Levosimendan reduces mortality in patients with severe sepsis and septic shock: A meta-analysis of randomized trials

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Alberto Zangrillo, MD[a, b]
zangrillo.alberto@hsr.it

Alessandro Putzu, MD[a]
alessandroputzu@ymail.com

Fabrizio Monaco, MD[a]
monaco.fabrizio@hsr.it

Alessandro Oriani, MD[a]
oriani.alessandro@hsr.it

Giovanna Frau, MD[a]
frau.giovanna@hsr.it

Monica De Luca, MD[a]
monica.deluca@libero.it

Nora Di Tomasso, MD[a]
noramed@hotmail.it

Elena Bignami, MD[a]
bignami.elena@hsr.it

Vladimir Lomivorotov, MD, PhD[c]
vvlom@mail.ru

Valery Likhvantsev, MD, PhD[d]
lik0704@gmail.com

Giovanni Landoni, MD[a, b, *]
landoni.giovanni@hsr.it

[a]Department of Anesthesia and Intensive Care, IRCCS San Raffaele Scientific Institute, Milan, Italy

[b]Vita-Salute San Raffaele University of Milan, Milan, Italy

[c]Department of Anesthesiology and Intensive Care, State Research Institute of Circulation Pathology, Novosibirsk, Russia

[d]Anesthesiology & Intensive Care Department, Moscow Regional Clinical & Research Institute, Moscow, Russia

*Corresponding author at: Department of Cardiothoracic Anesthesia and Intensive Care, Ospedale San Raffaele, Via Olgettina 60, Milan 20132, Italy. Tel.: + 39 02 26436154; fax: + 39 02 26437178.

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Abstract

Purpose

There is controversy about the use of inotropes in the treatment of severe sepsis and septic shock. The objective of this study was to evaluate if levosimendan, as compared with standard inotropic therapy (eg, dobutamine), reduces mortality in septic patients.

Materials and Methods

BioMedCentral, PubMed, EMBASE, and the Cochrane Central Register were searched for pertinent studies, up to 1st May 2015. Randomized trials on the use of levosimendan in patients with severe sepsis and septic shock were included if reporting mortality data. The primary outcome was mortality, whereas secondary outcomes were blood lactate, cardiac index, total fluid infused, norepinephrine dosage, and mean arterial pressure.

Results

Seven studies for a total of 246 patients were included in the analysis. Levosimendan was associated with significantly reduced mortality compared with standard inotropic therapy (59/125 [47%] in the levosimendan group and 74/121 [61%] in the control group; risk difference = − 0.14, risk ratio = 0.79 [0.63-0.98], *P* for effect = .03, I^2 = 0%, numbers needed to treat = 7). Blood lactate was significantly reduced in the levosimendan group, whereas cardiac index and total fluid infused were significantly higher in the levosimendan group. No difference in mean arterial pressure and norepinephrine usage was noted.

Conclusions

In patients with severe sepsis and septic shock, levosimendan is associated with a significant reduction in mortality compared with standard inotropic therapy. A large ongoing multicenter randomized trial will have to confirm these findings.

Keywords: Intensive care; ~~Critical care~~; Anesthesia; Septic shock; Severe sepsis; ~~Shock~~; ~~Vasoactive drugs~~; ~~Inotropes~~; Levosimendan; Dobutamine

1 Introduction

Acute organ dysfunction due to severe infection is associated with a high mortality rate [1]. The mortality rate of patients with septic shock is decreasing [2,3], but still remains high, despite widespread adoption of international sepsis guidelines [4]. There are still several doubts about medical therapy in septic patients. For example, a recent randomized controlled trial showed that protocol-based resuscitation of patients in septic shock does not improve outcomes [5]. Further studies are needed to evaluate new therapeutic approaches to decrease mortality and morbidity of septic patients.

Hypotension associated with septic shock is predominantly due to a vasodilatory state secondary to infection and inflammatory response. In addition, the perfusion deficit may be worsened by new-onset cardiac dysfunction, a well-known manifestation of organ dysfunction in sepsis. This occurs in 40% to 50% of patients with prolonged septic shock and is associated with a higher mortality [6–8]. Nowadays, whether the addition of an inotropic agent improves clinical outcomes in septic shock remains unresolved. Current guidelines recommend a trial of dobutamine in case of myocardial dysfunction or tissue hypoperfusion [4].

Another inotropic agent is levosimendan [9], a calcium-sensitizer agent [10] with vasodilatory properties [11], exerting beneficial effects particularly in cardiac surgery, a setting where it recently showed a survival benefit when compared with dobutamine [12]. The absence of increase in myocardial oxygen consumption likely brings to a myocardial protective effect [13]. Furthermore, novel available data suggest that levosimendan can be useful in patients with renal impairment [9,14].

Experimental studies in septic animal models showed that levosimendan improves myocardial function [15], attenuates intestinal dysfunction [16], improves microvascular oxygenation [17], protects against endotoxemic acute renal failure [18], and exerts immunomodulatory effects [19–21]. However, results are still controversial [17,22–25].

In humans, several case series [26–29] and small single-center randomized control clinical trials [30–36] provide good evidence to sustain the hypothesis that levosimendan might be a promising therapy in severe sepsis and septic shock. However, in 2 subanalyses of a meta-analysis regarding levosimendan administration in critical care setting, investigators failed to find a significant difference in mortality in the septic group [37,38].

Because new randomized articles have been recently published [30–32,34,36], we decided to perform an updated meta-analysis of all the randomized clinical trials published so far to determine the impact of levosimendan on mortality in patients with severe sepsis and septic shock.

2 Materials and methods

2.1 Search strategy

Appropriate studies were independently searched in BioMedCentral, PubMed, EMBASE, and the Cochrane Central Register of clinical trials (updated 1st May 2015) by 3 trained investigators. The full PubMed search strategy is available in the supplementary material (Supplementary Material 1). We decided to use a basic search strategy in order to make the strategy as sensitive as possible.

Abstracts from recent international conferences were searched for additional relevant studies. In addition, we used backward snowballing (ie, scanning of references of retrieved articles and pertinent reviews). The search strategy aimed to include any randomized study ever performed with levosimendan administration in severe sepsis and septic shock in adult humans. *Severe sepsis* was defined as an acute organ dysfunction secondary to documented or suspected infection, and *septic shock* was defined as severe sepsis with hypotension not reversed with fluid resuscitation [4]. No language restriction was enforced.

2.2 Study selection

References obtained from searches were first independently examined at an abstract level by 3 investigators and then, if potentially relevant, collected as complete articles. If the complete article was not available in the database, the corresponding author was contacted for further material.

The following inclusion criteria were used for potentially relevant studies: administration of levosimendan in patients with severe sepsis or septic shock, random allocation to treatment, comparison of levosimendan vs control, and mortality data availability. There were no restrictions on dose or time of administration. The exclusion criteria were as follows: duplicate publications, pediatric studies, and nonintravenous administration of levosimendan. Three investigators independently assessed compliance to selection criteria and selected studies for the final analysis, with divergences finally resolved by consensus.

If the article did not include appropriate data for the meta-analysis (eg, lack of data on mortality), the corresponding author was contacted.

2.3 Data abstraction and study characteristics

Three trained investigators abstracted baseline, procedural, and outcome data using a data-recording form developed for this purpose. In details, we collected potential sources of significant clinical heterogeneity, such as study design, sample size, clinical setting, inclusion and exclusion criteria, levosimendan dose and length, control treatment, mean arterial pressure (MAP) target, follow-up duration, and authors' conflicts of interests, as well as primary study end point and other secondary end points.

The primary end point of the present review was mortality at the longest follow-up available. The secondary end points were blood lactate, cardiac index (CI), total fluid infused, norepinephrine requirement, and MAP, after randomization. The time points of the collection of these variables followed what reported by the authors.

2.4 Internal validity and risk of bias assessment

The internal validity of each trial included in this review was critically evaluated for bias according to The Cochrane Collaboration methods [39]. We assessed the risk of bias associated with the sequence generation method, allocation concealment, blinding of participants and personnel, similarity of the concurrent therapy, completeness of outcome data, free of selective reporting, and free of other bias. We rated the risk of bias by applying a rating of “Yes,” “No,” or “Unclear” to determine whether adequate measures were taken to protect against each potential source of bias in each study. The overall risk of bias was expressed as low, moderate, or high.

2.5 Data analysis and synthesis

To analyze the binary outcome, we calculated the natural logarithms (ln) of risk ratios (RRs) and its SD. Standardized mean difference (SMD) and 95% confidence intervals were computed for continuous variables. Furthermore, we calculate risk difference and numbers needed to treat. To assess the between-study heterogeneity, we used Cochran Q statistic and the I^2 statistic ($I^2 > 25\%$ was used as a threshold indicating significant heterogeneity). We pooled the study-specific estimate using the inverse variance method and a fixed-effect model in case of low statistical inconsistency ($I^2 < 25\%$) or with random-effect model (which better accommodates clinical and statistical variations) in case of moderate or high statistical inconsistency ($I^2 > 25\%$). Publication bias was assessed by visually inspecting funnel plots of the primary outcome, by analytical appraisal based on the Begg adjusted-rank correlation test, and on Egger linear regression test (a 2-sided P value of .10 or less was regarded as significant).

Sensitivity analyses were done to quantify the effect on mortality when restricted to trials with low risk of bias and to trials reporting 30-day mortality. We also investigated the influence of a single study on the overall risk, estimated by sequentially removing the studies to test the robustness of the main results. To explore the influence of length of follow-up and year of publication on mortality, we performed univariate meta-regression analyses of log-risk against these variables.

Statistical significance was set at the 2-tailed .05 level for hypothesis testing. Data analysis was performed using STATA 11.0 Software (StataCorp LP, College Station, Tex). The present systematic review was conducted in keeping with Preferred Reporting Items for Systematic Reviews and Meta-analysis guidelines [40,41].

3 Results

3.1 Literature search

The search strategy yielded 106 abstracts (Fig. 1). Twenty-five studies were reviewed in complete form. Major exclusions were due to lack of mortality data (n = 2) [42,43] or of a randomized design (n = 14) [29,27,44–47,28,48–53,21,54,55]. Finally, 7 articles (246 participants) were included in the meta-analysis [30–36] (Table 1).

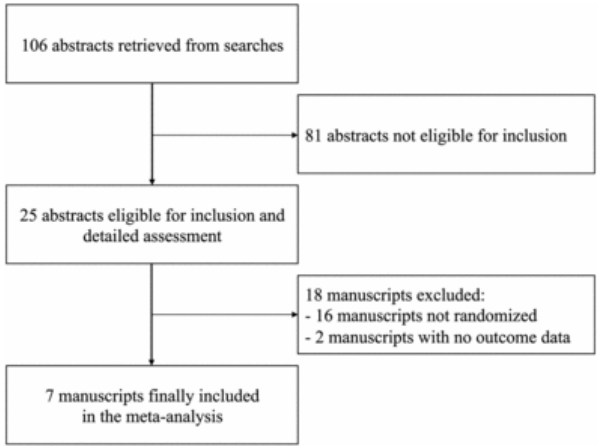

Fig. 1 Flow diagram for the selection of studies.

Table 1 Description of the studies included in the meta-analysis

First author	Year	Setting	Additional cardiocirculatory inclusion criteria	Levosimendan patients	Control patients	Primary outcome
Alhashemi	2009	Severe Sepsis or septic shock	–	21	21	$Scvo_2$ and serum lactate
Fang	2014	Septic shock	LVEF ≤ 45%	18	18	Hemodynamics and cardiac function
Memis	2012	Septic shock	MAP ≤ 65	15	15	Liver function
Morelli	2005	Septic shock	LVEF ≥ 45% and PAOP ≥ 12	15	15	Cardiac function
Morelli	2010	Septic shock	MAP ≥ 65	20	20	Sublingual microcirculatory blood flow
Torraco	2014	Septic shock	MAP ≥ 65	13	13	Mitochondria performance
Vaitsis	2009	Severe sepsis or septic shock	CI ≤ 2.2 or LVEF ≤ 35	23	19	Mortality

LVEF indicates left ventricular ejection fraction; PAOP, pulmonary artery occlusion pressure; $ScVO_2$, central venous oxygen saturation.

3.2 Study characteristics

The characteristics of the 7 selected studies are shown in Tables 1 and 2. Six studies had dobutamine as comparator [31–36], whereas 1 study evaluated levosimendan vs guideline-guided therapy [30]. All studies administered levosimendan as a 24-hour continuous infusion (0.17 ± 0.05 μg kg^{-1} min^{-1}) without bolus. The mean age was 63 ± 5 years in the entire group, and the percentage of female patients was 45% ± 4%. The assessment of the study quality is exposed in the supplementary material (Supplementary Material 2). Two studies showed a low risk of bias [33,34]; 3 studies, a moderate risk [30–32]; and 2 studies, a high risk [35,36].

Table 2 Description of the studies included in the meta-analysis

First author	Year	Control	Levosimendan infusion dose (μg kg^{-1} min^{-1})	Dobutamine infusion dose (μg kg^{-1} min^{-1})	Length of infusion (h)	MAP target (mm Hg)	Follow-up	Conflict of interests
Alhashemi	2009	Dobutamine	0.05-0.2	5-20	24	≥ 65	ICU mortality	NR
Fang	2014	Dobutamine	0.2	5	24	–	28 d	No

Memis	2012	Dobutamine	0.1	10	24	≥ 65	NR	No
Morelli	2005	Dobutamine	0.2	5	24	70-80	30 d	NR
Morelli	2010	Dobutamine	0.2	5	24	≥ 65	ICU mortality	No
Torraco	2014	Standard therapy	0.2	NR	24	65-75	28 d	No
Vaitsis	2009	Dobutamine	0.1	5-10	24	≥ 65	30 d	No

ICU indicates intensive care unit; NR, not reported.

3.3 Levosimendan in septic patients: mortality

The analysis showed that the use of levosimendan in septic patients was associated with a significant reduction in mortality at the longest follow-up available (59/125 [47%] in the levosimendan group and 74/121 [61%] in the control group, risk difference = – 0.14, RR = 0.79 [0.63-0.98], *P* for effect = .03, *P* for heterogeneity = .8, I^2 = 0%, numbers needed to treat = 7; Fig. 2), with 7 studies included (Supplementary Material 3).

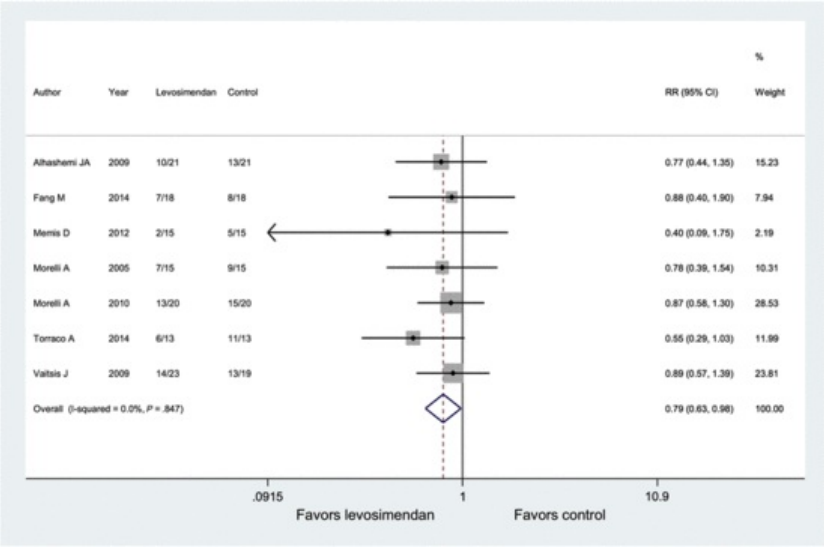

Fig. 2 Forest plot for the risk of mortality at longest follow-up available.

3.4 Levosimendan in septic patients: secondary end points

Four studies [31,33–35] reported blood lactate which was significantly lower in the levosimendan group when compared with the control group (SMD = – 2.09 [– 3.93 to – 0.24], *P* for effect = .03, *P* for heterogeneity < .001, I^2 = 95%; Table 3 and Supplementary Material 4).

Table 3 Secondary end points after randomization

Outcome of interest	No. of studies	Levosimendan group	Control group	RR (95% CI)	*P*	I^2 (%)
Blood lactate (mmol/L)	4	2.78 ± 0.76	3.88 ± 1.37	– 2.085 (– 3.932 to – 0.238)	.027	94.9
CI (L min^{-1} m^{-2})	4	3.75 ± 0.56	3.33 ± 0.57	1.389 (0.255 to 2.523)	.016	89.1
Total fluid infused (mL)	3	5785 ± 284	4439 ± 376	4125 (1154 to 7096)	.007	94.4
Norepinephrine dosage (μg kg^{-1} min^{-1})	4	0.28 ± 0.28	0.37 ± 0.29	– 0.203 (– 0.546 to 0.140)	.245	0.0

MAP (mm Hg)	5	74 ± 5	71 ± 5	0.652 (– 0.344 to 1.649)	.200	88.5

Four studies [31,33,34,36] reported CI, and this analysis showed that it was significantly higher in the levosimendan group when compared with the comparator group (SMD = 1.39 [0.26-2.52], *P* for effect = .02, *P* for heterogeneity < .001, I^2 = 89%; Table 3 and Supplementary Material 5).

Three studies [31,33,34] reported the total fluid infused, and this was significantly higher in the levosimendan group when compared with the control group (SMD = 4.13 [1.15-7.1], *P* for effect = .01, *P* for heterogeneity < .001, I^2 = 94%; Table 3; Supplementary Material 6).

No differences in norepinephrine requirements [30,31,33,34] (SMD = – 0.20 [– 0.55-0.14], *P* for effect = .2, *P* for heterogeneity = 0.7, I^2 = 0%; Table 3; Supplementary Material 7) and in MAP values [30–34] (SMD = 0.65 [– 0.34-1.65], *P* for effect = .2, *P* for heterogeneity < .001, I^2 = 89%; Table 3; Supplementary Material 8) were noted between groups at the end of the treatment period.

3.5 Risk of bias

The funnel plot revealed a possible presence of residual heterogeneity (Fig. 3). However, the Egger test (*P* = .1) and Peters test (*P* = .4) did not suggest the presence of publication bias.

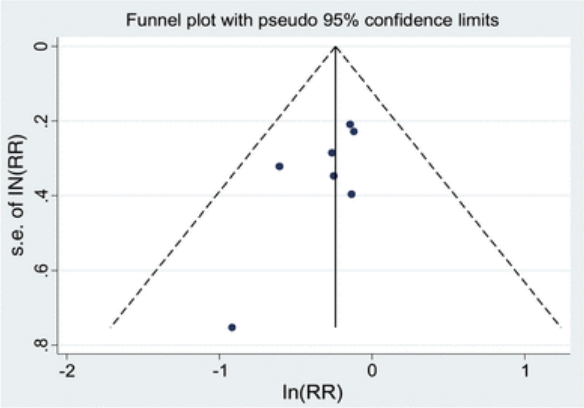

Fig. 3 Funnel plot for the risk of mortality at longest follow-up available.

3.6 Sensitivity analysis

Sensitivity analyses considering only data from studies with low risk of bias did not confirm the mortality reduction findings. Similarly, removal of the study by Torraco et al [30] made the overall estimated effect of levosimendan on mortality nonsignificant (*P* for effect = .12). Removal of any other study did not alter the results of mortality (Supplementary Material 9).

The univariate meta-regressions on log-risk of mortality showed no significant effect for the length of follow-up (*P* value = .9) and for the year of publication (*P* value = .8; Supplementary Material 10).

4 Discussion

The most important finding of our analysis is that levosimendan might reduce mortality in a population of patients with severe sepsis and septic shock when compared with conventional inotropic therapy. Interestingly, secondary analyses showed that levosimendan use was significantly associated with a lower level of blood lactate and higher CI and fluid requirement, when compared with control. Notably, this is the first meta-analysis performed on levosimendan administration in septic patients, and these results are in agreement with subgroup analyses of 2 meta-analyses [37,38], which showed a nonsignificant decreased risk of death in septic patients.

Six of the 7 trials included in the present meta-analysis used dobutamine as control inotropic agent. Only one study [30] included standard therapy according to current guidelines [4] as control, where administration of a dobutamine infusion can be used to achieve the resuscitation goals. This decreases study variability, making it acceptable to consider this meta-analysis a comparison between the 2 inotropic agents, levosimendan and dobutamine.

All the studies included in our analysis were individually very small in size and therefore statistically underpowered for clinically relevant outcomes such as mortality. However, as seen in Fig. 2, all of them showed the similar direction in the findings.

Current guidelines recommend a trial of dobutamine in case of low cardiac output or tissue hypoperfusion despite sufficient fluid resuscitation and perfusion pressure [4]. However, a recent meta-analysis found that the addition of dobutamine to norepinephrine did not significantly affect mortality compared with epinephrine or norepinephrine alone [56], and a recent retrospective study found that the use of inotropic treatment in septic shock was associated with increased 90-day mortality [48]. Nevertheless, it must be considered that myocardial responsiveness to β-adrenergic stimulation is decreased in septic patients [57,58]. Alteration of intracellular calcium influx is a fundamental pathophysiological event in the decreased ventricular contractility, so pharmacologic calcium sensitization might be an inviting therapy in these patients. Considering these pathophysiological events, levosimendan can find a role in the treatment of severe sepsis and septic shock.

Levosimendan may decrease mortality in septic patients, thanks to its immunomodulatory and anti-inflammatory properties, properties well described in experimental studies [18–21].

Unfortunately, there are few randomized trials analyzing the effect of levosimendan in septic patients. Ideally, a large randomized controlled trial on the lookout for the eventual beneficial effects of levosimendan in septic setting should be performed. In this direction, a large randomized controlled trial, including 516 patients, was commenced in the United Kingdom in January 2014, assessing the role of Levosimendan for the Prevention of Acute oRgan Dysfunction in Sepsis (LeoPARDS) [59]. The authors expect to end enrollment in late 2016. Consequently, our findings, if confirmed by the ongoing LeoPARDS, suggest that levosimendan could become a suitable choice in the treatment of severe sepsis and septic shock. Nowadays, it should be acknowledged that there are no guidelines for levosimendan use in this setting.

4.1 Strengths and limitations of the study

A major strength of this analysis is that we assessed the most important clinical outcome (mortality). Furthermore, there was no small study publication bias for the primary end point mortality and the statistical heterogeneity of the included trials was as low as I^2 = 0%. This is the first time that the beneficial effects of this inodilator are translated into the septic setting. Six of the 7 trials showed a trend toward mortality reduction. Recent findings of the presence of myocardial dysfunction in septic patients have biological plausibility of the results of this meta-analysis. We performed a systematic review on several databases and international congresses and without language restriction, aiming to reduce the possibility of missing minor publications. Finally, this is the only meta-analysis yet performed about levosimendan administration in septic setting. Among limitations, we recognized that the data set is quite small, including just 7 studies with an average of 35 patients each. Second, considering severe sepsis and septic shock part of the same entity could have led to heterogeneity. Third, the length of follow-up for mortality was not identical among trials, and we decided to use the longest follow-up reported. Two studies reported intensive care unit mortality [34,35], 2 reported 28-day mortality [30,31], 2 reported 30-day mortality [33,36], and 1 did not provide the time of mortality evaluation [32]. Nonetheless, a meta-regression suggested that the length of follow-up had no effect on the interpretation of the results. Also, the time points of secondary outcome collection were not homogenous among trials. Fourth, several included randomized controlled trials were of suboptimal quality. Furthermore, traditional limitations of meta-analyses due to variations in the treatment regimens, in populations or major subgroups within trials, and in the conduct of the trials apply to this study.

5 Conclusions

This meta-analysis suggests that the administration of levosimendan instead of usual inotropic therapy is associated with a significant reduction in mortality in septic patients. More studies are necessary to clarify whether levosimendan is beneficial for the treatment of severe sepsis and septic shock.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jcrc.2015.05.017.

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Appendix A. Supplementary data

Multimedia Component 1

Supplementary Material 1 - Search strategy for PubMed.

Multimedia Component 2

Supplementary Material 2 - Methodological quality: authors' judgments about each methodological quality item for each included study.

Multimedia Component 3

Supplementary Material 3 - Data on mortality.

Multimedia Component 4

Supplementary Material 4 - Forest plot for blood lactate after randomization.

Multimedia Component 5

Supplementary Material 5 - Forest plot for cardiac index after randomization.

Multimedia Component 6

Supplementary Material 6 - Forest plot for total fluid infused after randomization.

Multimedia Component 7

Supplementary Material 7 - Forest plot for norepinephrine dosage after randomization.

Multimedia Component 8

Supplementary Material 8 - Forest plot for mean arterial pressure after randomization.

Multimedia Component 9

Supplementary Material 9 - Sensitivity analysis of the studies included in the meta-analysis.

Multimedia Component 10

Supplementary Material 10 - Meta-regressions.

Queries and Answers

Query:

Journal style requires a maximum of 6 keywords. Please check and provide the necessary correction.

Answer: We are sorry for the mistake, now the keywords are six: Intensive care, Anesthesia, Septic shock, Severe sepsis, Levosimendan, Dobutamine

Query:

Supplementary caption was not provided. Please check suggested data if appropriate and correct if necessary.

Answer: We are sorry for the mistake, now the keywords are six: Intensive care, Anesthesia, Septic shock, Severe sepsis, Levosimendan, Dobutamine

Query:

Supplementary caption was not provided. Please check suggested data if appropriate and correct if necessary.

Answer: We are sorry for the mistake, now the keywords are six: Intensive care, Anesthesia, Septic shock, Severe sepsis, Levosimendan, Dobutamine

Query:

Supplementary caption was not provided. Please check suggested data if appropriate and correct if necessary.

Answer: Supplementary 3 caption: Data on mortality

Query:

Supplementary caption was not provided. Please check suggested data if appropriate and correct if necessary.

Answer: Supplementary 4 caption: Forest plot for blood lactate after randomization

Query:

Supplementary caption was not provided. Please check suggested data if appropriate and correct if necessary.

Answer: Supplementary 5 caption: Forest plot for cardiac index after randomization

Query:

Supplementary caption was not provided. Please check suggested data if appropriate and correct if necessary.

Answer: Supplementry 6 caption: Forest plot for total fluid infused after randomization

Query:

Supplementary caption was not provided. Please check suggested data if appropriate and correct if necessary.

Answer: Supplementary 7 caption: Forest plot for norepinephrine dosage after randomization

Query:

Supplementary caption was not provided. Please check suggested data if appropriate and correct if necessary.

Answer: Supplementary 8 caption: Forest plot for mean arterial pressure after randomization

Query:

Supplementary caption was not provided. Please check suggested data if appropriate and correct if necessary.

Answer: Supplementary 9 caption: Sensitivity analysis of the studies included in the meta-analysis

Query:

Supplementary caption was not provided. Please check suggested data if appropriate and correct if necessary.

Answer: Supplementary 10 caption: Meta-regressions

Query:

Refs 29 and 44 were identical; thus, the latter was deleted, and renumbering was done. Please check.

Answer: We are sorry for the mistake, we have checked the text, it is correct now.

Query:

Please provide the volume number and page range for the bibliography in Ref. [12].

Answer: Ref. [12] : Volume number 114 (5). Page range 746-756. We have changed Ref 12, now it reads: T. Greco, M.G. Calabrò, R.D. Covello, M.Greco, L.Pasin, A.Morelli, G.Landoni, et al., A Bayesian network meta-analysis on the effect of inodilatatory agents on mortality, Br J Anaesth **114**(5), 2015, 746-756.

Query:

Please provide last page.

Answer: The ref. 21 is correct.

Query:

As Refs. [21] and [57] were identical, the latter has been removed from the reference list and subsequent references have been renumbered. Please check and confirm if appropriate.

Answer: We are sorry for the mistake, we have checked the text, it is correct now.

Query:

As Refs. [27] and [45] were identical, the latter has been removed from the reference list and subsequent references have been renumbered. Please check and confirm if appropriate.

Answer: We are sorry for the mistake, we have checked the text, it is correct now.

Query:

As Refs. [28] and [50] were identical, the latter has been removed from the reference list and subsequent references have been renumbered. Please check and confirm if appropriate.

Answer: We are sorry for the mistake, we have checked the text, it is correct now.

Query:

Please confirm that given names and surnames have been identified correctly.

Answer: We confirm that given names and surnames are correct now.

Query:

Please provide last page.

Answer: The ref. 34 is correct.

Query:

Please provide last page.

Answer: The ref. 36 is correct.

Query:

Please confirm that given names and surnames have been identified correctly.

Answer: The correct names and surnames are: J. Vaitsis, H. Michalopoulou, C. Thomopoulos, S. Massias and P. Stamatis

Query:

Please provide last page.

Answer: The ref. 40 is correct.

Query:

Please provide last page.

Answer: The ref. 43 is correct. It is a single page reference

Query:

Please confirm that given names and surnames have been identified correctly.

Answer: The correct names and surnames are: H. Michalopoulou, P. Stamatis, A. Bakhal, T. Kelgiorgis, A. Foundouli, A. Basile, J. Vaitsis, E. Reinou, P. Batika, and D. Pragastis

Query:

As Refs. [51] and [61] were identical, the latter has been removed from the reference list and subsequent references have been renumbered. Please check and confirm if appropriate.

Answer: We are sorry for the mistake, we have checked the text, it is correct now.

Query:

Please provide last page.

Answer: The ref. 49 is correct.

Query:

Please provide last page.

Answer: The ref. 50 is correct.

Query:

Please provide last page.

Answer: The ref. 51 is correct.

Query:

Please confirm that given names and surnames have been identified correctly.

Answer: We confirm that given names and surnames are correct now.

Query:

Please provide last page.

Answer: The ref. 57 is correct.

Query:

Please provide last page.

Answer: The ref. 59 is correct.