0.12, 0.04]	0.3	0.08	55
Fluid infusion in 24-hr	[23], [26], [30]	104	3.78 [0.51, 7.05] *	0.02	< 0.00001	95
LOS	[23], [24], [27-29]	863	-1.36 [-3.87, 1.14]	0.29	0.02	65

Note: Subscript TRT stands for variables after treatment; Δ stands for change range of variables (value after treatment subscribes value at baseline); CI: cardiac index; HR: heart rate; LVSWI: left ventricular stroke work index; LVEF: left ventricular ejection fraction; NE: Norepinephrine; LOS: length of ICU stay; *Standard mean difference (SMD) is used in this case due to large difference in means [MD (95% CI) 1464.35 (1182.13-1746.58)].



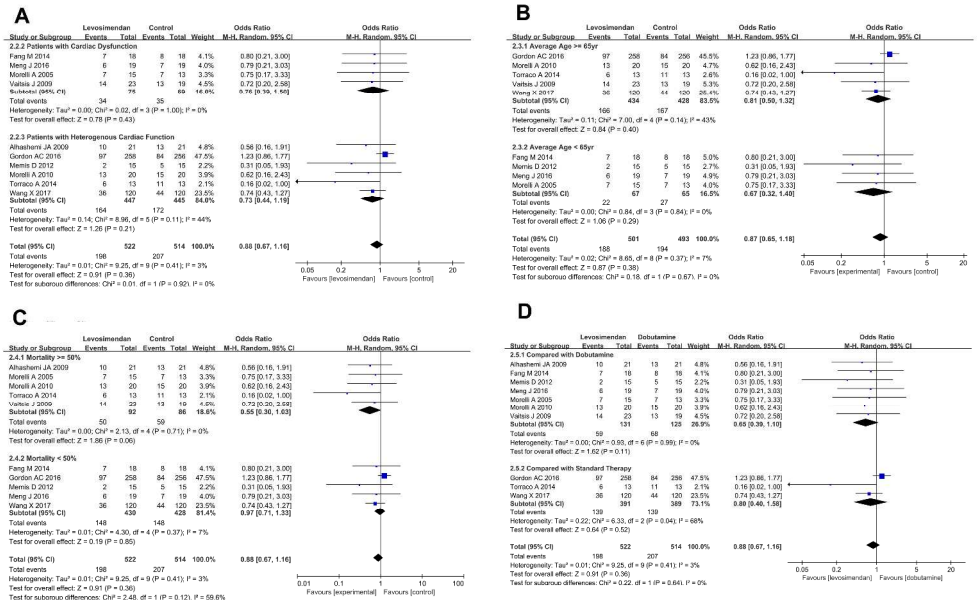
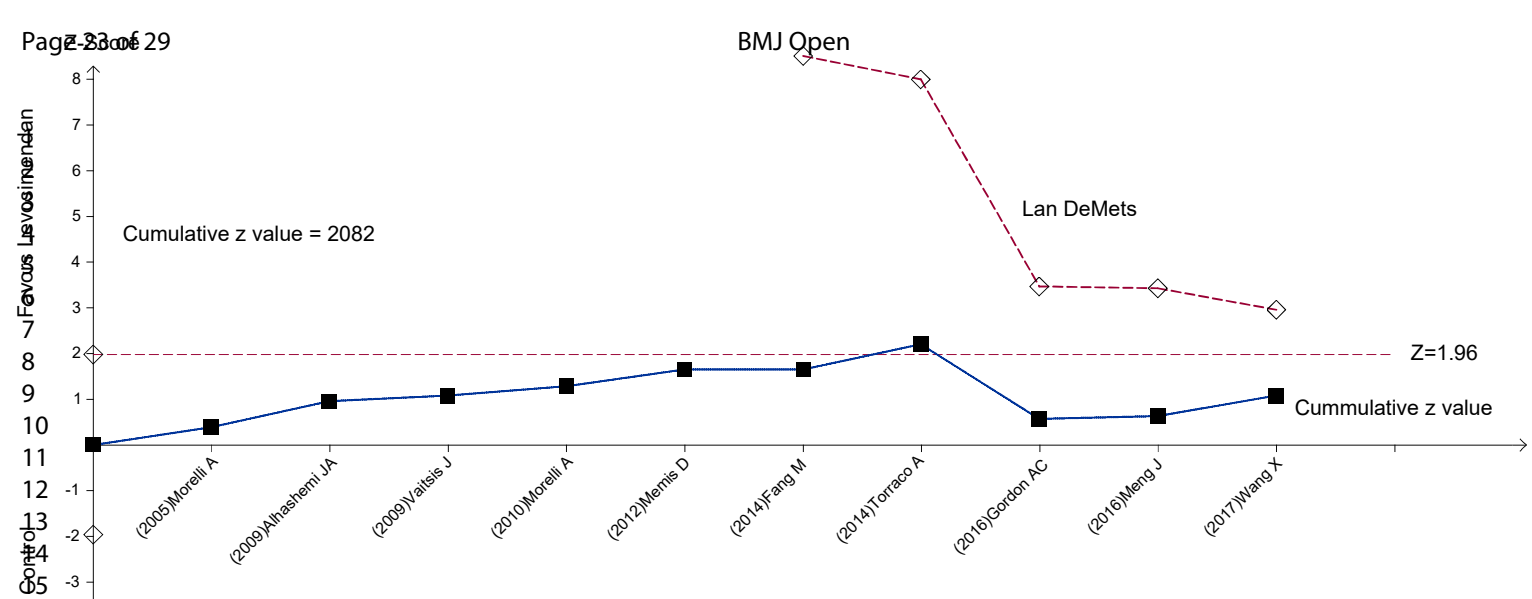
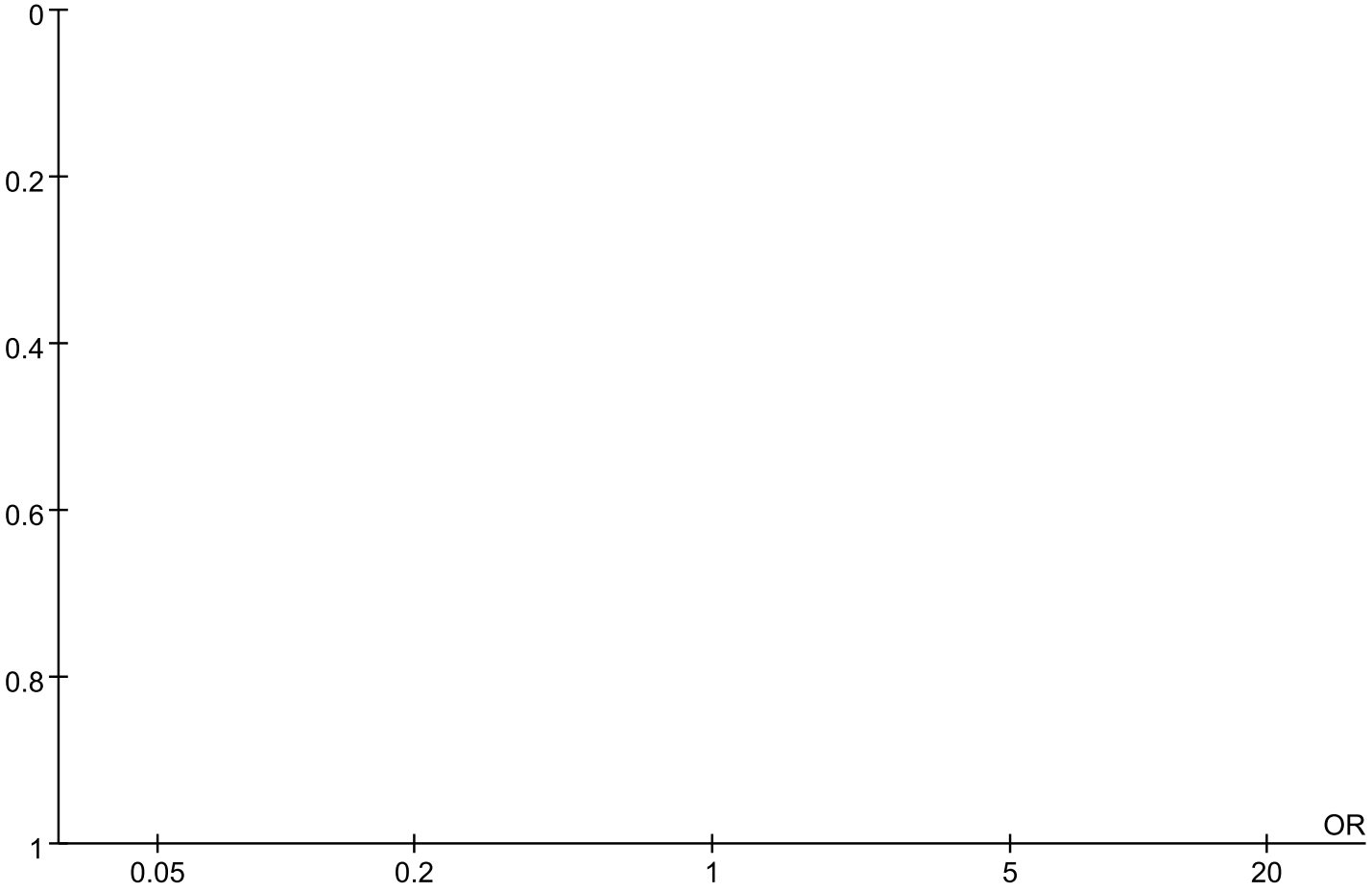
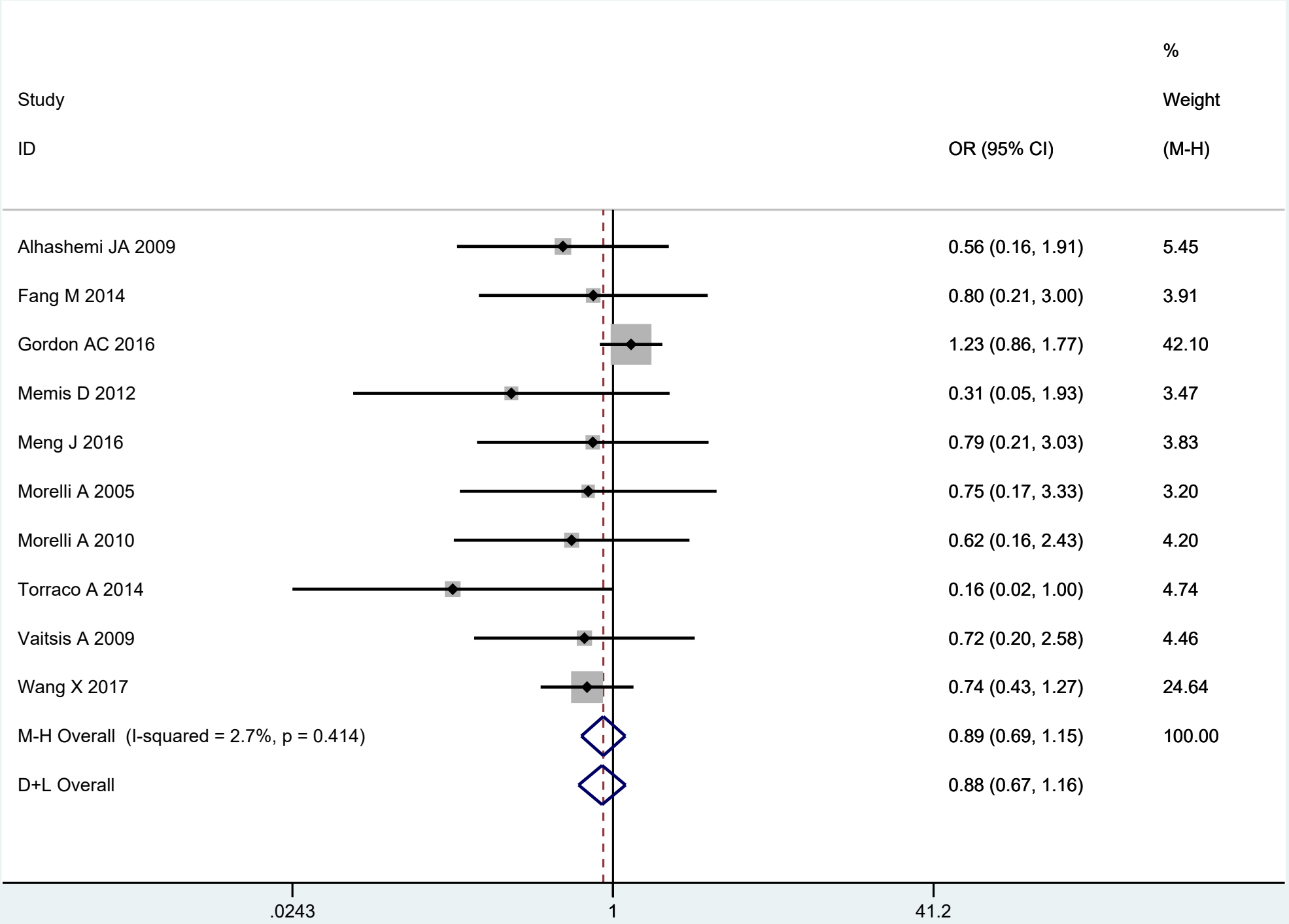


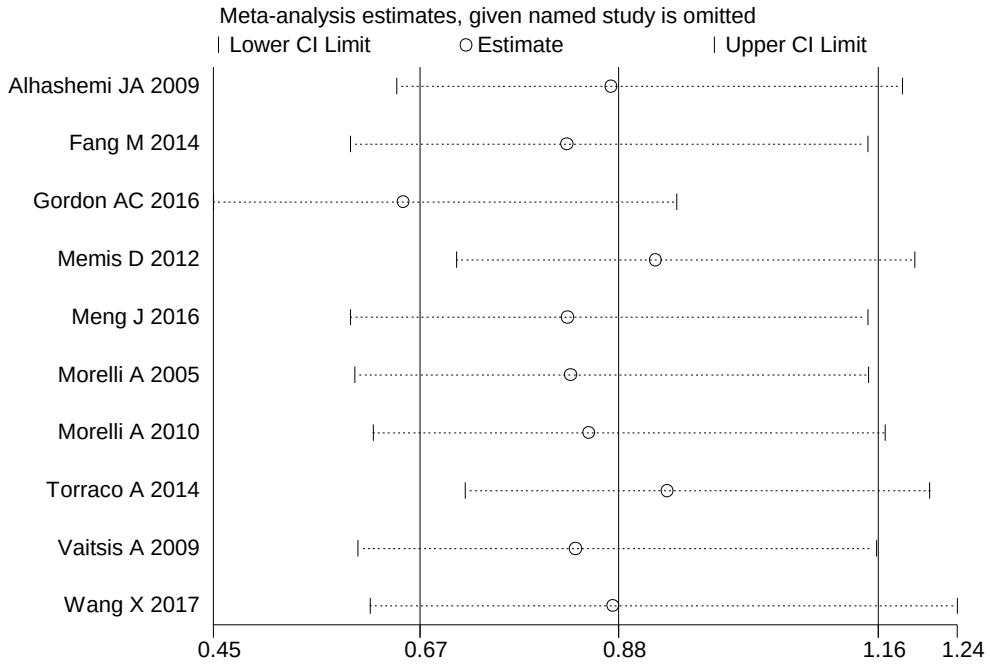
Fig 3 Sub-group analysis. (A) Levosimendan in patients with definite cardiac dysfunction vs. patients with heterogeneous cardiac function [OR (95% CI) 0.76 (0.35-1.50), P = 0.43 vs. 0.73 (0.44-1.19), P = 0.21]; (B) Levosimendan in patients with age >= 65-year-old vs. age < 65-year-old [OR (95% CI) 0.81(0.50-1.32), P = 0.40 vs. 0.67(0.32-1.40), P = 0.29]; (C) Levosimendan in patients with mortality >=50% vs. morality < 50% [OR (95% CI) 0.55 (0.30-1.03), P = 0.06 vs. 0.97 (0.71-1.33), P = 0.85]; (D) Levosimendan vs. dobutamine [OR (95% CI) 0.65 (0.39-1.10), P = 0.11] or standard therapy [OR (95%CI) 0.80 (0.40-1.58), P = 0.52].

1678x1044mm (120 x 120 DPI)









Study omitted	Estimate	[95% Conf.	Interval]
Alhashemi JA 2009	0.874	0.646	1.182
Fang M 2014	0.827	0.597	1.145
Gordon AC 2016	0.653	0.452	0.943
Memis D 2012	0.921	0.710	1.195
Meng J 2016	0.827	0.598	1.145
Morelli A 2005	0.831	0.602	1.146
Morelli A 2010	0.850	0.621	1.164
Torraco A 2014	0.933	0.719	1.211
Vaitsis A 2009	0.836	0.605	1.155
Wang X 2017	0.876	0.618	1.240
Combined	0.881	0.671	1.157

(((((levosimendan) OR simendan) OR Simadax) OR dextrosimendan)) AND ((((sepsis) OR septicemia) OR severe sepsis) OR septic shock)

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Pg. 1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Pg. 2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Pg. 4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Pg. 4-5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	-
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Pg. 5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Pg. 5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Pg. 5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Pg. 5-6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Pg. 6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Pg. 6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Pg. 6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Pg. 6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Pg. 7

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Pg. 7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Pg. 7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Pg. 7
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Pg. 7-8
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Pg. 8
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Pg. 8
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Pg. 8-9
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Pg. 9-10
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Pg. 10
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Pg. 10-11
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Pg. 11-12
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Pg. 12
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Pg. 13

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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BMJ Open

The effect of levosimendan on mortality in severe sepsis and septic shock: a meta-analysis of randomized trials

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Primary Subject Heading:	Intensive care
Secondary Subject Heading:	Intensive care, Cardiovascular medicine, Infectious diseases
Keywords:	sepsis, septic shock, septic cardiomyopathy, levosimendan, dobutamine

Title: The effect of levosimendan on mortality in severe sepsis and septic shock: a meta-analysis of randomized trials

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ABSTRACT

Objective We aim to synthesize the up-to-date randomized trials to investigate the effects of levosimendan on mortality and clinical outcomes in severe sepsis and septic shock.

Methods A collection of databases including PubMed, EMBASE, Cochrane Central register and Web of Science were searched updated to August, 2017. Randomized trials were included when relevant to the use of levosimendan in severe sepsis or septic shock compared with any category of inotropes, or as an adjunct to standard therapy with mortalities reported. The primary outcome was the mortality, and the secondary outcomes were clinical performances including serum lactate, cardiac function, vasopressor requirements, fluid infusion and length of intensive care unit (ICU) stay.

Results A final of 10 studies with 1036 patients were included in this meta-analysis. The results revealed that levosimendan could not reduce mortality significantly in septic shock (odds ratio 0.89, 95% CI 0.69-1.16, P = 0.39). Levosimendan could reduce serum lactate more effectively, enhance cardiac contractibility with increased cardiac index and left ventricular ejection fraction. However, it could also increase fluid infusion, and no differences in norepinephrine requirement and length of ICU stay were noted. No significant benefit in mortality could be observed of levosimendan vs. dobutamine use, or in patients with definite cardiac dysfunction.

Conclusions Current evidence is not sufficient to support levosimendan as superior to dobutamine or as an optimal adjunct in severe sepsis and septic shock. More large-scale randomized trials were necessary for the validation of the levosimendan use in sepsis.

Key words sepsis; septic shock; levosimendan; dobutamine; septic cardiomyopathy

Strengths and Limitations of this Study

1. This article synthesized the up-to-date random trials for comprehensive analysis of the effect of levosimendan on mortality in severe sepsis and septic shock.

2. Furthermore, a serious of sub-group analyses were conducted for investigation of the sub-population of patients who were likely to benefit most in levosimendan use.

3. Heterogeneity and biases were appraised between each study, and the optimal of sample size was also calculated.

4. However, the trials included were of limited sample size and quality, and were potentially high biased.

BACKGROUND

Sepsis is still a great challenge to the public health and its mortality increases tremendously when severe sepsis or septic shock occurs[1]. The incidence of cardiac dysfunction in severe sepsis and septic shock remains as high as 40%-60%[2] resulted from infectious process, cytokine storm[3], decreased myocardial perfusion and pulmonary injuries[4], and is associated with worse outcomes[5, 6].

Surviving Sepsis Campaign International Guidelines (2016) recommended the usage of dobutamine infusion in patients with persistent hypo-perfusion despite adequate fluid loading and the use of vasopressor agents (weak recommendation, low quality of evidence)[7]. However, its effect on mortality in sepsis is still under debate[8], and its adverse effects including increased myocardial oxygen consumption and risks of dysrhythmia could not be neglected.

Levosimendan, a calcium sensitizer with vasodilatory properties which could improve myocardial contractibility in the absence of increased oxygen consumption, is regarded as a promising adjunct in the treatment of both cardiac systolic and diastolic dysfunctions[9] and was demonstrated to have a beneficial effect on mortality in various clinical settings[10, 11].

Levosimendan was demonstrated superior to dobutamine and milrinone in restoring cardiac function in septic animal model[12]. It could also alleviate inflammatory response by NF-κB-dependent transcription down-regulation[13] and decreased inducible NO synthetase (iNOS) promoter activity and NO expression in vitro[14].

Several meta-analyses were conducted to investigate the effects of levosimendan on mortality in sepsis which revealed a beneficial effect on survival, however with limited sample size[15]. In this study, we aim to make an up-to-date meta-analysis to investigate the effects of levosimendan on

mortality in severe sepsis and septic shock.

METHODS

The manuscript was prepared according to the preferred reporting items for systematic review and meta-analysis (PRISMA) statement[16, 17].

Eligibility Criteria

We aimed to include all the randomised control trials (RCT) studying levosimendan use versus any categories of inotropes or as an adjunct to standard management in severe sepsis and septic shock. The articles would be included in our study if fulfilling the following criteria: (1) study population of severe sepsis or septic shock in adults, (2) randomized allocation of treatment, (3) comparison of levosimendan with any category of inotropic agents or placebo, with no restrictions on dose regimen or time limits of levosimendan infusion, (4) data on mortality reported; and exclusion criteria were as follows: (1) duplicates, (2) pediatric subjects, (3) animal experiments or *in vitro* studies, (4) no sepsis population and (5) lack of data on mortality.

Information Sources

Two investigators searched a collection of data-bases including PubMed, EMBASE, Cochrane Central register and Web of Science updated to July 31, 2017 separately with no language restrictions. When relevant systemic reviews or meta-analyses were found, we ran a backward snowballing to obtain further studies.

Search

Following key words were used as search terms: "levosimendan", "simendan", "Simdax", "dextrosimendan", "sepsis", "severe sepsis", "septicemia" and "septic shock". [Supplementary File 1]

Study Selection

Abstracts and titles of the articles were initially viewed separately by two investigators, if potentially pertinent, the complete articles were retrieved. Articles were assessed and selected separately by two investigators with disagreements solved by consensus.

Data Items

Information was extracted from each of the included trials on: (1) characteristics of the participants (including gender, age and diagnosis); (2) interventions (including the duration and dose regimen of the levosimendan or other inotropes); (3) outcome measurements with primary outcome determined as the mortality (follow-up time was tailored at the approximate duration by the reviewer's consensus), and secondary outcomes as clinical outcomes including serum lactate level, cardiac function including cardiac index (CI), left ventricular ejection fraction (LVEF) and left ventricular stroke work index (LVSWI); fluid infusion, vasopressor requirement and length of ICU stay (LOS).

Assessment of Risk of Bias

Internal validity and risks of bias were evaluated by two investigators separately following Cochrane Collaboration Methods protocols[18]. Risks of bias were assessed by scrutinizing the articles and rated as "Yes", "No" or "Unclear" according to the procedures taken in the articles.

Summary Measures

Dichotomous outcomes were measured as proportions and odds ratio (OR) were calculated. Continuous outcomes were described as mean ± standard deviation (SD) and calculated by mean difference (MD) or standard mean difference (SMD). The end-point and change range were both compared if the continuous variables were measured at baseline and after treatment. Missing data

were imputed from other information whenever possible[19][Supplementary File 2].

Statistical Analysis

The data retrieved from the pertinent articles were computerized and analyzed by Review Manager 5.3 (The Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen). We used Mantel-Haenszel statistic method for dichotomous variable (mortality) measurements and inverse variance for continuous variables (lactate level, CI, LVEF, LVSWI, fluid infusion, norepinephrine dose and LOS). Random-effects model was used for better accommodation of heterogeneity. Cochrane I^2 statistic was used for heterogeneity assessment between the studies, with a range of 0% to 30% representing no or mild heterogeneity, 30%-60% moderate heterogeneity, whereas > 60% as high heterogeneity. Publication bias was tested by visual inspection of funnel plots. As for sensitivity analysis, the dataset was analyzed in both fixed and randomized-effects models and the favoring directions were inspected, and each study was removed sequentially and the remaining data-set re-analyzed to assess the robustness of the results.

Trial sequential analysis was performed to estimate the optimal sample size for the plausible effects of levosimendan in sepsis[20]. Statistical significance was set at 2-lateral 0.05 level as hypothesis establishment.

Sub-group Analysis

Sub-group analyses were conducted dividing studies enrolling the patients with cardiac dysfunction vs. heterogeneous cardiac function. The use of levosimendan vs. dobutamine and vs. standard therapy was also compared. We further attempt to separate the studies with the patients with average age ≥ 65-years vs. < 65-years and mortality ≥ 50% and < 50% in the hope of finding the sub-population who would potentially benefit from the levosimendan use.

RESULTS

Study Selection

A total of 336 abstracts were yielded from the search strategy, with 218 duplicates were excluded and 125 excluded due to no eligible abstracts. Complete manuscripts of 93 abstracts were retrieved for further assessment, within which 46 were animal experiments, 4 non-RCTs, 16 non-septic patients, 8 pediatric patients, 3 with no mortality reported, 6 case reports and 1 study design. A final of 10 studies were included in this meta-analysis[21-30], within which two were conference abstracts[21, 22], and one was written in Chinese[26] [Fig 1].

Study Characteristics

Within the 10 studies enrolling 1036 patients, no differences were present in age and APACHE II scores between the treatment and control group at the baseline. Patients diagnosed as septic shock or severe sepsis after adequate fluid resuscitation were included in each study, and four studies set explicit criteria of cardiac dysfunctions during patient recruitment[21, 26, 27, 30]. Norepinephrine was used as necessary to achieve the target MAP ranging from 65 to 80mmHg during inotropic therapy depending on the study design. Seven studies used dobutamine (dose ranges from 5μg/kg per min to 20μg/kg per min) as a comparator[21-24, 26, 27, 30] and three used levosimendan as an adjunct to standard therapy[25, 28, 29]. Levosimendan was administered as continuous infusion (dose ranges from 0.05μg/kg per min to 2.0μg/kg per min) over 24 hours with no bolus. Parameters reflecting cellular metabolism, microcirculation, hemodynamics, cardiac function and target organ perfusion were measured in individual studies [Tab 1].

Syntheses of Results

Mortality data were randomized and calculated from the ten studies, and the final result in

mortality at the longest follow-up day revealed no statistical difference (total events 187/522 vs. 197/514 in levosimendan and control group respectively, OR 0.89, 95% CI 0.69-1.16, $P = 0.39$), with no evidence of heterogeneity ($I^2 = 0\%$, $P = 0.52$) [Fig 2].

We conducted a serious of sub-group analyses according to the patients' characteristics. No statistical significance could be observed dividing the studies enrolling patients with definite clinical cardiac dysfunction[21, 26, 27, 30] (OR 0.76, 95% CI 0.39-1.50, $P = 0.43$) or those with homogenous cardiac functions[22-25, 28, 29] (OR 0.75, 95% CI 0.48-1.19, $P = 0.23$).

We also compared the effects of levosimendan vs. dobutamine on mortality in sepsis and find no statistical difference in mortality between levosimendan and dobutamine group (OR 0.65, 95% CI 0.39-1.10, $P = 0.11$) [21-24, 26, 27, 30], neither of levosimendan in comparison of standard therapy[25, 28, 29] (OR 0.82, 95% CI 0.44-1.55, $P = 0.54$) [Fig 3].

We attempted to divide the studies according to the patients' average age (< 65yr vs. ≥ 65yr) and mortality (< 50% vs. ≥ 50%), and found no statistical significance between each sub-group [Supplementary Fig 1].

We also extracted and compared the data of lactate reduction[22, 23, 26, 28, 30], measurements reflecting cardiac functions including CI[23, 25-28, 30], LVEF[21, 26, 27, 30] and LVSWI[23, 26, 27, 30], fluid infusion[23, 26, 28, 30], norepinephrine dosage[23, 25-27, 30] and LOS[23, 24, 27-29]. The results revealed that lactate was more profoundly reduced, and cardiac function significantly improved (with increased CI, LVEF and LVSWI) in levosimendan group. Norepinephrine dose was reduced slightly, however total fluid infusion over 24 hours was tremendously increased in levosimendan group. LOS in levosimendan group was slightly shortened ($P = 0.29$) [Tab 2, Supplementary Fig 2].

Risk of Bias and Sensitivity Analyses

The funnel plot was drawn for testing the bias, and visual inspection of the funnel plot revealed potential asymmetry [Supplementary Fig 3].

The data-set was analyzed both in the fixed and random-effects model for sensitivity analysis and the result revealed no shift of favouring directions [Supplementary Fig 4]. Each trial was removed and remaining dataset re-analyzed subsequently, and the result indicated that the statistical significance obscured only when the trial by Gordon AC et al. [28], was put into analysis [Supplementary Fig 5].

Trial Sequential Analysis

A trial sequential analysis (TSA) was performed to determine the optimal information size. We estimated a 26% mortality based on the recent epidemiologic data of severe sepsis[31], and an assumed an average of 20% relative risk reduction in reference to the effect of levosimendan on overall mortality reduction in hospitalized patients[32] with 80% power and $\alpha = 0.05$ two-sided. The calculation indicated the optimal information size of 2082 patients for detection of the plausible treatment effect of levosimendan in sepsis. The Lan DeMets sequential monitoring boundary constructed by the optimal information size was not crossed, indicating that the cumulative evidence was not conclusive and reliable [Fig 4].

DISSCUSSION

The main finding of this study was that levosimendan could not reduce the mortality in severe sepsis and septic shock patients significantly. Furthermore, levosimendan could reduce serum lactate level more effectively, improve cardiac function. However, no change in norepinephrine dose but profound increase in fluid infusion, and no difference in LOS has been noted.

We noticed that, albeit improved cardiac function more fluid was infused after levosimendan use

for maintenance of the target MAP probably due to its vasodilatory effect, which could exacerbate pulmonary and peripheral edema and potentially impeding oxygen uptake and exchange. The use of levosimendan was also suggested to be accompanied with higher incidence of life-threatening arrhythmias like supraventricular tachyarrhythmia, which could bring hemodynamic instability and risks to the patients[28].

The previous study by Zangrillo et al. enrolling a series of RCTs yielded a significantly reduced mortality in levosimendan group in septic shock[15]. However, it should be concerned that, in our study, statistical significance was obscured after a large, multi-center RCT with a sample size of 514 patients by Gordon AC et al.[28] were included.

We thought that there may be several seasons for this. The percentage of patients in the trial by Gordon et al. that underwent cardiac function assessment was rather low (30%), so Gordon and co-workers might have enrolled the patients with heterogenous cardiac function[33]. Although the prevalence of septic cardiomyopathy is high (40-60%), but the discriminative enrollment could still obliviate the potential benefit of levosimendan, considering that there might be patients recruited who did not have cardiac dysfunction, and may not benefit from inotropic use as indicated by the SSC (2016) Guideline in which the increase of cardiac function to supranormal level is discouraged[7].

We synthesized the studies with patients who had definite cardiac dysfunction, however the result revealed no statistical significance (OR 0.76, 95% CI 0.39-1.50, $P = 0.43$). We then ran a TSA and yielded an optimal sample size of 1719, suggesting more trials are needed focusing on the patients with cardiac dysfunction for the plausible effects of levosimendan in sepsis.

The patients enrolled in the trial by Gordon et al. might be relatively at low risk[33, 34]. Although

the 28-day mortality in that trial was 31%, which was markedly high, however, according to previous studies, the mortality decreased from 61% to 47% after levosimendan use[15]. It should be noted that the baseline mortality is very high (61% in control group), suggesting that the patients at "extremely" high risk may be most benefited from levosimendan use.

We also attempted to synthesized the studies dividing the studies with patients at high (≥ 50%) and low (< 50%) risks and found with OR 0.55, 95% 0.30-1.03 vs. OR 0.89, 95% 0.69-1.16, respectively, suggesting patients with high-risk were possibly more likely to benefit from levosimendan use, still, more trials are definitely needed.

Limitations

Our study had several limitations. The trials included in this meta-analysis were of limited sample size, 8 out of 10 studies included less than 50 patients[21-27, 30], and were potentially high biased. Follow-up duration was not reported in one study[24], only ICU mortality was reported in two studies[22, 23], and the inconsistency in follow-up duration could potentially bring bias to the results. The dose regimen of levosimendan ranged from 0.05 to 0.2 μg/kg per min, which could cause different hemodynamic effects.

CONCLUSION

Although levosimendan could improve clinical outcomes including cardiac function and tissue perfusion compared with dobutamine or standard therapy, it also increases fluid infusion and has no significance on vasopressor requirements, still, it failed to bring significant benefits to mortality in sepsis. More RCTs are necessary for further elucidation of the effects of levosimendan in sepsis, particularly in those with cardiac dysfunctions.

LIST OF ABBREVIATIONS

APACHE Acute Physiology and Chronic Health Evaluation;

CI cardiac index;

ICU intensive care unit;

iNOS inducible NO synthetase;

IQR inter-quartile range;

LOS length of ICU stay;

LV left ventricle;

LVEF left ventricle ejection fraction;

LVSWI left ventricular stroke work index;

MAP mean arterial pressure;

MD mean difference;

NE norepinephrine;

OR odds ratio;

RCT randomized control trial;

ROS reactive oxygen species;

SD standard deviation;

SMD standard mean difference;

TSA trial sequential analysis.

DECLARATIONS

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

WC carried out the analysis and interpretation of data and participated in drafting, editing and submitting the manuscript. The articles were reviewed by two reviewers (WC and JFX) independently in accordance with the inclusion criteria. Disagreements were resolved and by consensus and discussion including a third reviewer (JYX). The quality of the articles was assessed by WC and JFX independently, with disagreements resolved by consulting a third reviewer (JYX). YY was responsible for conception, design and coordination of the study, and revising the manuscript for important intellectual content. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

Fig 1 Flow diagram of search process and study selection

Fig 2 The effect of levosimendan on mortality in severe sepsis and septic patients.

Fig 3 Sub-group analysis. (A) Levosimendan in patients with definite cardiac dysfunction vs. patients with heterogeneous cardiac function (OR 0.76, 95% CI 0.35-1.50, *P* = 0.43 vs. OR 0.75, 95% CI 0.48-1.19, *P* = 0.39); (B) Levosimendan vs. dobutamine (OR 0.65, 95% CI 0.39-1.10, *P* = 0.11) or standard therapy (OR 0.82, 95% CI 0.44-1.55, *P* = 0.54).

Fig 4 Trial sequential analysis. The optimal information size of 2082 patients for detection of the plausible treatment effect of levosimendan in sepsis, and the Lan DeMets sequential monitoring boundary constructed by the optimal information size was not crossed

Supplementary Fig 1 Sub-group analysis. (A) Levosimendan in patients with mortality >=50% vs. morality < 50% (OR 0.55, 95% CI 0.30-1.03, *P* = 0.06 vs. OR 0.99 95% CI 0.74-1.32, *P* = 0.92); (B) Levosimendan in patients with age >= 65-year-old vs. age < 65-year-old (OR 0.84 95% CI 0.54-1.30, *P* = 0.44 vs. OR 0.67 95% CI 0.32-1.40, *P* = 0.49).

Supplementary Fig 2 Forest plots for secondary outcomes.

Supplementary Fig 3 Funnel plot for inspection of bias

Supplementary Fig 4 Sensitivity analysis with data-set analyzed in fixed and random-effects models

Supplementary Fig 5 Sensitivity analysis with single study omitted sequentially

Tab 1 Characteristics of the included trials. MAP mean artery pressure; LVEF left ventricular ejection fraction; PAOP: pulmonary artery occlusion pressure; CI cardiac index; NR not reported; SOFA Sequential Organ Failure Assessment; * A total of 256 patients were finally included for 28-day mortality analysis; ** Two patients in control group failed to complete the study and were excluded.

Study	Year	Subjects No	Levosimendan group	Control group	Inclusion criteria	Cardiovascular criteria	Levosimendan therapy	Control therapy	Target MAP (mm Hg)	Follow-up (day)	Primary outcome
Alh	20	42	21	21	Severe	NR	0.05 to 2μg/kg per min, 24hr	Dobutamine 5 to	⩾65	IC	ScvO2 and serum lactate

ashemi JA [22]	09				sepsis/ septic shock			20μg/kg per min, 7 days		U stay	
Fang M [26]	2014	36	18	18	Septic shock	LVEF≤45%	Dobutamine 0.5μg/kg per min for 24hr; levosimendan 0.2μg/kg per min 24hr subsequently	Dobutamine 5μg/kg per min, 48hr	NR	28	Hemodynamics and cardiac function
Gordon AC [28]	2016	515	258	257*	Septic shock	MAP 60 to 70mmHg	0.05 to 0.2μg/kg per min, 24hr	Standard therapy	65 to 70	28	Daily SOFA score
Memis D [24]	2012	30	15	15	Septic shock	MAP≤ 65mmHg	0.1μg/kg per min, 24hr	Dobutamine 10μg/kg per min, 24hr	>65	NR	Liver function
Meng J [27]	2016	38	19	19	Septic shock	MAP≥ 65mmHg and LVEF≤45%	0.2μg/kg per min, 24hr	Dobutamine 5μg/kg per min, 24hr	≥65	28	Hemodynamics and myocardial injury biomarkers
Morelli A [30]	2005	28	15	13**	Septic shock	MAP 70 to 80mmHg, PAOP≥ 12mmHg and LVEF<45%	0.2μg/kg per min, 24hr	Dobutamine 5μg/kg per min, 24hr	70 to 80	30	Hemodynamics and cardiac function
Morelli A [23]	2010	40	20	20	Septic shock	MAP≥ 65mmHg	0.2μg/kg per min, 24hr	Dobutamine 5μg/kg per min, 24hr	70 ± 5	ICU stay	Systemic and microvascular hemodynamics
Torraco A [25]	2014	26	13	13	Septic shock	MAP≥ 65mmHg	0.2μg/kg per min, 24hr	Standard therapy	65 to 75	28	Mitochondrial function
Vaitsis J [21]	2009	42	23	19	Sepsis	CI<2.2, LVEF<35%	0.1μg/kg per min, 24hr	Dobutamine 5 to 10μg/kg per min, 24hr	>65	30	Mortality at 7 and 30 days
Wang X [29]	2017	240	120	120	Septic shock	MAP≥65mm Hg	0.1-0.2 μg/kg per min, 24 hours	Standard care	≥65	28	Mortality at 28 days, ICU discharge and hospital discharge

Note: MAP: mean artery pressure; LVEF: left ventricular ejection fraction; PAOP: pulmonary artery occlusion pressure; CI: cardiac index; NR: not reported; SOFA: Sequential Organ Failure Assessment; * A total of 256 patients were finally included for 28-day mortality analysis; ** Two patients in control group failed to complete the study and were excluded.

Tab 2 Clinical outcomes after randomization. Subscript TRT stands for outcomes after treatment; Δ stands for change range of outcomes (value after treatment subscribes value at baseline); CI cardiac index; LVSWI left

ventricular stroke work index; LVEF left ventricular ejection fraction; NE Norepinephrine; LOS length of ICU stay; * Standard mean difference (SMD) is used in this case due to large difference in means (MD 1048.74, 95% CI 303.21-1794.27).

Outcomes	References	No. of subjects	MD (95% CI)	*P* for overall effect	*P* for heterogeneity	I^2 (%)
$Lactate_{TRT}$	[22], [23], [26], [27], [28], [30]	656	-0.89 (-1.48, -0.29)	0.003	< 0.00001	87
ΔLactate	[23], [26], [27], [28], [30]	614	-0.98 (-1.59, -0.37)	0.002	0.03	62
CI_{TRT}	[23], [26], [27], [28], [30]	277	0.39 (0.17, 0.62)	0.0005	0.05	59
ΔCI	[21], [23], [26], [27], [28], [30]	319	0.46 (0.28, 0.64)	< 0.00001	0.01	65
$LVSWI_{TRT}$	[26], [27], [30]	102	3.73 (0.49, 6.98)	0.02	0.0009	86
ΔLVSWI	[23], [26], [27], [30]	142	5.00 [3.95, 6.06]	< 0.00001	0.83	0
$LVEF_{TRT}$	[26], [27], [30]	102	6.76 [3.53, 10.00]	< 0.0001	0.75	0
ΔLVEF	[21], [26], [27], [30]	144	4.98 [0.75, 9.21]	0.02	0.001	81
$Norepinephrine\ dose_{TRT}$	[23], [26], [27], [30]	142	-0.08 [-0.21, 0.06]	0.26	< 0.00001	95
ΔNE dose	[23], [25], [27], [30]	132	-0.04 [-0.12, 0.04]	0.3	0.08	55
Fluid infusion in 24-hr	[23], [26], [28], [30]	581	2.72 [0.75, 4.69]*	0.007	< 0.00001	97
LOS	[23], [24], [27-29]	863	-1.36 [-3.87, 1.14]	0.29	0.02	65

Note: Subscript TRT stands for outcomes after treatment; Δ stands for change range of outcomes (value after treatment subscribes value at baseline); CI cardiac index; LVSWI left ventricular stroke work index; LVEF left ventricular ejection fraction; NE Norepinephrine; LOS length of ICU stay; * Standard mean difference (SMD) is used in this case due to large difference in means (MD 1048.74, 95% CI 303.21-1794.27).

Supplementary File 1 Full electronic search strategy for PubMed.

Supplementary File 2 Methods of the imputation of missing data.

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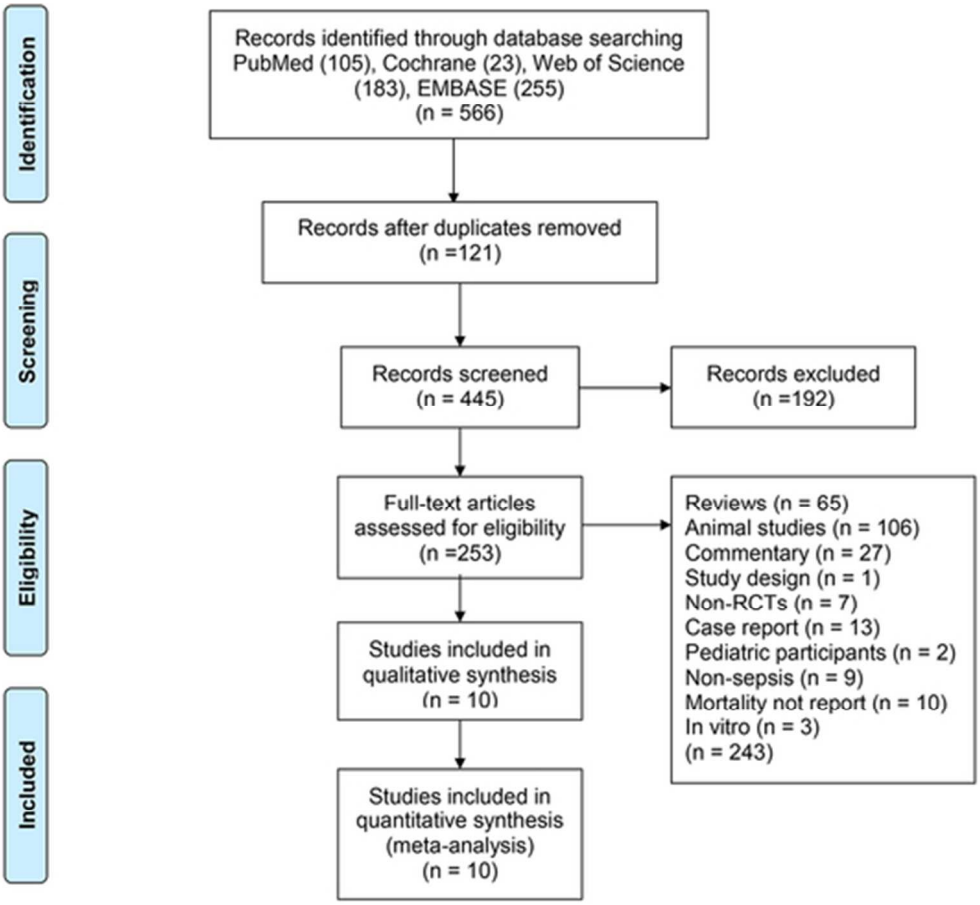

Fig 1 Flow diagram of search process and study selection

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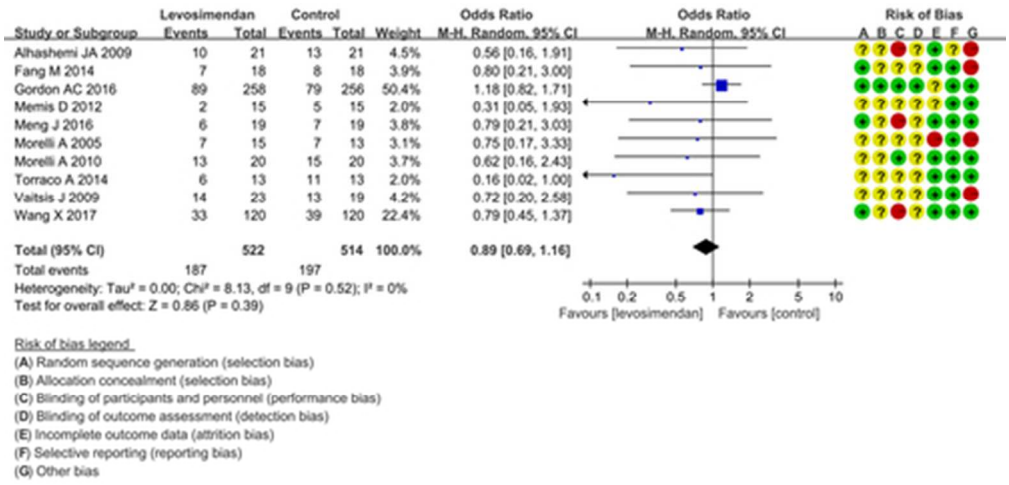

Fig 2 The effect of levosimendan on mortality in severe sepsis and septic patients.

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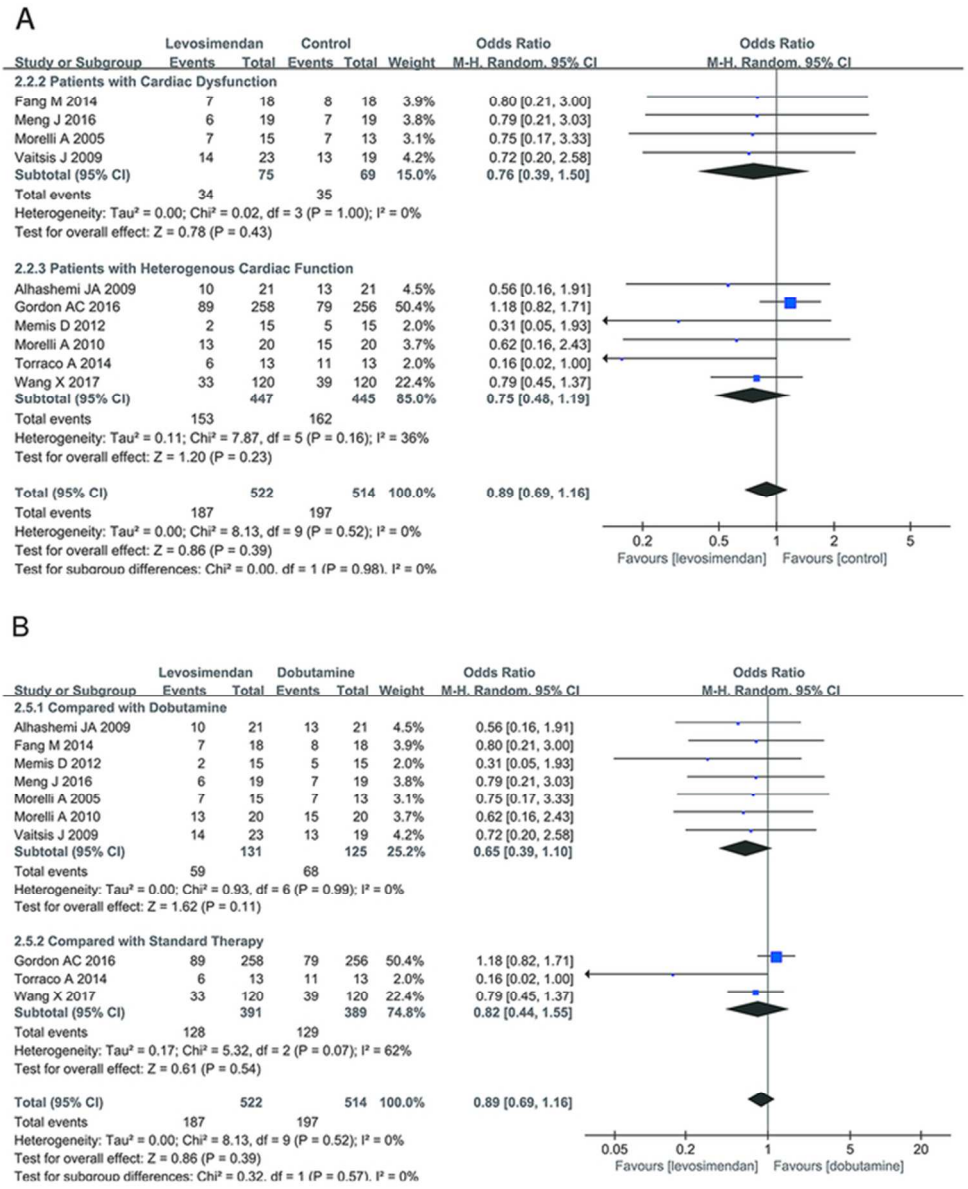

Fig 3 Sub-group analysis. (A) Levosimendan in patients with definite cardiac dysfunction vs. patients with heterogeneous cardiac function (OR 0.76, 95% CI 0.35-1.50, P = 0.43 vs. OR 0.75, 95% CI 0.48-1.19, P = 0.39); (B) Levosimendan vs. dobutamine (OR 0.65, 95% CI 0.39-1.10, P = 0.11) or standard therapy (OR 0.82, 95% CI 0.44-1.55, P = 0.54).

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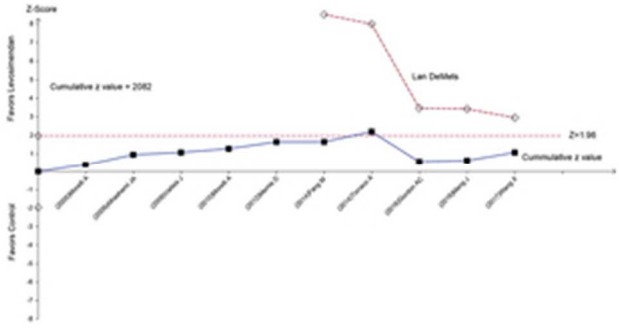

Fig 4 Trial sequential analysis. The optimal information size of 2082 patients for detection of the plausible treatment effect of levosimendan in sepsis, and the Lan DeMets sequential monitoring boundary constructed by the optimal information size was not crossed

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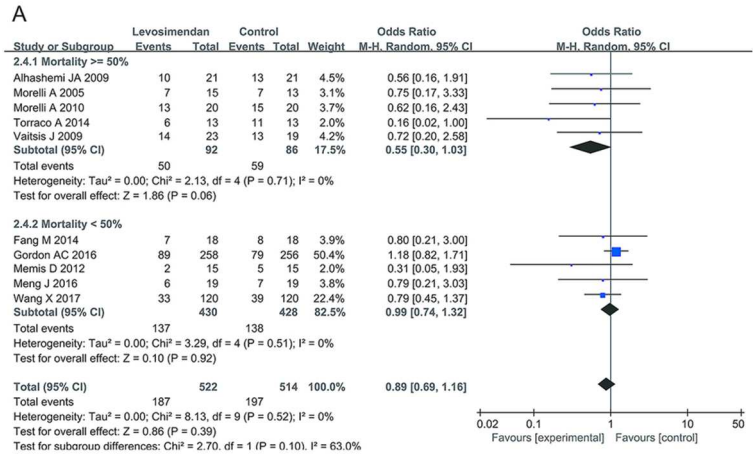


Study	Year	Age† (years)
Alhashemi JA [22]	2009	NR
Fang M [26]	2014	61.4±7.1 in levosimendan group; 61.7±7.3 in dobutamine group
Gordon AC [28]	2016	67 (58-75) in levosimendan group; 69 (58-77) in control group
Memis D [24]	2012	54.93±18.92 in levosimendan group; 56.27±14.93 in dobutamine group
Meng J [27]	2016	55.4±17.5 in levosimendan group; 50.2±13.6 in dobutamine group
Morelli A [30]	2005	61.5±7.0 in levosimendan group; 62.4±7.3 in dobutamine group
Morelli A [23]	2010	68 (55-74) in levosimendan group; 66 (54-78) in control group
Torraco A [25]	2014	70 (58-80) in levosimendan group; 68 (57-79) in control group
Vaitsis J [21]	2009	66.1±7.54
Wang X [29]	2017	70 (67-74) in levosimendan group; 69 (67-73) in control group

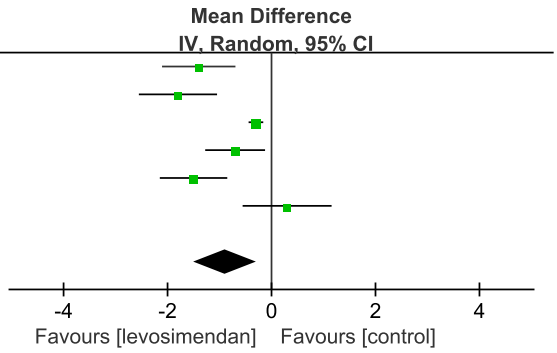
† Age presented as mean ± SD or median (IQR)

40x64mm (600 x 600 DPI)

1. The effect of levosimendan on lactate reduction. The lactate levels (mmol/L) after treatment were compared.

Study or Subgroup	Levosimendan Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Alhashemi JA 2009	2.1	0.92	21	3.5	1.37	21	16.0%	-1.40 [-2.11, -0.69]
Fang M 2014	3.4	1.1	18	5.2	1.2	18	15.5%	-1.80 [-2.55, -1.05]
Gordon AC 2016	1.4	0.74	236	1.7	0.82	236	20.3%	-0.30 [-0.44, -0.16]
Meng J 2016	3.6	0.8	19	4.3	1	19	17.3%	-0.70 [-1.28, -0.12]
Morelli A 2005	3.7	0.7	15	5.2	1	13	16.5%	-1.50 [-2.15, -0.85]
Morelli A 2010	1.9	0.96	20	1.6	1.7	20	14.4%	0.30 [-0.56, 1.16]
Total (95% CI)			**329**			**327**	**100.0%**	**-0.89 [-1.48, -0.29]**

Heterogeneity: Tau² = 0.45; Chi² = 37.36, df = 5 (P < 0.00001); I² = 87%
Test for overall effect: Z = 2.93 (P = 0.003)

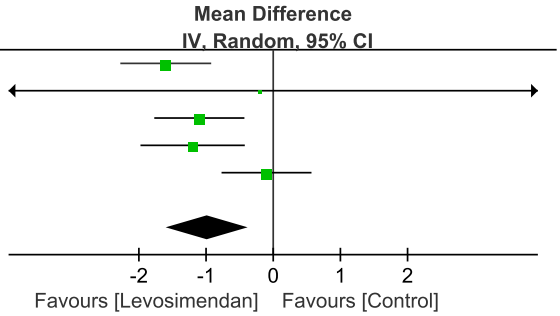


2. The effect of levosimendan on lactate reduction. The lactate level (mmol/L) changes were compared.

Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Fang M 2014	-1.7	1.01	18	-0.1	1.06	18	25.0%	-1.60 [-2.28, -0.92]
Gordon AC 2016	-0.8	21.85	236	-0.6	24.94	236	2.0%	-0.20 [-4.43, 4.03]
Meng J 2016	-1.5	1.06	19	-0.4	1.05	19	25.1%	-1.10 [-1.77, -0.43]
Morelli A 2005	-1.2	1.04	15	0	1.05	13	22.8%	-1.20 [-1.98, -0.42]
Morelli A 2010	-0.4	1.09	20	-0.3	1.07	20	25.2%	-0.10 [-0.77, 0.57]
Total (95% CI)			**308**			**306**	**100.0%**	**-0.98 [-1.59, -0.37]**

Heterogeneity: Tau² = 0.27; Chi² = 10.43, df = 4 (P = 0.03); I² = 62%

Test for overall effect: Z = 3.15 (P = 0.002)

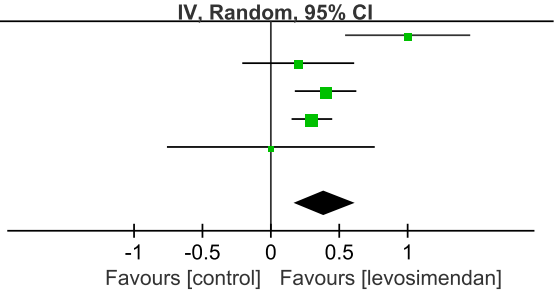


3. The effect of levosimendan on cardiac index (CI). The CIs (L/min/m^2) after treatment were compared.

	levosimendan			Control				Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI
Fang M 2014	4.6	0.7	18	3.6	0.7	18	14.8%	1.00 [0.54, 1.46]
Gordon AC 2016	3.5	1.4	69	3.3	1	66	16.9%	0.20 [-0.21, 0.61]
Meng J 2016	3.5	0.3	19	3.1	0.4	19	28.0%	0.40 [0.18, 0.62]
Morelli A 2005	4.5	0.2	15	4.2	0.2	13	33.4%	0.30 [0.15, 0.45]
Morelli A 2010	4.1	1.19	20	4.1	1.26	20	7.0%	0.00 [-0.76, 0.76]
Total (95% CI)			**141**			**136**	**100.0%**	**0.39 [0.17, 0.62]**

Heterogeneity: Tau² = 0.03; Chi² = 9.71, df = 4 (P = 0.05); I² = 59%

Test for overall effect: Z = 3.47 (P = 0.0005)

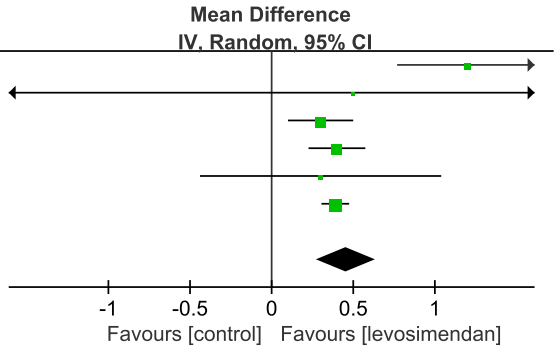


4. The effect of levosimendan on cardiac index (CI). The CI (L/min/m^2) changes after treatment were compared.

Study or Subgroup	Levosimendan Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Fang M 2014	1.4	0.66	18	0.2	0.66	18	11.6%	1.20 [0.77, 1.63]
Gordon AC 2016	0.6	11.78	69	0.1	9.59	66	0.2%	0.50 [-3.12, 4.12]
Meng J 2016	0.5	0.26	19	0.2	0.36	19	24.4%	0.30 [0.10, 0.50]
Morelli A 2005	0.4	0.2	15	0	0.26	13	26.3%	0.40 [0.23, 0.57]
Morelli A 2010	0.5	1.12	20	0.2	1.26	20	5.0%	0.30 [-0.44, 1.04]
Vaitsis J 2009	1.79	0.16	23	1.4	0.12	19	32.4%	0.39 [0.31, 0.47]
Total (95% CI)			**164**			**155**	**100.0%**	**0.46 [0.28, 0.64]**

Heterogeneity: Tau² = 0.02; Chi² = 14.31, df = 5 (P = 0.01); I² = 65%

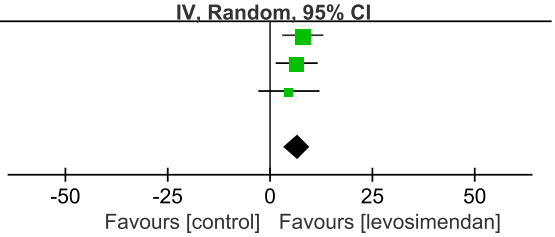
Test for overall effect: Z = 5.03 (P < 0.00001)



5. The effect of levosimendan on left ventricular ejection fraction (LVEF). The LVEF (%) after treatment were compared.

Study or Subgroup	Levosimendan Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Fang M 2014	46.3	6.8	18	38.3	8.5	18	41.4%	8.00 [2.97, 13.03]
Meng J 2016	45.6	7.6	19	39.1	8.5	19	39.8%	6.50 [1.37, 11.63]
Morelli A 2005	45.4	8.4	15	40.8	11.3	13	18.8%	4.60 [-2.87, 12.07]
Total (95% CI)			**52**			**50**	**100.0%**	**6.76 [3.53, 10.00]**

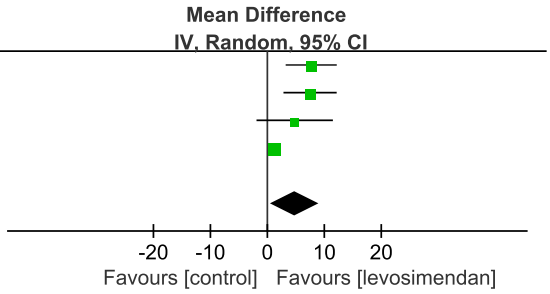
Heterogeneity: Tau² = 0.00; Chi² = 0.56, df = 2 (P = 0.75); I² = 0%
Test for overall effect: Z = 4.10 (P < 0.0001)



6. The effect of levosimendan on left ventricular ejection fraction (LVEF). The LVEF (%) changes were compared

Study or Subgroup	Levosimendan Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Fang M 2014	9.2	6.01	18	1.5	7.62	18	24.4%	7.70 [3.22, 12.18]
Meng J 2016	9.4	6.71	19	1.9	7.93	19	23.8%	7.50 [2.83, 12.17]
Morelli A 2005	8.3	7.37	15	3.5	10.25	13	18.2%	4.80 [-1.90, 11.50]
Vaitsis J 2009	4.8	0.2	23	3.5	0.7	19	33.5%	1.30 [0.97, 1.63]
Total (95% CI)			**75**			**69**	**100.0%**	**4.98 [0.75, 9.21]**

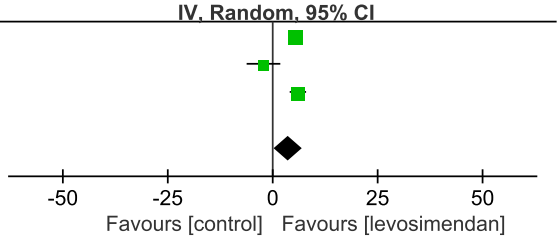
Heterogeneity: Tau² = 13.88; Chi² = 15.46, df = 3 (P = 0.001); I² = 81%
Test for overall effect: Z = 2.31 (P = 0.02)



7. The effect of levosimendan on left ventricular stroke work index (LVSWI). The LVSWIs ($g*m/m^2$) after treatment were compared

	Levosimendan			Control				Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI
Fang M 2014	33.7	2.4	18	28.2	1.2	18	38.9%	5.50 [4.26, 6.74]
Meng J 2016	36.9	2.7	19	39.1	8.5	19	25.3%	-2.20 [-6.21, 1.81]
Morelli A 2005	33.9	3.7	15	27.9	1	13	35.9%	6.00 [4.05, 7.95]
Total (95% CI)			**52**			**50**	**100.0%**	**3.73 [0.49, 6.98]**

Heterogeneity: Tau² = 6.65; Chi² = 13.94, df = 2 (P = 0.0009); I² = 86%
Test for overall effect: Z = 2.25 (P = 0.02)

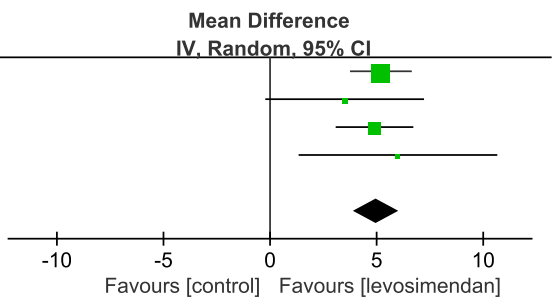


8. The effect of levosimendan on left ventricular stroke work index (LVSWI). The LVSWI (g*m/m2) changes were compared

Study or Subgroup	Levosimendan Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Fang M 2014	4.6	2.82	18	-0.6	1.37	18	53.2%	5.20 [3.75, 6.65]
Meng J 2016	5.4	2.38	19	1.9	7.93	19	8.1%	3.50 [-0.22, 7.22]
Morelli A 2005	4.3	3.34	15	-0.6	1.25	13	33.6%	4.90 [3.08, 6.72]
Morelli A 2010	8	7.52	20	2	7.52	20	5.1%	6.00 [1.34, 10.66]
Total (95% CI)			**72**			**70**	**100.0%**	**5.00 [3.95, 6.06]**

Heterogeneity: Tau² = 0.00; Chi² = 0.89, df = 3 (P = 0.83); I² = 0%

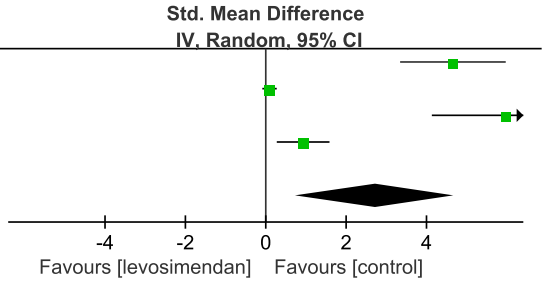
Test for overall effect: Z = 9.28 (P < 0.00001)



9. The effect of levosimendan on fluid infusion. The standard mean difference of fluid infsuion were compared.

Study or Subgroup	Levosimendan Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Fang M 2014	5,746	420	18	4,156.7	215	18	24.3%	4.66 [3.34, 5.97]
Gordon AC 2016	1,847	1,664	239	1,718	1,010	238	27.2%	0.09 [-0.09, 0.27]
Morelli A 2005	5,907	330	15	4,311	136	13	22.0%	5.98 [4.13, 7.82]
Morelli A 2010	5,700	1,000	20	4,850	777.78	20	26.5%	0.93 [0.27, 1.59]
Total (95% CI)			**292**			**289**	**100.0%**	**2.72 [0.75, 4.69]**

Heterogeneity: Tau² = 3.70; Chi² = 87.19, df = 3 (P < 0.00001); I² = 97%
Test for overall effect: Z = 2.71 (P = 0.007)

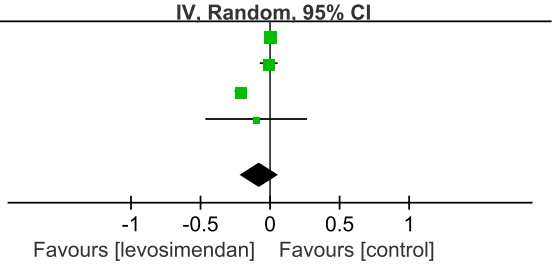


10. The effect of levosimendan on norepinephrine dose. The norepinephrine doses (μg/kg/min) after treatment were compared.

Study or Subgroup	Levosimendan Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Fang M 2014	0.33	0.05	18	0.33	0.06	18	30.8%	0.00 [-0.04, 0.04]
Meng J 2016	0.36	0.11	19	0.37	0.09	19	29.4%	-0.01 [-0.07, 0.05]
Morelli A 2005	0.02	0.06	15	0.23	0.06	13	30.4%	-0.21 [-0.25, -0.17]
Morelli A 2010	0.3	0.59	20	0.4	0.59	20	9.4%	-0.10 [-0.47, 0.27]
Total (95% CI)			**72**			**70**	**100.0%**	**-0.08 [-0.21, 0.06]**

Heterogeneity: Tau² = 0.01; Chi² = 55.77, df = 3 (P < 0.00001); I² = 95%

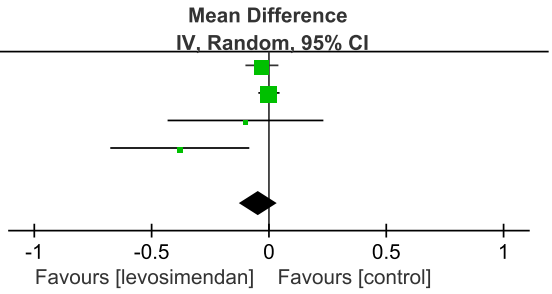
Test for overall effect: Z = 1.12 (P = 0.26)



11. The effect of levosimendan on norepinephrine dose. The norepinephrine dose (μg/kg/min) changes were compared.

Study or Subgroup	Levosimendan Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Meng J 2016	-0.06	0.12	19	-0.03	0.1	19	39.8%	-0.03 [-0.10, 0.04]
Morelli A 2005	0	0.066	15	0	0.056	13	48.2%	0.00 [-0.05, 0.05]
Morelli A 2010	-0.1	0.56	20	0	0.51	20	5.4%	-0.10 [-0.43, 0.23]
Torraco A 2014	-0.15	0.37	13	0.23	0.4	13	6.6%	-0.38 [-0.68, -0.08]
Total (95% CI)			**67**			**65**	**100.0%**	**-0.04 [-0.12, 0.04]**

Heterogeneity: Tau² = 0.00; Chi² = 6.68, df = 3 (P = 0.08); I² = 55%
Test for overall effect: Z = 1.03 (P = 0.30)

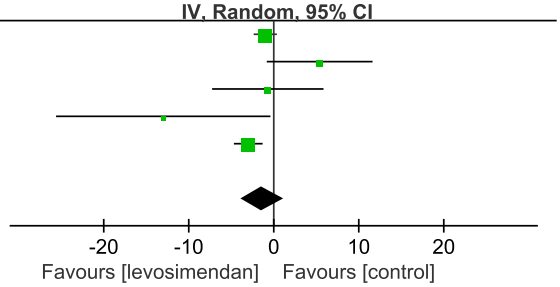


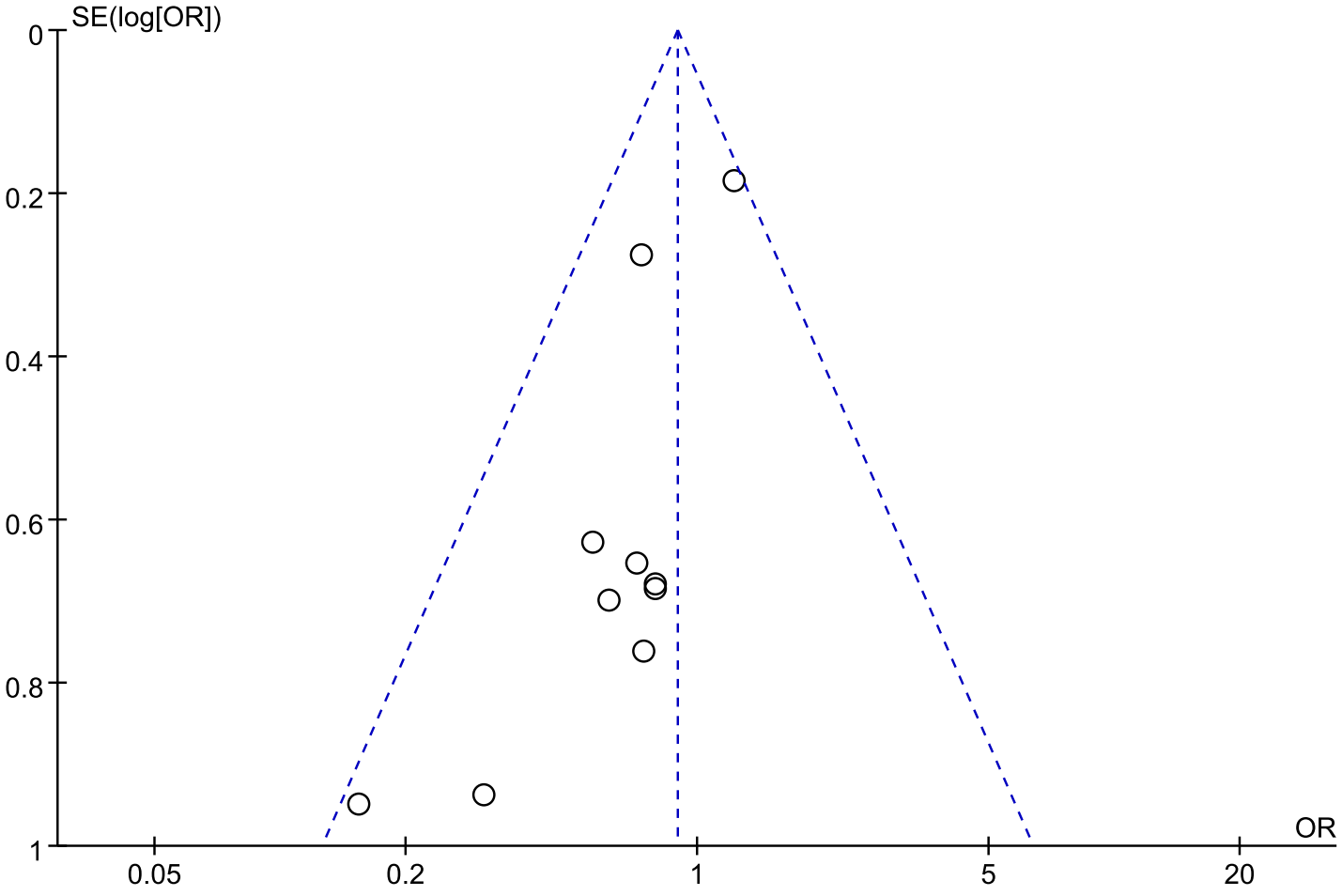
12. The effect of levosimendan on length of ICU stay. The length of ICU stay (day) were compared.

	Levosimendan			Control				Mean Difference	Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI	IV, Random, 95% CI
Gordon AC 2016	7.3	8.59	258	8.3	7.11	257	37.9%	-1.00 [-2.36, 0.36]	
Memis D 2012	15.6	11.05	15	10.2	5.38	15	11.8%	5.40 [-0.82, 11.62]	
Meng J 2016	12.6	10.1	19	13.3	10.5	19	10.9%	-0.70 [-7.25, 5.85]	
Morelli A 2010	14	5.93	20	27	28.15	20	3.6%	-13.00 [-25.61, -0.39]	
Wang X 2017	17	6.67	120	20	6.67	120	35.8%	-3.00 [-4.69, -1.31]	
Total (95% CI)			**432**			**431**	**100.0%**	**-1.36 [-3.87, 1.14]**	

Heterogeneity: Tau² = 3.83; Chi² = 11.46, df = 4 (P = 0.02); I² = 65%

Test for overall effect: Z = 1.07 (P = 0.29)




SE(log[OR])
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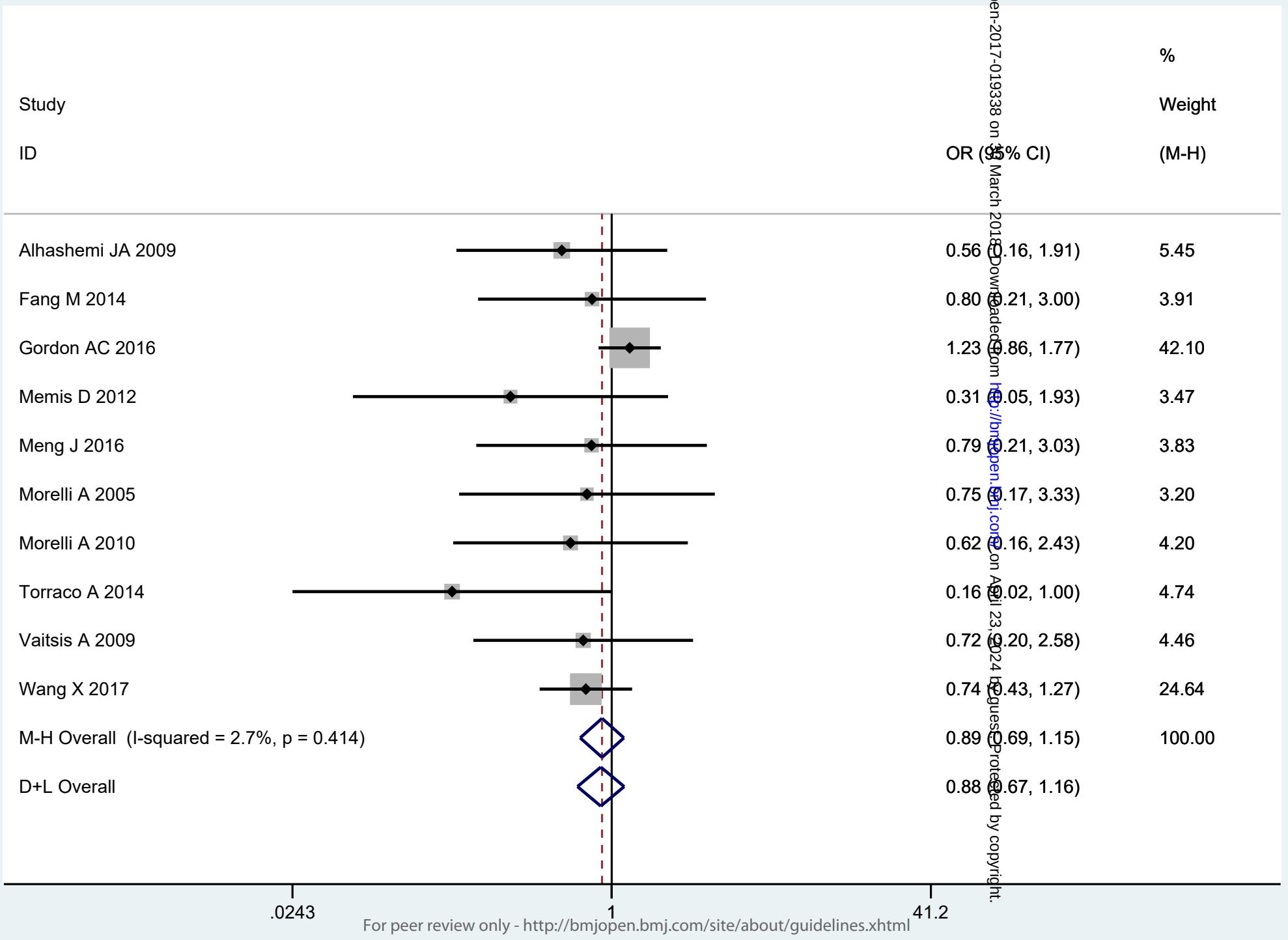
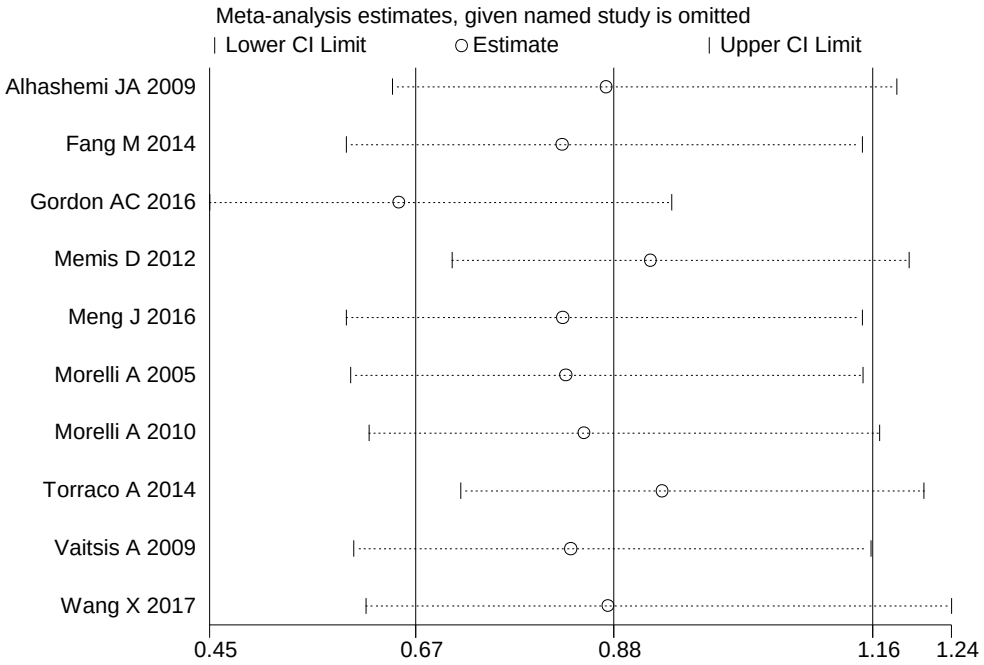
0.2
0.4
0.6
0.8
1
0.05
0.2
1
5
20
OR





Study omitted	Estimate	[95% Conf.	Interval]
Alhashemi JA 2009	0.874	0.646	1.182
Fang M 2014	0.827	0.597	1.145
Gordon AC 2016	0.653	0.452	0.943
Memis D 2012	0.921	0.710	1.195
Meng J 2016	0.827	0.598	1.145
Morelli A 2005	0.831	0.602	1.146
Morelli A 2010	0.850	0.621	1.164
Torraco A 2014	0.933	0.719	1.211
Vaitsis A 2009	0.836	0.605	1.155
Wang X 2017	0.876	0.618	1.240
Combined	0.881	0.671	1.157

(((((levosimendan) OR simendan) OR Simdax) OR dextrosimendan)) AND ((((sepsis) OR septicemia) OR severe sepsis) OR septic shock)

Methods of imputation of missing data

1. In studies outcomes are presented as median (IQR):

The distribution of outcome is assumed to be normal. Mean is substituted by median, and SD is calculated by the following formula:

$$SD = \frac{IQ_{up} - IQ_{down}}{1.35}$$

2. In studies when baseline and final outcomes are told and presented as mean±SD ($mean_B \pm SD_B$ and $mean_F \pm SD_F$), and the changes are unknown. The mean ($mean_C$) and SD (SD_C) of the changes are calculated by the following formulas:

$$mean_C = mean_F - mean_B$$

$$SD_c = \sqrt{SD_B^2 + SD_F^2 - 2 \times R \times SD_B \times SD_F}$$

Within which, *R* is called correlation coefficient and is regarded as 0.4 or 0.5 during the calculation, and more values of R (0.2 and 0.8) is used during the sensitivity analysis.

Abbreviations: IQR inter-quartile range, SD standard deviation

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Pg. 1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Pg. 2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Pg. 4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Pg. 4-5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	-
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Pg. 5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Pg. 5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Pg. 5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Pg. 5-6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Pg. 6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Pg. 6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Pg. 6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Pg. 6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Pg. 7

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Pg. 7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Pg. 7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Pg. 7
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Pg. 7-8
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Pg. 8
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Pg. 8
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Pg. 8-9
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Pg. 9-10
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Pg. 10
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Pg. 10-11
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Pg. 11-12
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Pg. 12
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Pg. 13

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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BMJ Open

The effect of levosimendan on mortality in severe sepsis and septic shock: a meta-analysis of randomized trials

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Complete List of Authors:	Chang, Wei; Southeast University Zhongda Hospital, Department of Critical Care Medicine Xie, Jianfeng; School of Medicine, Southeast University, Department of Critical Care Medicine Xu, Jing-Yuan; School of Medicine, Southeast University, Department of Critical Care Medicine Yang, Yi; School of Medicine, Southeast University, Department of Critical Care Medicine
Primary Subject Heading:	Intensive care
Secondary Subject Heading:	Intensive care, Cardiovascular medicine, Infectious diseases
Keywords:	sepsis, septic shock, septic cardiomyopathy, levosimendan, dobutamine

Title: The effect of levosimendan on mortality in severe sepsis and septic shock: a meta-analysis of randomized trials

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ABSTRACT

Objective We aim to synthesize the up-to-date randomized trials to investigate the effects of levosimendan on mortality and clinical outcomes in severe sepsis and septic shock.

Methods A collection of databases including PubMed, EMBASE, Cochrane Central register and Web of Science were searched updated to August, 2017. Randomized trials were included when pertaining the use of levosimendan in severe sepsis or septic shock compared with any category of inotropes, or as an adjunct to standard therapy with mortality reported. The primary outcome was the mortality, and the secondary outcomes were clinical performances including serum lactate, cardiac function, vasopressor requirement and fluid infusion.

Results A final of 10 studies with 1036 patients were included in this meta-analysis. The results revealed that levosimendan could not reduce mortality significantly in severe sepsis and septic shock (odds ratio 0.89, 95% CI 0.69-1.16, P = 0.39). Levosimendan use could reduce serum lactate more effectively, enhance cardiac contractibility with increased cardiac index and left ventricular ejection fraction. However, its use could also increase fluid infusion but not reduce norepinephrine dose. No significant benefit in mortality could be observed of levosimendan vs. dobutamine use, or in patients with proved cardiac dysfunction.

Conclusions Current evidence is not sufficient to support levosimendan as superior to dobutamine or as an optimal adjunct in severe sepsis and septic shock. More large-scale randomized trials are necessary for the validation of the levosimendan use in sepsis.

Key words sepsis; septic shock; levosimendan; dobutamine; septic cardiomyopathy

Strengths and Limitations of this Study

1. This article synthesized the up-to-date randomized trials for quantitative analysis of the effect of levosimendan on mortality in severe sepsis and septic shock.

2. Sub-group analyses were conducted to investigate the sub-population of patients who were likely to benefit most from levosimendan use.

3. Heterogeneity and biases were appraised between each study, and the optimal sample size was calculated.

4. However, the trials included were of limited sample size and quality, and potentially high biased.

BACKGROUND

Sepsis is still a great challenge to the public health and its mortality increases tremendously when severe sepsis or septic shock occurs[1]. The incidence of cardiac dysfunction in severe sepsis and septic shock remains as high as 40%-60%[2], resulted from infectious process, cytokine storm[3], decreased myocardial perfusion and pulmonary injuries[4], and is associated with poor outcomes[5, 6].

Surviving Sepsis Campaign (SSC) International Guidelines (2016) recommended the usage of dobutamine infusion in patients with persistent hypo-perfusion despite adequate fluid loading and the use of vasopressor agents (weak recommendation, low quality of evidence)[7]. However, its effect on mortality in sepsis is still under debate[8], and its adverse effects including increased myocardial oxygen consumption and risks of dysrhythmia could not be neglected.

Levosimendan, a calcium sensitizer which could improve myocardial contractibility in the absence of increased oxygen consumption, is regarded as a promising adjunct in the treatment of both cardiac systolic and diastolic dysfunctions[9] and has been demonstrated to have a beneficial effect on mortality in cardiac peri-operative patients and patients with advanced heart failure [10, 11].

Levosimendan was demonstrated as superior to dobutamine and milrinone in restoring cardiac function in septic animal models[12]. It could also alleviate inflammatory response by down-regulating NF-κB-dependent transcription [13], inhibiting inducible NO synthetase (iNOS) promoter activity, and reducing NO expression *in vitro*[14].

Several meta-analyses were conducted to investigate the effect of levosimendan on mortality in sepsis, which revealed a beneficial effect, however with limited sample size[15]. In this study, we aim to perform an up-to-date meta-analysis to investigate the effect of levosimendan on mortality

in severe sepsis and septic shock.

METHODS

The manuscript was prepared according to the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) statement[16, 17].

Eligibility Criteria

We aimed to include all the randomized control trials (RCT) studying levosimendan use versus any category of inotropes or as an adjunct to standard management in severe sepsis and septic shock. The articles would be included in our study if fulfilling the following criteria: (1) study population of severe sepsis or septic shock in adults, (2) randomized allocation of treatment, (3) comparison of levosimendan with any category of inotropic agents or placebo, with no restrictions on dose regimen or time limits of levosimendan infusion, (4) data on mortality reported; and exclusion criteria were as follows: (1) duplicates, (2) pediatric subjects, (3) animal experiments or *in vitro* studies, (4) no sepsis population and (5) lack of data on mortality.

Information Sources

Two investigators searched a collection of data-bases including PubMed, EMBASE, Cochrane Central register and Web of Science updated to August 1, 2017 separately with no language restrictions. When relevant systemic reviews or meta-analyses were found, we ran a backward snowballing to obtain further studies.

Search

Following key words were used as search terms: "levosimendan", "simendan", "Simdax", "dextrosimendan", "sepsis", "severe sepsis", "septicemia" and "septic shock". [Supplementary File 1]

Study Selection

Abstracts and titles of the articles were initially viewed separately by two investigators, if potentially pertinent, the complete articles were retrieved. Articles were assessed and selected separately by two investigators with disagreements solved by consensus.

Data Items

Information was extracted from each of the included trials on: (1) characteristics of the participants (including gender, age and diagnosis); (2) interventions (including the infusion duration and dose regimen of the levosimendan or other inotropes); (3) outcome measurements with primary outcome determined as the mortality (follow-up time was tailored at the approximate duration by the reviewers' consensus), and secondary outcomes as clinical outcomes including serum lactate level, cardiac function including cardiac index (CI), left ventricular ejection fraction (LVEF) and left ventricular stroke work index (LVSWI); fluid infusion and vasopressor requirement.

Assessment of Risk of Bias

Internal validity and risks of bias were evaluated by two investigators separately following Cochrane Collaboration Methods protocols[18]. Risks of bias were assessed by scrutinizing the articles and rated as "Yes", "No" or "Unclear" according to the procedures taken in the articles.

Summary Measures

Dichotomous outcomes were measured as proportions and calculated by odds ratio (OR). Continuous outcomes were described as mean ± standard deviation (SD) and calculated by mean difference (MD) or standard mean difference (SMD). The end-point and change range were both compared if the continuous variables were measured at baseline and after treatment. Missing data

were imputed from other information whenever possible[19][Supplementary File 2].

Statistical Analysis

The data retrieved from the relevant articles were computerized and analyzed by Review Manager 5.3 (The Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen). We used Mantel-Haenszel statistic method for dichotomous variable (mortality) measurements and inverse variance for continuous variables (lactate level, CI, LVEF, LVSWI, fluid infusion and norepinephrine dose). Random-effects model was used for better accommodation of heterogeneity. Cochrane I^2 statistic was used for heterogeneity assessment between the studies, with a range of 0% to 30% representing no or mild heterogeneity, 30%-60% as moderate heterogeneity, whereas > 60% as high heterogeneity. Publication bias was tested by visual inspection of funnel plots. As for sensitivity analysis, the dataset was analyzed in both fixed and randomized-effects models and the favoring directions were inspected. Each study was removed sequentially and the remaining data-set re-analyzed to assess the robustness of the results. Trial sequential analysis (TSA) was performed to estimate the optimal sample size for the plausible effects of levosimendan in sepsis[20]. Statistical significance was set at a 2-tailed 0.05 level as hypothesis establishment.

Sub-group Analysis

We pre-specified the sub-group analyses. Studies enrolling the patients with proved cardiac dysfunction vs. heterogeneous cardiac function were compared, and also the use of levosimendan vs. dobutamine and vs. standard therapy. We further attempted to separate the studies enrolling the patients with average age ≥ 65-years vs. < 65-years and mortality ≥ 50% vs. < 50% in the hope of finding the sub-population who would potentially benefit from the levosimendan use.

RESULTS

Study Selection

A total of 566 abstracts were retrieved from the search strategy, with 121 duplicates excluded and 199 excluded due to no eligible abstracts. Complete manuscripts of 246 abstracts were retrieved for further assessment, within which 92 were reviews or commentaries, 106 were animal experiments, 3 in vitro studies, 7 non-RCTs, 9 non-septic patients, 2 pediatric patients, 3 with mortality not reported, 13 case reports and 1 study design. A final of 10 studies were included in this meta-analysis[21-30], within which two were conference abstracts[21, 22], and one was written in Chinese[26] [Fig 1].

Study Characteristics

Within the 10 studies enrolling 1036 patients, no differences were present in age and APACHE II scores between the treatment and control group at the baseline. Patients diagnosed as septic shock or severe sepsis after adequate fluid resuscitation were included in the studies. Four studies set explicit criteria of cardiac dysfunctions during patients recruitment[21, 26, 27, 30]. Norepinephrine was used as necessary to achieve the target MAP ranging from 65 to 80mmHg during levosimendan therapy depending on the study design. Seven studies used dobutamine (dose ranged from 5μg/kg per min to 20μg/kg per min) as a comparator[21-24, 26, 27, 30] and three used levosimendan as an adjunct to standard therapy[25, 28, 29]. Levosimendan was administered as continuous infusion (dose ranged from 0.05μg/kg per min to 2.0μg/kg per min) over 24 hours with no bolus. Parameters reflecting cellular metabolism, microcirculation, hemodynamics, cardiac function and target organ perfusion were measured in individual studies [Tab 1].

Syntheses of Results

The data on mortality were randomized and calculated from the ten studies, and the final result

revealed no statistical difference (total events 187/522 vs. 197/514 in levosimendan and control group respectively, OR 0.89, 95% CI 0.69-1.16, $P = 0.39$), with no evidence of heterogeneity ($I^2 = 0\%$, $P = 0.52$) [Fig 2].

We conducted a series of sub-group analyses according to the patients' characteristics. No statistical significance could be observed in the studies enrolling patients with proved clinical cardiac dysfunction[21, 26, 27, 30] (OR 0.76, 95% CI 0.39-1.50, $P = 0.43$) or those with heterogenous cardiac functions[22-25, 28, 29] (OR 0.75, 95% CI 0.48-1.19, $P = 0.23$).

We compared the effect of levosimendan vs. dobutamine on mortality in sepsis and find no statistical difference in mortality between levosimendan and dobutamine group (OR 0.65, 95% CI 0.39-1.10, $P = 0.11$) [21-24, 26, 27, 30], neither of levosimendan in comparison with standard therapy[25, 28, 29] (OR 0.82, 95% CI 0.44-1.55, $P = 0.54$) [Fig 3].

We attempted to divide the studies according to the patients' average age (< 65yr or ≥ 65yr) and mortality (< 50% or ≥ 50%), and found no statistical significance between each sub-group [Supplementary Fig 1].

We also extracted and compared the data of lactate reduction[22, 23, 26, 28, 30], measurements reflecting cardiac function including CI[23, 25-28, 30], LVEF[21, 26, 27, 30] and LVSWI[23, 26, 27, 30], fluid infusion[23, 26, 28, 30] and norepinephrine dosage[23, 25-28, 30]. The results revealed that lactate was more profoundly reduced, and cardiac function significantly improved (with increased CI, LVEF and LVSWI) in levosimendan group. Norepinephrine dose was reduced slightly, however total fluid infusion over 24 hours was tremendously increased in levosimendan group [Tab 2, Supplementary Fig 2].

Risk of Bias and Sensitivity Analyses

The funnel plot was drawn for testing the bias, and visual inspection of the funnel plot revealed potential asymmetry [Supplementary Fig 3].

The data-set was analyzed both in the fixed and random-effects model for sensitivity analysis, and the result revealed no shift of favouring directions [Supplementary Fig 4]. Each trial was removed and remaining dataset re-analyzed subsequently, and the result indicated that the statistical significance obscured only when the trial by Gordon AC *et al.*[28], was put into analysis [Supplementary Fig 5].

Trial Sequential Analysis

The trial sequential analysis (TSA) was performed to determine the optimal information size. We estimated a 26% mortality based on the recent epidemiologic data of severe sepsis[31], and an assumed an average of 20% relative risk reduction in reference to the effect of levosimendan on overall mortality reduction in hospitalized patients[32] with 80% power and $\alpha = 0.05$ two-sided. The calculation indicated the optimal information size of 2082 patients for detection of the plausible treatment effect of levosimendan in sepsis. The Lan DeMets sequential monitoring boundary constructed by the optimal information size was not crossed, indicating that the cumulative evidence was not conclusive and reliable [Fig 4].

DISSCUSSION

The main finding of this study was that levosimendan could not significantly reduce the mortality in severe sepsis and septic shock. Levosimendan could reduce serum lactate level more effectively, improve cardiac function. However, no change in norepinephrine dose but profound increase in fluid infusion could be observed.

We noticed that, albeit cardiac function was improved after levosimendan use, more fluid was

infused for maintenance of the target MAP probably due to the vasodilatory effect of levosimendan, which could exacerbate pulmonary and peripheral edema and potentially impeding oxygen uptake and exchange. The use of levosimendan was also suggested to be accompanied with higher incidence of life-threatening arrhythmias like supraventricular tachyarrhythmia, which could cause hemodynamic instability and bring risks to the patients[28].

The previous study by Zangrillo *et al.* enrolling a series of RCTs yielded a significantly reduced mortality in levosimendan group in septic shock[15]. However, it should be noted that, in our study, statistical significance obscured after a large, multi-center RCT with a sample size of 514 patients by Gordon AC *et al*.[28] were included.

We thought that there may be several reasons for this. The percentage of patients in the trial by Gordon *et al*. that underwent cardiac function assessment was rather low (30%), so Gordon and co-workers might have enrolled the patients with heterogenous cardiac functions[33]. Although the prevalence of septic cardiomyopathy is high (40-60%), the discriminative enrollment could still mask the potential benefit of levosimendan, considering that there might be patients recruited who did not have cardiac dysfunction, and may not benefit from inotropic use as indicated by the SSC (2016) Guidelines in which the increase of cardiac function to supranormal level is discouraged[7].

We attempted to synthesize the studies with patients who had proved cardiac dysfunction, however the result revealed no statistical significance (OR 0.76, 95% CI 0.39-1.50, P = 0.43). We then performed a TSA and yielded an optimal sample size of 1719, suggesting that more trials focusing on the patients with cardiac dysfunction are probably needed, for the determination of the plausible effects of levosimendan in sepsis.

The patients enrolled in the trial by Gordon *et al.* might be relatively at low risk (with the 28-day

mortality of 31%).[33, 34] In the study by Zangrillo *et al*, the mortality decreased from 61% to 47% after levosimendan use[15], and in that study, the baseline mortality is very high (61% in control group), suggesting that the patients at "extremely" high risk may be most benefited from levosimendan use.

We also attempted to synthesize the studies dividing the studies with patients at high (≥ 50%) or low (< 50%) risks and found the OR of 0.55, 95% CI 0.30-1.03 and OR 0.99, 95% CI 0.74-1.32, respectively. Although no statistical significance could be observed, we found the group of studies with high-risk patients were more likely to benefit from levosimendan use. Still, more trials are definitely needed.

Limitations

Our study had several limitations. The randomized trials included in this meta-analysis were of limited sample size, 8 out of 10 studies included less than 50 patients[21-27, 30], and were potentially high biased. Follow-up duration was not reported in one study[24]; only ICU mortality was reported in two studies[22, 23], and the inconsistency in follow-up duration could potentially bring bias to the results. Also, the dose regimen of levosimendan varied from 0.05 to 0.2 μg/kg per min, which could cause different hemodynamic effects to the patients.

CONCLUSION

Although levosimendan could improve clinical outcomes including cardiac function and tissue perfusion compared with dobutamine or standard therapy, it also increased fluid infusion but did not reduce vasopressor requirements. Still, it failed to bring significant benefit to mortality in sepsis. More RCTs are necessary for further elucidation of the effects of levosimendan in sepsis, particularly in those with cardiac dysfunctions.

LIST OF ABBREVIATIONS

APACHE Acute Physiology and Chronic Health Evaluation;

CI cardiac index;

HR heart rate;

ICU intensive care unit;

iNOS inducible NO synthetase;

IQR inter-quartile range;

LV left ventricle;

LVEF left ventricle ejection fraction;

LVSWI left ventricular stroke work index;

MAP mean arterial pressure;

MD mean difference;

NE norepinephrine;

OR odds ratio;

RCT randomized control trial;

ROS reactive oxygen species;

SD standard deviation;

SSC Surviving Sepsis Campaign;

SMD standard mean difference;

TSA trial sequential analysis.

DECLARATIONS

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

WC carried out the analysis and interpretation of data and participated in drafting, editing and submitting the manuscript. The articles were reviewed by two reviewers (WC and JFX) independently in accordance with the inclusion criteria. Disagreements were resolved and by consensus and discussion including a third reviewer (JYX). The quality of the articles was assessed by WC and JFX independently, with disagreements resolved by consulting a third reviewer (JYX). YY was responsible for conception, design and coordination of the study, and revising the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Not applicable.

Fig 1 Flow diagram of search process and study selection

Fig 2 The effect of levosimendan on mortality in severe sepsis and septic patients.

Fig 3 Sub-group analysis. (A) Levosimendan in patients with proved cardiac dysfunction vs. patients with heterogeneous cardiac function (OR 0.76, 95% CI 0.39-1.50, *P* = 0.43 vs. OR 0.75, 95% CI 0.48-1.19, *P* = 0.23); (B) Levosimendan vs. dobutamine (OR 0.65, 95% CI 0.39-1.10, *P* = 0.11) or standard therapy (OR 0.82, 95% CI 0.44-1.55, *P* = 0.54).

Fig 4 Trial sequential analysis. The optimal information size of 2082 patients for detection of the plausible treatment effect of levosimendan in sepsis, and the Lan DeMets sequential monitoring boundary constructed by the optimal information size was not crossed

Supplementary Fig 1 Sub-group analysis. (A) Levosimendan in patients with mortality >=50% vs. morality < 50% (OR 0.55, 95% CI 0.30-1.03, *P* = 0.06 vs. OR 0.99 95% CI 0.74-1.32, *P* = 0.92); (B) Levosimendan in patients with age >= 65-year-old vs. age < 65-year-old (OR 0.84 95% CI 0.54-1.30, *P* = 0.44 vs. OR 0.67 95% CI 0.32-1.40, *P* = 0.49).

Supplementary Fig 2 Forest plots for secondary outcomes.

Supplementary Fig 3 Funnel plot for inspection of bias

Supplementary Fig 4 Sensitivity analysis with data-set analyzed in fixed and random-effects models

Supplementary Fig 5 Sensitivity analysis with single study omitted sequentially

Tab 1 Characteristics of the included trials.

Study	Year	Subjects No	Levosimendan group	Control group	Inclusion criteria	Cardiovascular criteria	Levosimendan therapy	Control therapy	Target MAP (mmHg)	Follow-up (day)	Primary outcome
Alh	20	42	21	21	Severe	NR	0.05 to 2μg/kg per min, 24hr	Dobutamine 5	⩾ 65	IC	ScvO2 and serum

ashemi JA [22]	09				sepsis/septic shock			to 20μg/kg per min, 7 days		U stay	lactate
Fang M [26]	2014	36	18	18	Septic shock	LVEF≤ 45%	Dobutamine 0.5μg/kg per min for 24hr; levosimendan 0.2μg/kg per min 24hr subsequently	Dobutamine 5μg/kg per min, 48hr	NR	28	Hemodynamics and cardiac function
Gordon AC [28]	2016	515	258	257*	Septic shock	MAP 60 to 70mmHg	0.05 to 0.2μg/kg per min, 24hr	Standard therapy	65 to 70	28	Daily SOFA score
Memis D [24]	2012	30	15	15	Septic shock	MAP≤ 65mmHg	0.1μg/kg per min, 24hr	Dobutamine 10μg/kg per min, 24hr	>65	NR	Liver function
Meng J [27]	2016	38	19	19	Septic shock	MAP≥ 65mmHg and LVEF ≤45%	0.2μg/kg per min, 24hr	Dobutamine 5μg/kg per min, 24hr	≥ 65	28	Hemodynamics and myocardial injury biomarkers
Morelli A [30]	2005	28	15	13**	Septic shock	MAP 70 to 80mmHg, PAOP≥ 12mmHg and LVEF<45 %	0.2μg/kg per min, 24hr	Dobutamine 5μg/kg per min, 24hr	70 to 80	30	Hemodynamics and cardiac function
Morelli A [23]	2010	40	20	20	Septic shock	MAP≥ 65mmHg	0.2μg/kg per min, 24hr	Dobutamine 5μg/kg per min, 24hr	70 ± 5	ICU stay	Systemic and microvascular hemodynamics
Toracco A [25]	2014	26	13	13	Septic shock	MAP≥ 65mmHg	0.2μg/kg per min, 24hr	Standard therapy	65 to 75	28	Mitochondrial function
Vaitsis J [21	2009	42	23	19	Sepsis	CI<2.2, LVEF<35 %	0.1μg/kg per min, 24hr	Dobutamine 5 to 10μg/kg per min, 24hr	>65	30	Mortality at 7 and 30 days

]											
Wang X [29]	2017	240	120	120	Septic shock	MAP≥65mmHg	0.1-0.2 μg/kg per min, 24 hours	Standard care	≥65	28	Mortality at 28 days, ICU discharge and hospital discharge

Note: MAP: mean artery pressure; LVEF: left ventricular ejection fraction; PAOP: pulmonary artery occlusion pressure; CI: cardiac index; NR: not reported; SOFA: Sequential Organ Failure Assessment; * A total of 256 patients were finally included for 28-day mortality analysis; ** Two patients in control group failed to complete the study and were excluded.

Tab 2 Clinical outcomes after randomization.

Outcomes	References	No. of subjects	MD (95% CI)	*P* for overall effect	*P* for heterogeneity	I^2 (%)
$Lactate_{TRT}$	[22], [23], [26], [27], [28], [30]	656	-0.89 (-1.48, -0.29)	0.003	< 0.00001	87
ΔLactate	[23], [26], [27], [28], [30]	614	-0.80 (-1.41, -0.20)	0.009	0.0002	82
CI_{TRT}	[23], [26], [27], [28], [30]	277	0.39 (0.17, 0.62)	0.0005	0.05	59
ΔCI	[21], [23], [26], [27], [28], [30]	319	0.46 (0.30, 0.63)	< 0.00001	0.01	66
$LVSWI_{TRT}$	[26], [27], [30]	102	3.73 (0.49, 6.98)	0.02	0.0009	86
ΔLVSWI	[23], [26], [27], [30]	142	5.00 [3.95, 6.06]	< 0.00001	0.83	0
$LVEF_{TRT}$	[26], [27], [30]	102	6.76 [3.53, 10.00]	< 0.0001	0.75	0
ΔLVEF	[21], [26], [27], [30]	144	4.98 [0.75, 9.21]	0.02	0.001	81
Norepinephrine $dose_{TRT}$	[23], [26], [27], [28], [30]	547	-0.04 [-0.16, 0.09]	0.58	< 0.00001	96
ΔNE dose	[23], [25], [27], [28], [30]	537	-0.06 [-0.13, 0.01]	0.08	0.006	72
Fluid infusion in 24-hr	[23], [26], [28], [30]	581	2.72 [0.75, 4.69]*	0.007	< 0.00001	97

Note: Subscript TRT stands for outcomes after treatment; Δ stands for change range of outcomes (value after treatment subscribes value at baseline); CI cardiac index; LVSWI left ventricular stroke work index; LVEF left ventricular ejection fraction; NE Norepinephrine; * Standard mean difference (SMD) is used in this case due to large difference in means (MD 1048.74, 95% CI 303.21-1794.27).

Supplementary File 1 Full electronic search strategy for PubMed.

Supplementary File 2 Methods of the imputation of missing data.

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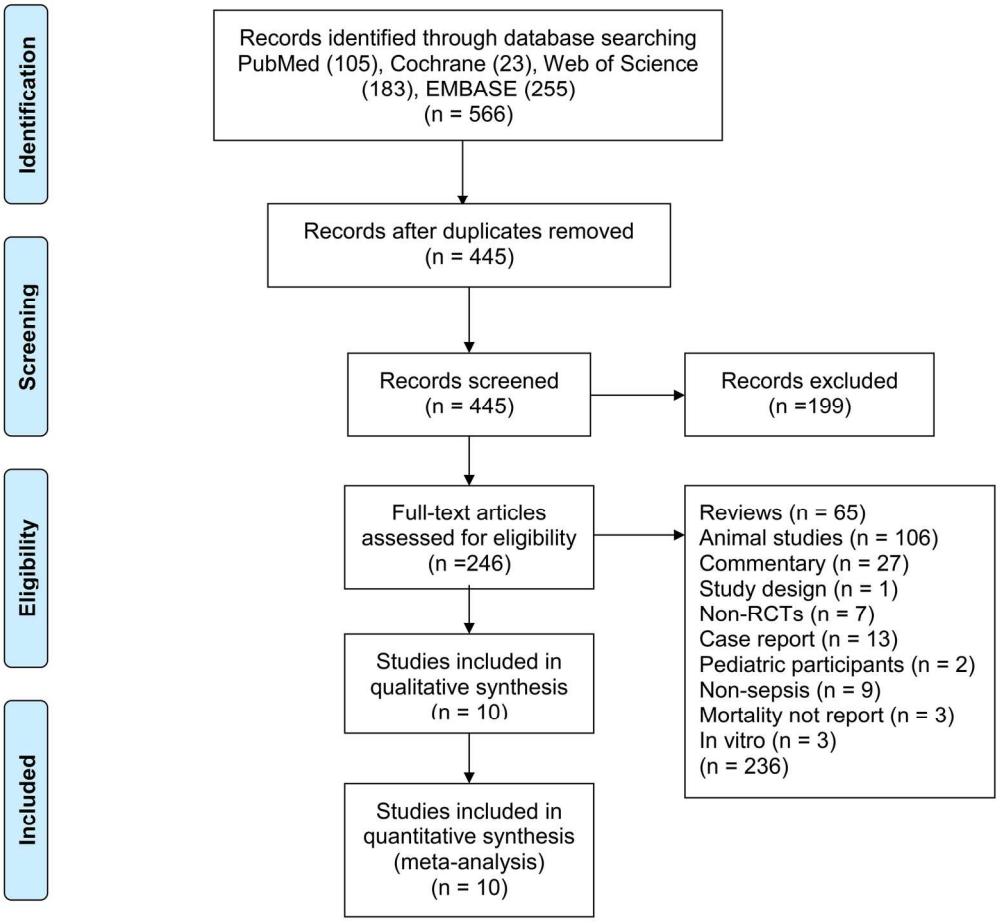

Fig 1 Flow diagram of search process and study selection

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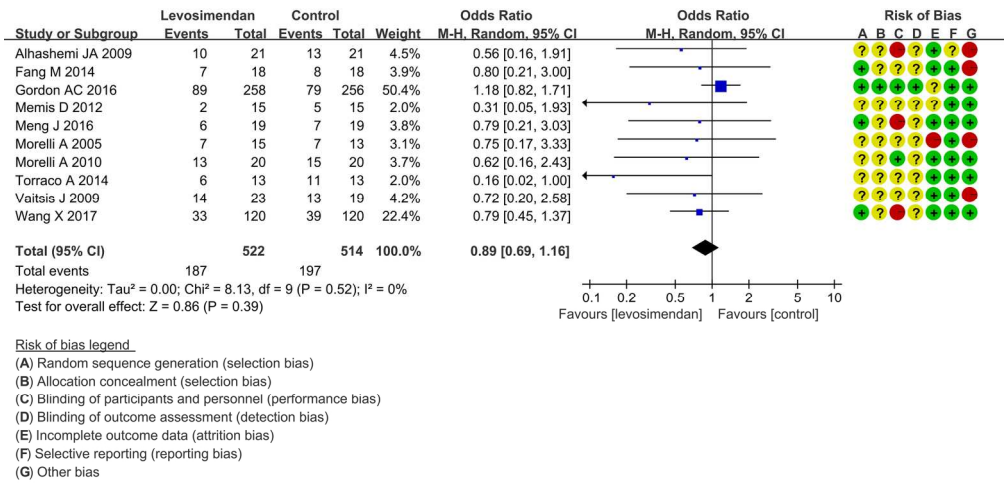

Fig 2 The effect of levosimendan on mortality in severe sepsis and septic patients.

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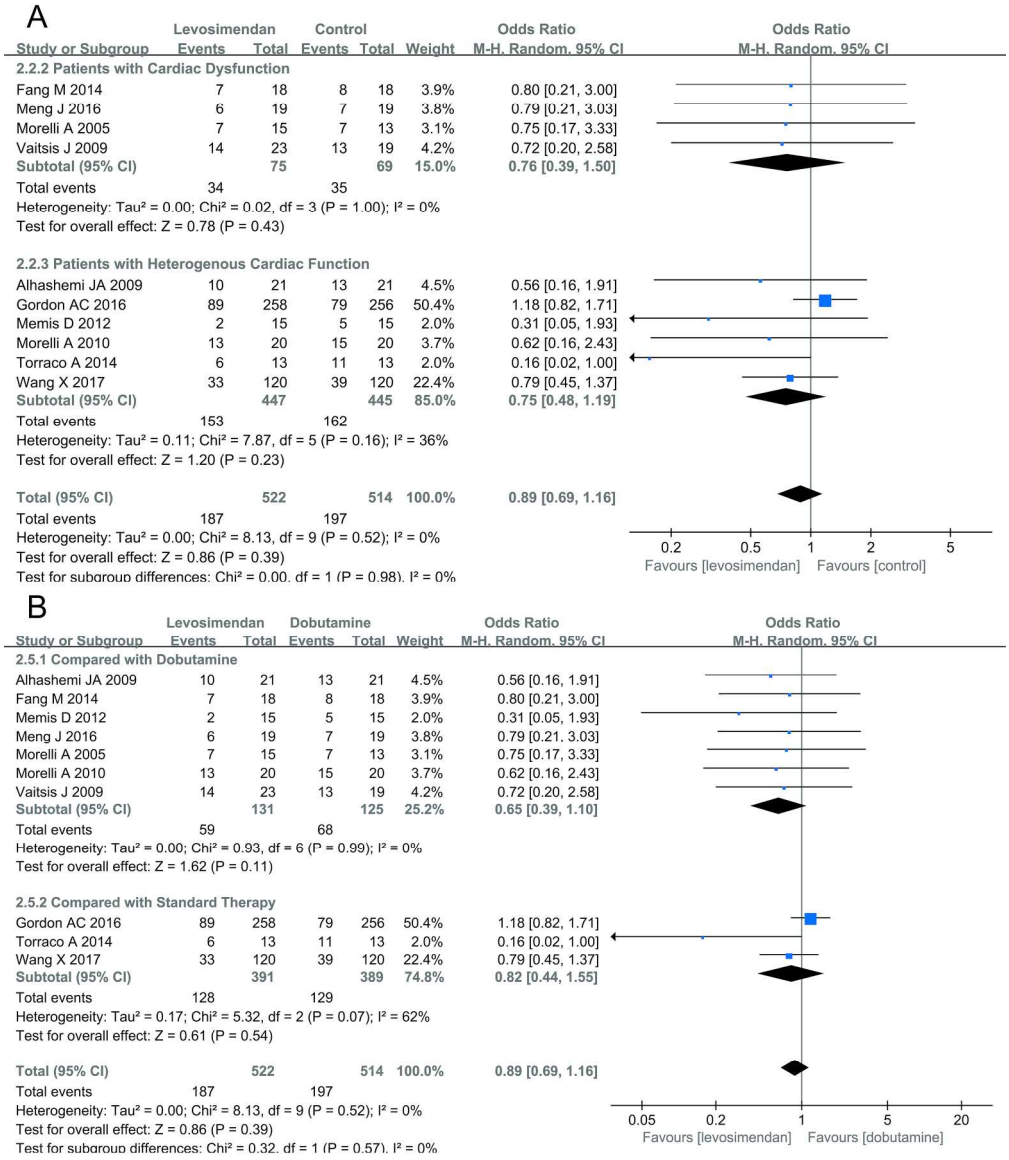

Fig 3 Sub-group analysis. (A) Levosimendan in patients with proved cardiac dysfunction vs. patients with heterogeneous cardiac function (OR 0.76, 95% CI 0.39-1.50, P = 0.43 vs. OR 0.75, 95% CI 0.48-1.19, P = 0.23); (B) Levosimendan vs. dobutamine (OR 0.65, 95% CI 0.39-1.10, P = 0.11) or standard therapy (OR 0.82, 95% CI 0.44-1.55, P = 0.54).

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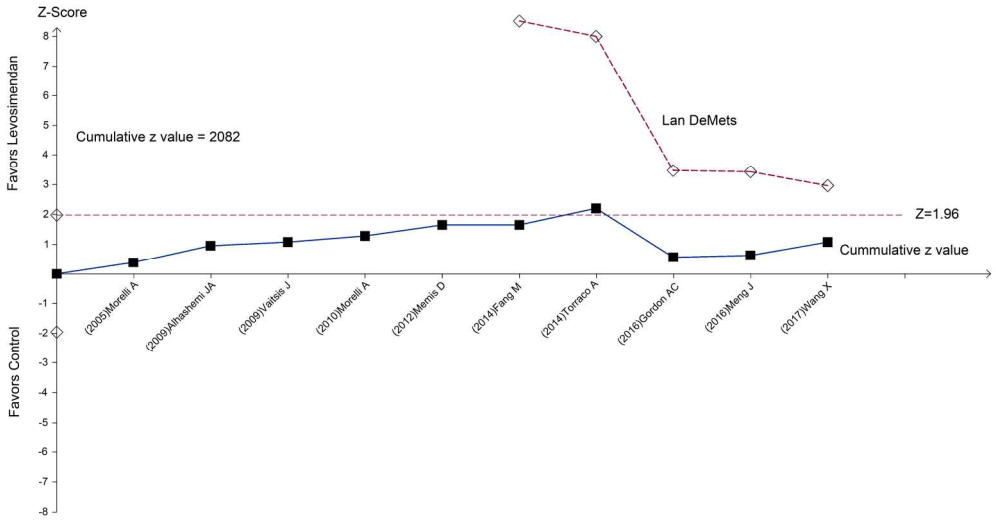

Fig 4 Trial sequential analysis. The optimal information size of 2082 patients for detection of the plausible treatment effect of levosimendan in sepsis, and the Lan DeMets sequential monitoring boundary constructed by the optimal information size was not crossed

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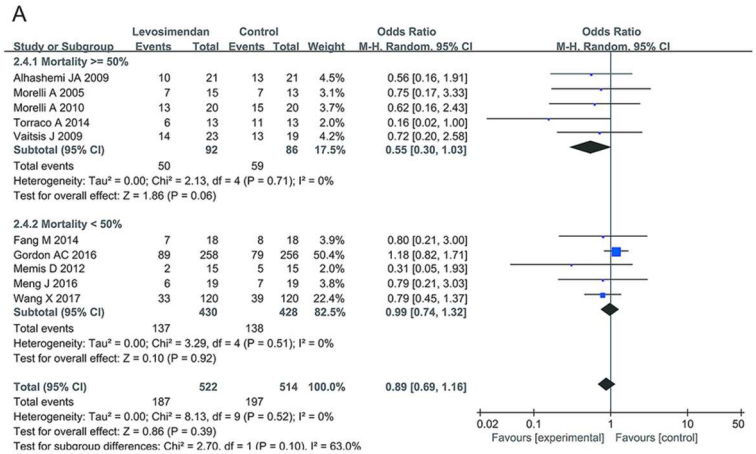


Study	Year	Age† (years)
Alhashemi JA [22]	2009	NR
Fang M [26]	2014	61.4±7.1 in levosimendan group; 61.7±7.3 in dobutamine group
Gordon AC [28]	2016	67 (58-75) in levosimendan group; 69 (58-77) in control group
Memis D [24]	2012	54.93±18.92 in levosimendan group; 56.27±14.93 in dobutamine group
Meng J [27]	2016	55.4±17.5 in levosimendan group; 50.2±13.6 in dobutamine group
Morelli A [30]	2005	61.5±7.0 in levosimendan group; 62.4±7.3 in dobutamine group
Morelli A [23]	2010	68 (55-74) in levosimendan group; 66 (54-78) in control group
Torraco A [25]	2014	70 (58-80) in levosimendan group; 68 (57-79) in control group
Vaitsis J [21]	2009	66.1±7.54
Wang X [29]	2017	70 (67-74) in levosimendan group; 69 (67-73) in control group

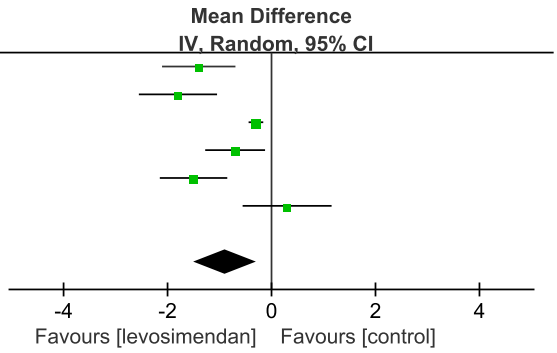
† Age presented as mean ± SD or median (IQR)

40x64mm (600 x 600 DPI)

1. The effect of levosimendan on lactate reduction. The lactate levels (mmol/L) after treatment were compared.

Study or Subgroup	Levosimendan Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Alhashemi JA 2009	2.1	0.92	21	3.5	1.37	21	16.0%	-1.40 [-2.11, -0.69]
Fang M 2014	3.4	1.1	18	5.2	1.2	18	15.5%	-1.80 [-2.55, -1.05]
Gordon AC 2016	1.4	0.74	236	1.7	0.82	236	20.3%	-0.30 [-0.44, -0.16]
Meng J 2016	3.6	0.8	19	4.3	1	19	17.3%	-0.70 [-1.28, -0.12]
Morelli A 2005	3.7	0.7	15	5.2	1	13	16.5%	-1.50 [-2.15, -0.85]
Morelli A 2010	1.9	0.96	20	1.6	1.7	20	14.4%	0.30 [-0.56, 1.16]
Total (95% CI)			**329**			**327**	**100.0%**	**-0.89 [-1.48, -0.29]**

Heterogeneity: Tau² = 0.45; Chi² = 37.36, df = 5 (P < 0.00001); I² = 87%
Test for overall effect: Z = 2.93 (P = 0.003)

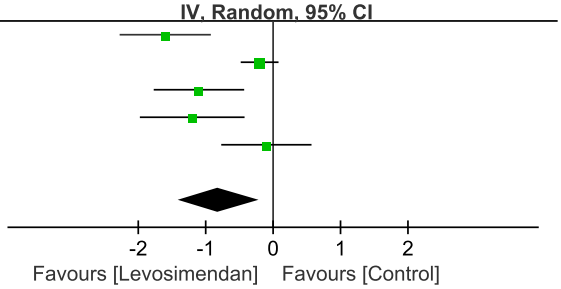


2. The effect of levosimendan on lactate reduction. The lactate level (mmol/L) changes were compared.

Study or Subgroup	Experimental Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Fang M 2014	-1.7	1.01	18	-0.1	1.06	18	19.3%	-1.60 [-2.28, -0.92]
Gordon AC 2016	-0.8	1.34	236	-0.6	1.742	236	24.1%	-0.20 [-0.48, 0.08]
Meng J 2016	-1.5	1.06	19	-0.4	1.05	19	19.4%	-1.10 [-1.77, -0.43]
Morelli A 2005	-1.2	1.04	15	0	1.05	13	17.9%	-1.20 [-1.98, -0.42]
Morelli A 2010	-0.4	1.09	20	-0.3	1.07	20	19.4%	-0.10 [-0.77, 0.57]
Total (95% CI)			**308**			**306**	**100.0%**	**-0.80 [-1.41, -0.20]**

Heterogeneity: Tau² = 0.38; Chi² = 22.12, df = 4 (P = 0.0002); I² = 82%

Test for overall effect: Z = 2.60 (P = 0.009)

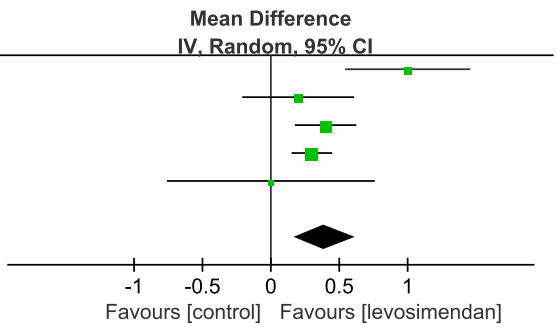


3. The effect of levosimendan on cardiac index (CI). The CIs (L/min/m2) after treatment were compared.

Study or Subgroup	levosimendan Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Fang M 2014	4.6	0.7	18	3.6	0.7	18	14.8%	1.00 [0.54, 1.46]
Gordon AC 2016	3.5	1.4	69	3.3	1	66	16.9%	0.20 [-0.21, 0.61]
Meng J 2016	3.5	0.3	19	3.1	0.4	19	28.0%	0.40 [0.18, 0.62]
Morelli A 2005	4.5	0.2	15	4.2	0.2	13	33.4%	0.30 [0.15, 0.45]
Morelli A 2010	4.1	1.19	20	4.1	1.26	20	7.0%	0.00 [-0.76, 0.76]
Total (95% CI)			**141**			**136**	**100.0%**	**0.39 [0.17, 0.62]**

Heterogeneity: Tau² = 0.03; Chi² = 9.71, df = 4 (P = 0.05); I² = 59%

Test for overall effect: Z = 3.47 (P = 0.0005)

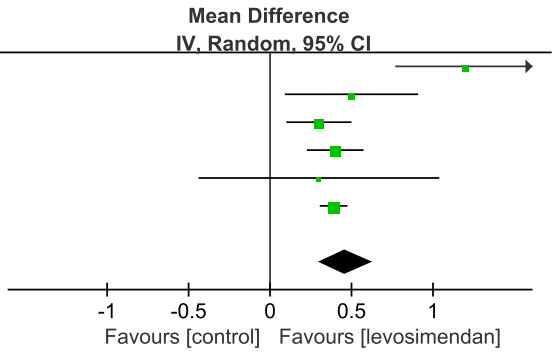


4. The effect of levosimendan on cardiac index (CI). The CI (L/min/m2) changes after treatment were compared.

Study or Subgroup	Levosimendan Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Fang M 2014	1.4	0.66	18	0.2	0.66	18	10.1%	1.20 [0.77, 1.63]
Gordon AC 2016	0.6	1.31	69	0.1	1.11	66	10.8%	0.50 [0.09, 0.91]
Meng J 2016	0.5	0.26	19	0.2	0.36	19	21.8%	0.30 [0.10, 0.50]
Morelli A 2005	0.4	0.2	15	0	0.26	13	23.6%	0.40 [0.23, 0.57]
Morelli A 2010	0.5	1.12	20	0.2	1.26	20	4.4%	0.30 [-0.44, 1.04]
Vaitsis J 2009	1.79	0.16	23	1.4	0.12	19	29.4%	0.39 [0.31, 0.47]
Total (95% CI)			**164**			**155**	**100.0%**	**0.46 [0.30, 0.63]**

Heterogeneity: Tau² = 0.02; Chi² = 14.53, df = 5 (P = 0.01); I² = 66%

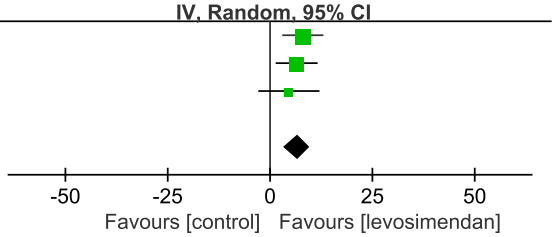
Test for overall effect: Z = 5.46 (P < 0.00001)



5. The effect of levosimendan on left ventricular ejection fraction (LVEF). The LVEF (%) after treatment were compared

Study or Subgroup	Levosimendan Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Fang M 2014	46.3	6.8	18	38.3	8.5	18	41.4%	8.00 [2.97, 13.03]
Meng J 2016	45.6	7.6	19	39.1	8.5	19	39.8%	6.50 [1.37, 11.63]
Morelli A 2005	45.4	8.4	15	40.8	11.3	13	18.8%	4.60 [-2.87, 12.07]
Total (95% CI)			**52**			**50**	**100.0%**	**6.76 [3.53, 10.00]**

Heterogeneity: Tau² = 0.00; Chi² = 0.56, df = 2 (P = 0.75); I² = 0%
Test for overall effect: Z = 4.10 (P < 0.0001)

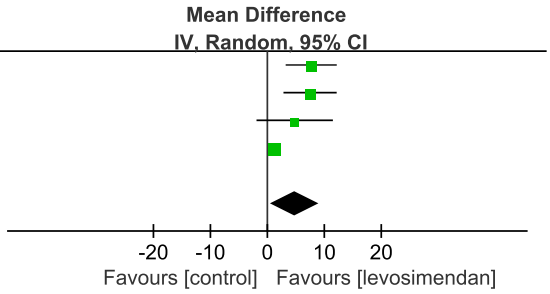


6. The effect of levosimendan on left ventricular ejection fraction (LVEF). The LVEF (%) changes were compared

Study or Subgroup	Levosimendan Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Fang M 2014	9.2	6.01	18	1.5	7.62	18	24.4%	7.70 [3.22, 12.18]
Meng J 2016	9.4	6.71	19	1.9	7.93	19	23.8%	7.50 [2.83, 12.17]
Morelli A 2005	8.3	7.37	15	3.5	10.25	13	18.2%	4.80 [-1.90, 11.50]
Vaitsis J 2009	4.8	0.2	23	3.5	0.7	19	33.5%	1.30 [0.97, 1.63]
Total (95% CI)			**75**			**69**	**100.0%**	**4.98 [0.75, 9.21]**

Heterogeneity: Tau² = 13.88; Chi² = 15.46, df = 3 (P = 0.001); I² = 81%

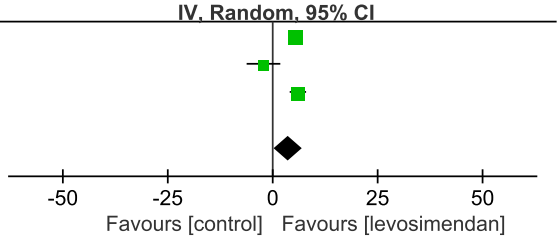
Test for overall effect: Z = 2.31 (P = 0.02)



7. The effect of levosimendan on left ventricular stroke work index (LVSWI). The LVSWIs (g*m/m2) after treatment were compared

Study or Subgroup	Levosimendan Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Fang M 2014	33.7	2.4	18	28.2	1.2	18	38.9%	5.50 [4.26, 6.74]
Meng J 2016	36.9	2.7	19	39.1	8.5	19	25.3%	-2.20 [-6.21, 1.81]
Morelli A 2005	33.9	3.7	15	27.9	1	13	35.9%	6.00 [4.05, 7.95]
Total (95% CI)			**52**			**50**	**100.0%**	**3.73 [0.49, 6.98]**

Heterogeneity: Tau² = 6.65; Chi² = 13.94, df = 2 (P = 0.0009); I² = 86%
Test for overall effect: Z = 2.25 (P = 0.02)

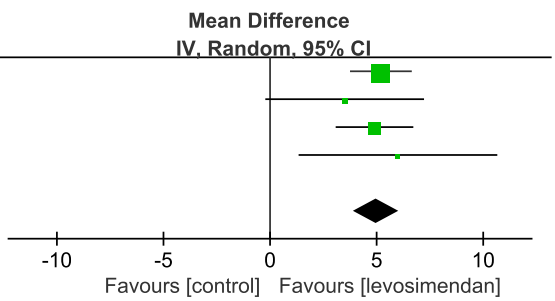


8. The effect of levosimendan on left ventricular stroke work index (LVSWI). The LVSWI (g*m/m2) changes were compared

Study or Subgroup	Levosimendan Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Fang M 2014	4.6	2.82	18	-0.6	1.37	18	53.2%	5.20 [3.75, 6.65]
Meng J 2016	5.4	2.38	19	1.9	7.93	19	8.1%	3.50 [-0.22, 7.22]
Morelli A 2005	4.3	3.34	15	-0.6	1.25	13	33.6%	4.90 [3.08, 6.72]
Morelli A 2010	8	7.52	20	2	7.52	20	5.1%	6.00 [1.34, 10.66]
Total (95% CI)			**72**			**70**	**100.0%**	**5.00 [3.95, 6.06]**

Heterogeneity: Tau² = 0.00; Chi² = 0.89, df = 3 (P = 0.83); I² = 0%

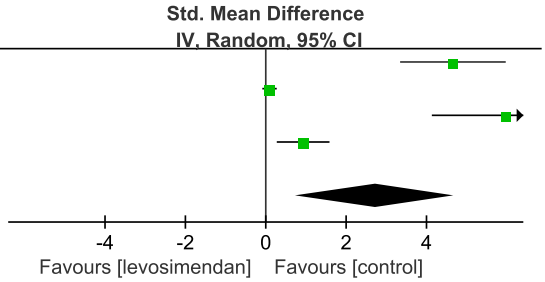
Test for overall effect: Z = 9.28 (P < 0.00001)



9. The effect of levosimendan on fluid infusion. The standard mean difference of fluid infsuion were compared.

Study or Subgroup	Levosimendan Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Fang M 2014	5,746	420	18	4,156.7	215	18	24.3%	4.66 [3.34, 5.97]
Gordon AC 2016	1,847	1,664	239	1,718	1,010	238	27.2%	0.09 [-0.09, 0.27]
Morelli A 2005	5,907	330	15	4,311	136	13	22.0%	5.98 [4.13, 7.82]
Morelli A 2010	5,700	1,000	20	4,850	777.78	20	26.5%	0.93 [0.27, 1.59]
Total (95% CI)			**292**			**289**	**100.0%**	**2.72 [0.75, 4.69]**

Heterogeneity: Tau² = 3.70; Chi² = 87.19, df = 3 (P < 0.00001); I² = 97%
Test for overall effect: Z = 2.71 (P = 0.007)

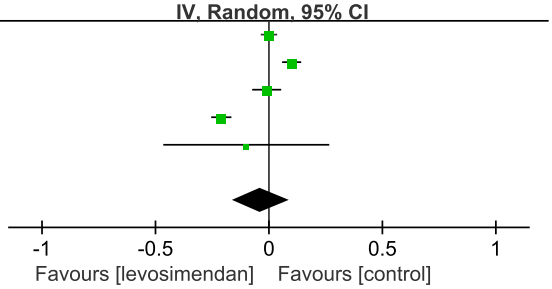


10. The effect of levosimendan on norepinephrine dose. The norepinephrine doses (μg/kg/min) after treatment were compared.

Study or Subgroup	Levosimendan Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Fang M 2014	0.33	0.05	18	0.33	0.06	18	23.4%	0.00 [-0.04, 0.04]
Gordon AC 2016	0.28	0.237	210	0.18	0.193	195	23.2%	0.10 [0.06, 0.14]
Meng J 2016	0.36	0.11	19	0.37	0.09	19	22.4%	-0.01 [-0.07, 0.05]
Morelli A 2005	0.02	0.06	15	0.23	0.06	13	23.1%	-0.21 [-0.25, -0.17]
Morelli A 2010	0.3	0.59	20	0.4	0.59	20	7.8%	-0.10 [-0.47, 0.27]
Total (95% CI)			**282**			**265**	**100.0%**	**-0.04 [-0.16, 0.09]**

Heterogeneity: Tau² = 0.02; Chi² = 102.51, df = 4 (P < 0.00001); I² = 96%

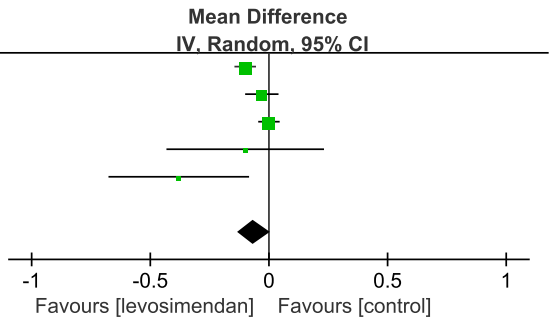
Test for overall effect: Z = 0.56 (P = 0.58)

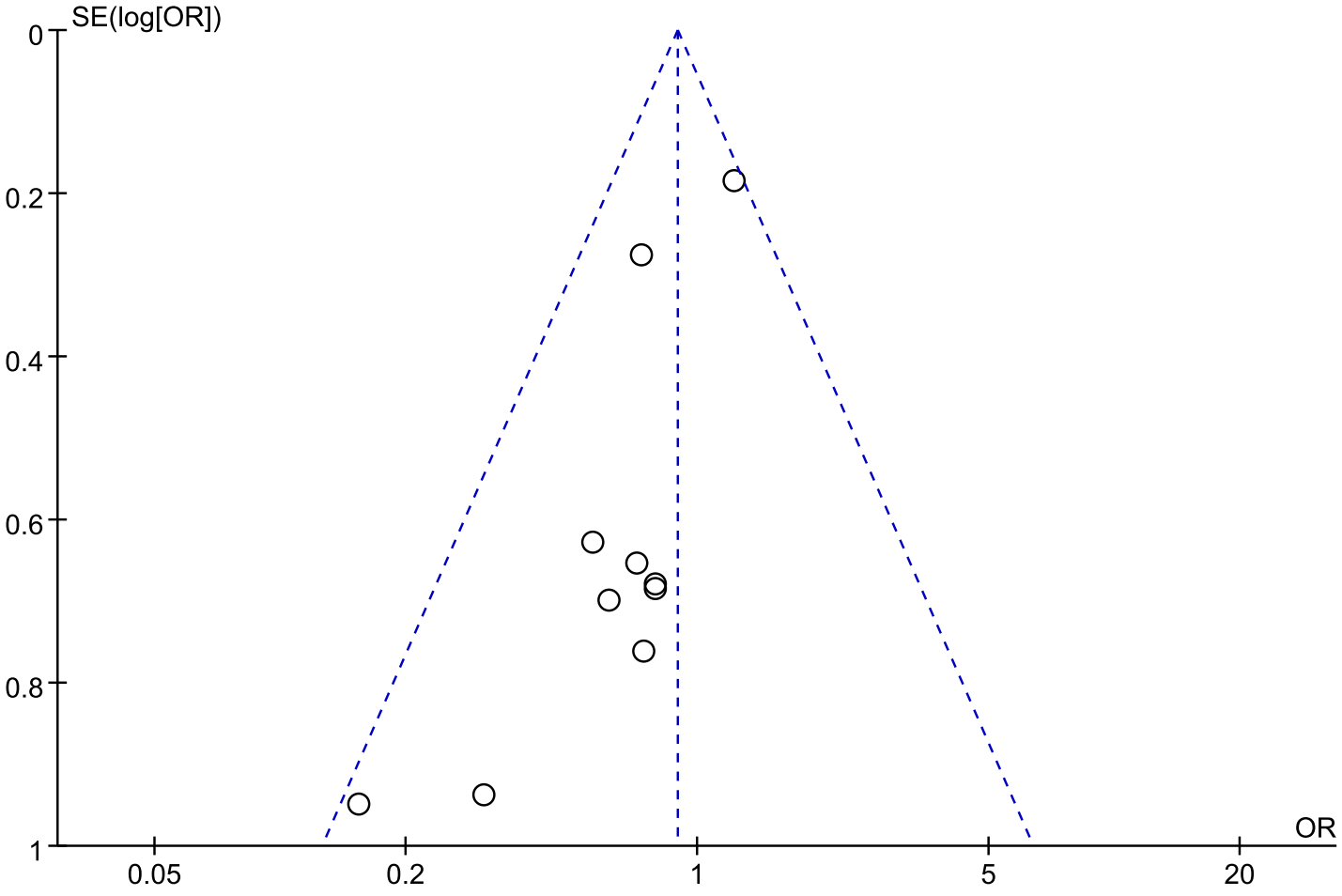


11. The effect of levosimendan on norepinephrine dose. The norepinephrine dose (μg/kg/min) changes were compared.

Study or Subgroup	Levosimendan Mean	SD	Total	Control Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Gordon AC 2016	-0.01	0.26	210	0.09	0.204	195	32.0%	-0.10 [-0.15, -0.05]
Meng J 2016	-0.06	0.12	19	-0.03	0.1	19	27.0%	-0.03 [-0.10, 0.04]
Morelli A 2005	0	0.066	15	0	0.056	13	32.0%	0.00 [-0.05, 0.05]
Morelli A 2010	-0.1	0.56	20	0	0.51	20	4.1%	-0.10 [-0.43, 0.23]
Torraco A 2014	-0.15	0.37	13	0.23	0.4	13	5.0%	-0.38 [-0.68, -0.08]
Total (95% CI)			**277**			**260**	**100.0%**	**-0.06 [-0.13, 0.01]**

Heterogeneity: Tau² = 0.00; Chi² = 14.54, df = 4 (P = 0.006); I² = 72%
Test for overall effect: Z = 1.74 (P = 0.08)

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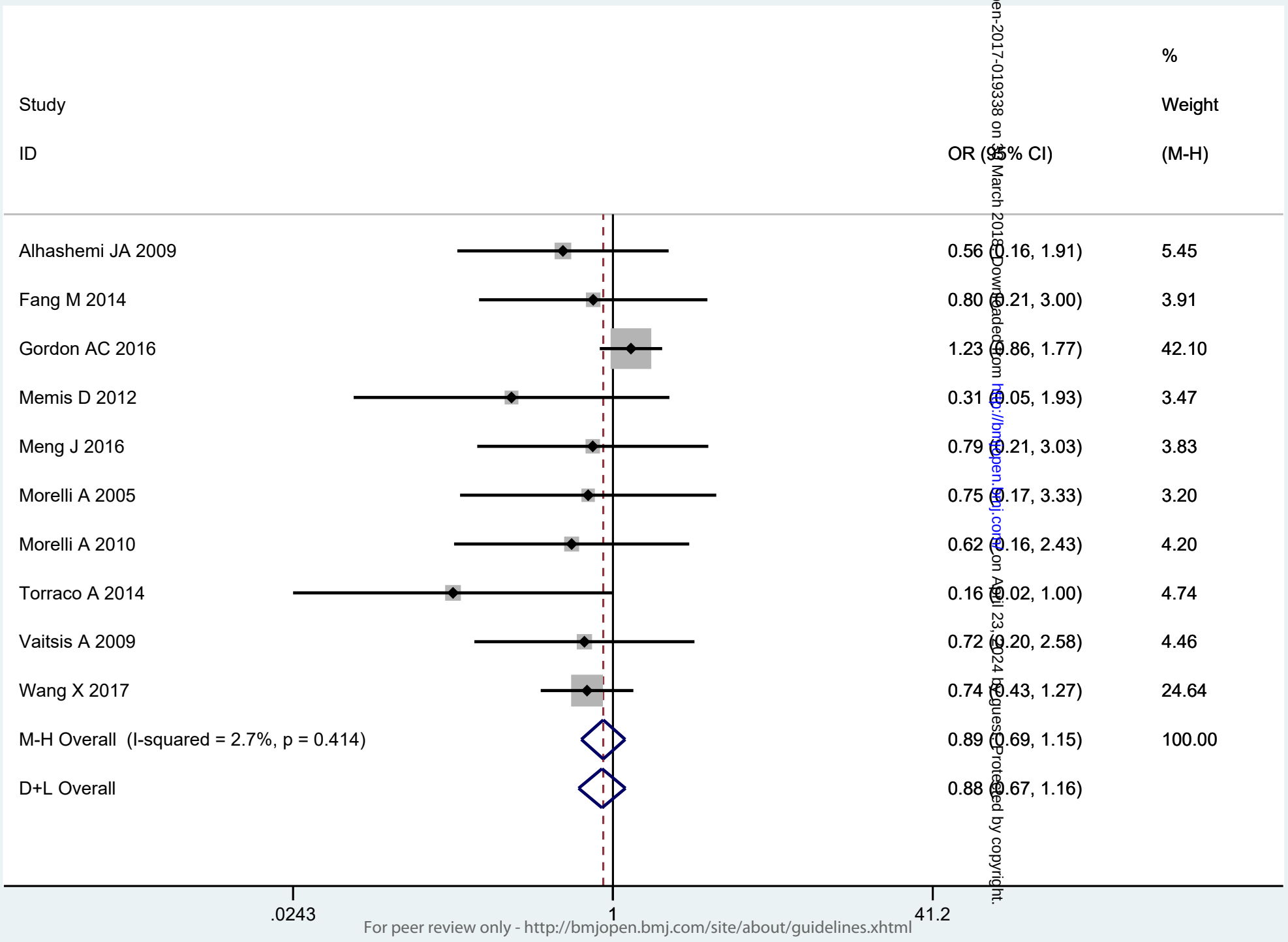

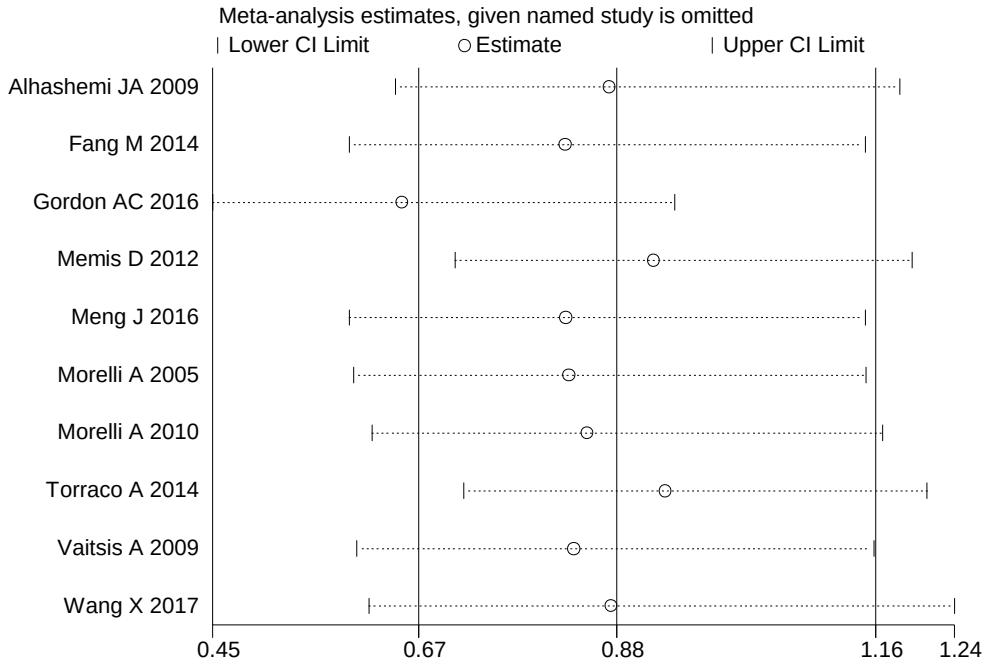

Study omitted	Estimate	[95% Conf.	Interval]
Alhashemi JA 2009	0.874	0.646	1.182
Fang M 2014	0.827	0.597	1.145
Gordon AC 2016	0.653	0.452	0.943
Memis D 2012	0.921	0.710	1.195
Meng J 2016	0.827	0.598	1.145
Morelli A 2005	0.831	0.602	1.146
Morelli A 2010	0.850	0.621	1.164
Torraco A 2014	0.933	0.719	1.211
Vaitsis A 2009	0.836	0.605	1.155
Wang X 2017	0.876	0.618	1.240
Combined	0.881	0.671	1.157

(((((levosimendan) OR simendan) OR Simdax) OR dextrosimendan)) AND ((((sepsis) OR septicemia) OR severe sepsis) OR septic shock)

Methods of imputation of missing data

1. In studies outcomes are presented as median (IQR):

The distribution of outcome is assumed to be normal. Mean is substituted by median, and SD is calculated by the following formula:

$$SD = \frac{IQ_{up} - IQ_{down}}{1.35}$$

2. In studies when baseline and final outcomes are told and presented as mean±SD ($mean_B \pm SD_B$ and $mean_F \pm SD_F$), and the changes are unknown. The mean ($mean_C$) and SD (SD_C) of the changes are calculated by the following formulas:

$$mean_C = mean_F - mean_B$$

$$SD_c = \sqrt{SD_B^2 + SD_F^2 - 2 \times R \times SD_B \times SD_F}$$

Within which, R is called correlation coefficient and is regarded as 0.4 or 0.5 during the calculation, and more values of R (0.2 and 0.8) is used during the sensitivity analysis.

Abbreviations: IQR inter-quartile range, SD standard deviation

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Pg. 1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Pg. 2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Pg. 4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Pg. 4-5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	-
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Pg. 5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Pg. 5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Pg. 5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Pg. 5-6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Pg. 6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Pg. 6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Pg. 6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Pg. 6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Pg. 7

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Pg. 7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Pg. 7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Pg. 7
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Pg. 7-8
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Pg. 8
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Pg. 8
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Pg. 8-9
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Pg. 9-10
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Pg. 10
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Pg. 10-11
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Pg. 11-12
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Pg. 12
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Pg. 13

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: www.prisma-statement.org.