

# Methylene blue as a vasopressor: a meta-analysis of randomised trials

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Hypotension is a common finding in critically ill patients. Hypotensive states below a critical threshold may cause inadequate tissue perfusion, resulting in organ system dysfunction, morbidity and mortality if not promptly treated.<sup>1</sup> Vasodilatory shock can develop as a consequence of sepsis and other conditions such as inadequate tissue oxygenation, ischaemia–reperfusion, prolonged hypotension or cardiopulmonary bypass.<sup>2</sup> Conventional treatments include administration of fluids, vasopressors and inotropes;<sup>3</sup> however, these treatments present significant side effects,<sup>4</sup> and refractory hypotension — a condition referred to as vasoplegic syndrome (VS) — and vascular hyporesponsiveness to vasopressors<sup>5</sup> can complicate this condition.

Recently, interest in catecholamine-sparing agents is increasing, and the negative results of landmark studies such as the Vasopressin and Septic Shock Trial<sup>6</sup> highlight the need for further research on this topic.

Among the many pathophysiological alterations responsible for the low-resistance state, the role of the nitric oxide (NO) pathway seems to be of paramount importance:<sup>7</sup> increased production or release of this ubiquitous mediator is believed to be responsible for both vasodilation and the reduced response to vasopressors. However, when inhibition of the synthesis of NO was used as a therapeutic strategy, it resulted in increased mortality both in animal<sup>8</sup> and human<sup>9</sup> studies. Methylene blue (MB) has been found to oppose NO-induced effects by inhibiting soluble guanylate cyclase,<sup>10,11</sup> one of the downstream effectors of NO. When used in situations of vasodilatory shock, it resulted in increased systemic blood pressure, systemic vascular resistance and myocardial contractility,<sup>12,13</sup> with no apparent major side effect. Due to the lack of adequately powered studies, we performed a meta-analysis of randomised controlled trials to evaluate the efficacy of MB to raise mean arterial pressure (MAP) in hypotensive patients and its effects on survival.

## Materials and methods

### Search strategy

Four trained investigators independently searched BioMedCentral, PubMed, Embase, and the Cochrane Central Register of clinical trials (updated 1 May 2012) for relevant studies. The full PubMed search strategy was developed according to

## ABSTRACT

**Objective:** To evaluate the efficacy of methylene blue in raising mean arterial pressure in hypotensive patients.

**Design:** A meta-analysis of randomised controlled trials.

**Data sources:** We searched BioMedCentral, PubMed, Embase and the Cochrane Central Register of clinical trials.

**Data extraction:** Inclusion criteria were random allocation to treatment and comparison of methylene blue versus any comparator. Exclusion criteria were duplicate publications, non-adult studies and no data on main outcomes. The primary end point was mean arterial blood pressure value 1 hour after the study drug administration; the secondary end points were mortality at the longest follow-up available, and cardiac index.

**Data synthesis:** Data from 174 patients in five randomised controlled studies were analysed. Mean arterial pressure rose in patients receiving methylene blue (weighted mean difference = 6.93 mmHg; 95% CI, 1.67 to 12.18; *P* for effect = 0.01; *P* for heterogeneity = 0.17; *I*<sup>2</sup> = 41%). Only two studies reported the values of cardiac index with a non-statistically significant improvement in the methylene blue group (mean difference = 0.76 L/min/m<sup>2</sup>; 95% CI, -0.32 to 1.84; *P* for effect = 0.2). The overall mortality rate was 16% (14/88) among methylene blue-treated patients and 23% (20/86) in the control group (odds ratio = 0.65; 95% CI, 0.21 to 2.08; *P* for effect = 0.5).

**Conclusions:** Methylene blue increases arterial blood pressure and systemic vascular resistances in vasoplegic patients without a detrimental effect on survival.

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Biondi-Zoccai et al,<sup>14</sup> and is available in the Appendix. In addition, we employed backward snowballing (ie, scanning of references of retrieved articles and pertinent reviews) and contacted international experts and the manufacturer for further studies. No language restriction was enforced.

### Study selection

Four investigators independently examined articles retrieved from database and literature searches at the title

and abstract level. Divergences were resolved by consensus, and potentially relevant articles were retrieved in full. Inclusion criteria were: clinical setting in humans, patients with hypotension (as per author definition), random allocation to treatment, and comparison of MB versus placebo. There were no restrictions on dose or time of administration. The exclusion criteria were: duplicate publications, non-adult studies, oral administration of MB, and lack of data on mortality. Two investigators independently assessed compliance with selection criteria and selected the studies for final analysis. Divergences were resolved by consensus.

### Data abstraction and study characteristics

Baseline, procedural and outcome data were independently abstracted by four trained investigators, with divergences resolved by consensus. Specifically, we extracted potential sources of significant clinical heterogeneity, such as study design, sample size, clinical setting or indication, MB bolus dose, infusion dose and duration, control treatment, and follow-up duration, as well as primary study end points and other key outcomes. Two separate attempts to contact original authors were made in cases of missing data.

### End points

Our primary end point was MAP value 1 hour after administration of the study drug. The secondary end points were mortality at the longest follow-up available and cardiac index.

### Internal validity and risk of bias assessment

The internal validity and risk of bias of the included trials were appraised by two independent reviewers according to Cochrane Collaboration methods,<sup>15</sup> with divergences resolved by consensus.

The possible evidence of publication bias was assessed by visual inspection of funnel plots and by analytical appraisal based on Begg's adjusted-rank correlation test<sup>16</sup> and Egger's linear regression test:<sup>17</sup> a two-sided  $P$  value of  $\leq 0.10$  was regarded as significant.

### Data analysis and synthesis

We performed computations with RevMan 5 (freeware available from the Cochrane Collaboration) and Stata, version 11 (StataCorp). Hypothesis of statistical heterogeneity was tested by means of the Cochran Q test, with statistical significance set at the two-tailed 0.10 level, whereas extent of statistical consistency was measured with  $I^2$  of Higgins and Thompson. We analysed all continuous variables from individual studies to compute individual mean differences with 95% confidence intervals, and pooled the data using the inverse variance method and

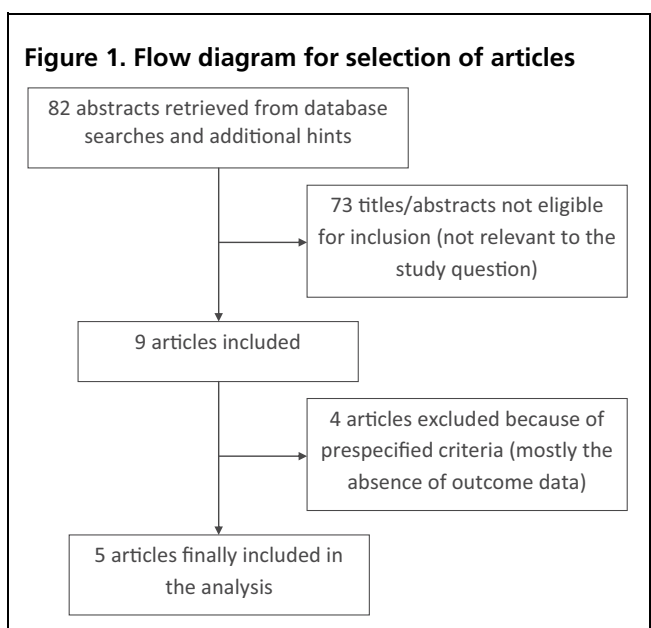
with a fixed-effect model if statistical inconsistency was low ( $I^2 < 25\%$ ) or with a random-effect model if statistical inconsistency was high ( $I^2 > 25\%$ ). We computed the individual risk ratio (RR) and 95% confidence interval for binary outcomes and pooled the data using the inverse variance method as just described.<sup>15</sup> We performed sensitivity analyses by analysing only data from studies with a low risk of bias. Statistical significance was set at the two-tailed 0.05 level for hypothesis testing. We report unadjusted  $P$  values throughout. This study was performed in compliance with the Cochrane Collaboration<sup>15</sup> and Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.<sup>18</sup>

## Results

Database searches, backward snowballing, and contacting experts yielded a total of 82 articles. Excluding 73 non-pertinent titles or abstracts according to the selection criteria, we retrieved and assessed nine studies (Figure 1). Four of these studies were excluded because the included patients were not hypotensive,<sup>19</sup> because there was no control group,<sup>13,20</sup> or because they were not randomised.<sup>21,22</sup> Ultimately, therefore, we identified five eligible randomised clinical trials for inclusion in the analysis.<sup>23-27</sup>

### Study characteristics

The five included trials had collectively allocated 174 patients at random to receive MB (88 patients) or placebo (86 patients) (Table 1). All studies reported data on mortality and four reported MAP data. Clinical heterogeneity was mostly due to setting, dose of MB and duration of follow-up (Table 1 and Table 2). Bolus dosing was used in three



**Table 1. Description of the five randomised studies included in the meta-analysis**

First author	Year	Setting	Multi-centre	Hypotension definition	Inclusion criteria	Methylene blue patients	Control patients	Control group
Kirov MY <sup>25</sup>	2001	ICU	No	MAP < 70 mmHg for at least 30 min	Patients admitted to ICU and diagnosed with severe sepsis and septic shock	10	10	Placebo
Koelzow H <sup>23</sup>	2002	Liver transplant	No	Decrease of MAP > 30%	Patients scheduled for elective OLTX	20	18	Placebo
Levin RL <sup>27</sup>	2004	Cardiac surgery	Yes	MAP < 50 mmHg	Elective cardiac surgery patients with VS	28	28	Placebo
Maslow AD <sup>24</sup>	2006	Cardiac surgery (during CPB)	No	MAP < 50 mmHg or systolic blood pressure < 85 mmHg	Patients taking ACEi within 24 h before elective heart surgery requiring CPB	15	15	Placebo
Memis D <sup>26</sup>	2002	ICU	No	Not specified	Critically ill patients with documented bacterial infections	15	15	Placebo

ICU = intensive care unit. MAP = mean arterial pressure. CPB = cardiopulmonary bypass. OLTX = orthotopic liver transplant. ACEi = angiotensin-converting-enzyme inhibitor. VS = vasoplegic syndrome.

studies, ranging between 1.5 mg/kg and 3 mg/kg,<sup>23-25</sup> and was followed by continuous infusion of MB (0.25–2 mg/kg/h for 4 hours) in only one study.<sup>27</sup> In two studies, MB was administered by continuous infusion only: 0.5 mg/kg/h for 6 hours<sup>26</sup> and 1.5 mg/kg in 1 hour.<sup>27</sup>

MAP value was measured 1 hour after bolus administration or completion of infusion.<sup>23-25,27</sup> In only one study, MAP was measured immediately after completion of a 6-hour infusion of MB.<sup>26</sup> Four of the five studies reported a reduction in vasopressor use after administration of MB,<sup>23-25,27</sup> but data were not comparable because of the use of different types of vasopressors and of different units of measurement. One study was a multicentre trial.<sup>27</sup> Studies appeared to be of variable quality. Four studies were considered to be at low or moderate risk of bias (Table 3).

### Quantitative data synthesis

Overall analysis showed that the use of MB was associated with a statistically significant increase in MAP (mean difference, 6.93 mmHg [95% CI, 1.67 to 12.18]; *P* for effect = 0.01; *P* for heterogeneity = 0.17; *I*<sup>2</sup> = 41%, with four studies included) (Figure 2). Only two studies reported cardiac index<sup>23,25</sup> with a non-statistically significant improvement in the MB group (mean difference, 0.76 L/min/m<sup>2</sup> [95% CI, -0.32 to 1.84]; *P* for effect = 0.2). Notably, mortality rate was lower but not statistically different in the MB group (14/88 [16%] as compared with the control group 20/86 [23%]; odds ratio, 0.65 [95% CI, 0.21 to 2.08]; *P* for effect = 0.5, with all five studies included) (Figure 3). No adverse event associated with the administration of MB was reported in the considered studies, except for a blue colour of skin and urine.

**Table 2. Doses and duration of of methylene blue treatment, follow-up and adverse events**

First author	Methylene blue bolus	Interval between bolus and continuous infusion	Methylene blue infusion	Overall duration of treatment	Follow-up	Adverse events
Kirov MY <sup>25</sup>	2 mg/kg in 15 min	2 hours	0.25–2 mg/kg/h for 4 hours	7 hours	28 days	Urine and skin colour
Koelzow H <sup>23</sup>	1.5 mg/kg immediately before reperfusion		No	Bolus during surgery	30 days	Not reported
Levin RL <sup>27</sup>		No interval	1.5 mg/kg/h for 1 hour	1 hour	4 days	Urine colour (blue or green)
Maslow AD <sup>24</sup>	3 mg/kg		No	Bolus during surgery	Surgery time	Transient reduction of SvO <sub>2</sub> at the monitor
Memis D <sup>26</sup>		No interval	0.5 mg/kg/hour for 6 hours	6 hours	28 days	No

Visual inspection of the funnel plot for MAP (Figure 4) did not identify a skewed or asymmetrical shape for mortality. Quantitative evaluation did not suggest a presence of publication bias, as measured by Begg's test ( $P=0.9$ ) and Egger's test ( $P=0.7$ ). The sensitivity analyses, performed in trials with low risk of bias, confirmed that the use of MB was associated with a significant increase in MAP (mean difference, 8.93 mmHg [95% CI, 1.55 to 16.32];  $P$  for effect=0.02;  $P$  for heterogeneity=0.40;  $I^2=0$ , with two studies included).

## Discussion

This meta-analysis collected all the available randomised controlled trials on the use of MB to restore haemodynamic stability in the context of VS: our most important finding is to suggest that MB raises MAP in hypotensive patients in a statistically significant way. Interestingly, unlike a previous study in which NO synthase inhibitors were used to treat vasoplegic patients,<sup>9</sup> this happened without negative effects on survival. The mean difference of 7 mmHg between the MAPs of the two different groups is probably not clinically relevant per se, but is of particular relevance in light of a possible use of MB as a catecholamine-sparing agent. Unfortunately, because of the differences in the type of catecholamines used and in the reporting of the dose administered, we could not collect consistent data to investigate whether a dose-response effect on haemodynamic parameters was present. However, four included studies reported a reduced need for catecholamine in patients treated with MB.<sup>23-25,27</sup> Since no severe adverse event related to MB administration was reported in all five studies, we considered that mortality would have been a reasonable surrogate for safety.

The main limitation of our analysis is that it included only five studies with a total of 174 hypotensive critically ill patients. These studies had different clinical settings (two

were among ICU patients, two among patients who had cardiac surgery, one among patients undergoing liver transplantation), all except one were single centre studies, and they all included a small number of patients. However, vasoplegic shock is a syndrome rather than a specific nosographic entity, and this reduces the significance of such heterogeneity: in fact, an alteration in the NO pathway has been suggested as a common feature of this condition in many different clinical settings.<sup>2</sup> In the selected studies there were differences in the timing and method of administration of MB (bolus or continuous infusion). However, the total dose of MB administered was comparable in all the studies, ranging from 1.5 to 3 mg/kg, further contributing to a reduction in the heterogeneity of the population.

Despite the small number of studies included, the funnel plot of MAP did not suggest a presence of publication bias. Four of five studies were considered to be at low or moderate risk of bias, and the sensitivity analyses we performed in two trials with low risk of bias confirmed the finding that the use of MB was associated with a significant increase in MAP.

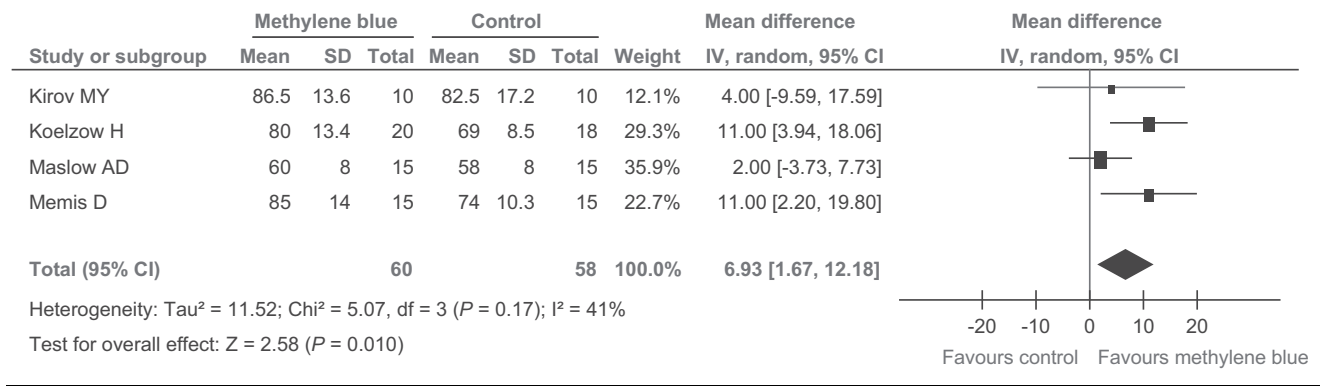
## Possible pathophysiological explanation

Systemic inflammatory response associated with profound vasodilation has been observed in various clinical settings.<sup>2</sup> VS has been associated with severe sepsis, cardiopulmonary bypass (CPB), anaphylaxis, ischaemia-reperfusion syndrome and haemodialysis.<sup>28-31</sup> Its incidence varies, but it can occur in as many as 10% of patients after cardiac surgery<sup>31-33</sup> and may be present in up to 50% of patients who die from sepsis.<sup>28,30</sup> VS is thought to be related to dysregulation of endothelial homeostasis and subsequent endothelial dysfunction. This results in an acute clinical syndrome of persistent hypotension and inadequate tissue oxygenation, leading to organ system dysfunction and, if not promptly treated, death.

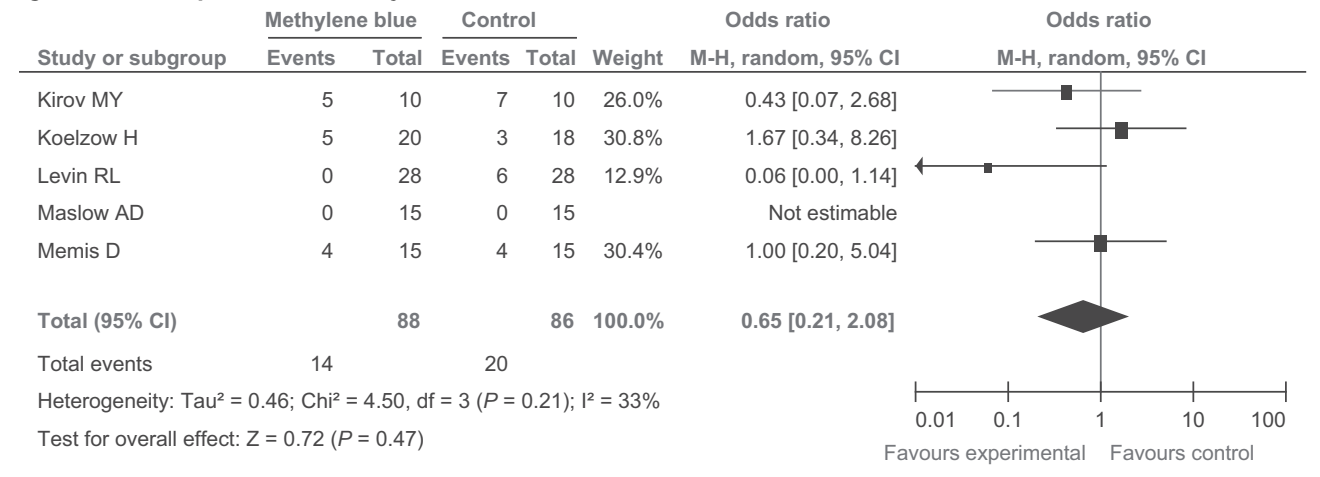
**Table 3. Methodological quality summary: review of authors' judgements about each methodological quality item for each included study**

First author	Domain/question								
	Adequate sequence generation	Allocation concealment used	Blinding	Concurrent therapies similar	Free of incomplete outcome data addressed	Uniform and explicit outcome definitions	Free of selective outcome reporting	Free of other bias	Overall risk of bias
Kirov MY <sup>25</sup>	Unclear	Yes	No	Yes	Yes	Yes	Yes	Yes	Low
Koelzow H <sup>23</sup>	Unclear	Unclear	No	Yes	Unclear	Yes	Unclear	Unclear	High
Levin RL <sup>27</sup>	Unclear	Unclear	No	Yes	Yes	Yes	Yes	Yes	Moderate
Maslow AD <sup>24</sup>	Unclear	Unclear	No	Yes	Yes	Yes	Yes	Yes	Moderate
Memis D <sup>26</sup>	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Yes	Low

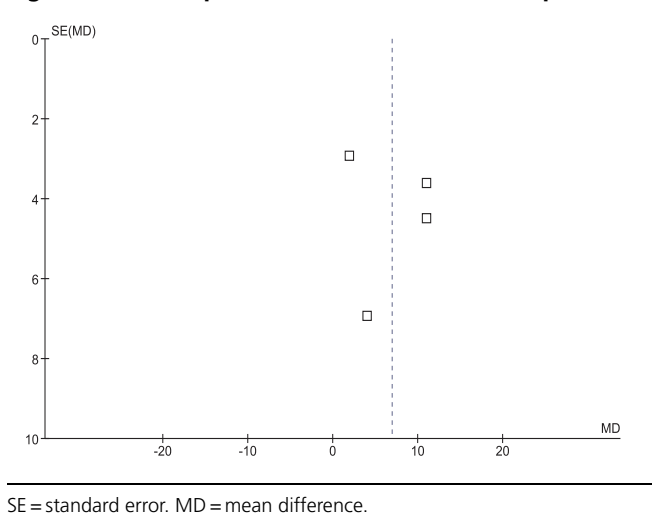
**Figure 2. Forest plot for mean arterial blood pressure**



**Figure 3. Forest plot for mortality**



**Figure 4. Funnel plot for mean arterial blood pressure**



Among the mechanisms thought to be contributory to VS, the NO pathway appears to play a prominent role.

The “classical” mechanism of action underlying the vasodilating effect of NO is mediated by nitrosylation of the haem iron within soluble guanylate cyclase, leading to increased synthesis of cyclic guanosine monophosphate (cGMP). This, in turn, can act on several targets, such as protein kinases that modulate myosin-light-chain kinase and phosphatase activities, or cGMP-mediated opening of calcium- and ATP-sensitive potassium channels.<sup>34</sup> However, it was recently found that many of the actions of NO are cGMP-independent, and happen through nitrosation of critical thiols of target proteins.<sup>35</sup>

Reduction of NO synthesis, by direct inhibition of NO synthase, has been proposed as an approach to attenuate hypotension and revert vascular hyporesponsiveness.<sup>36</sup> However, evidence from both animal and human studies found that treatment with non-selective NO synthase

inhibitor resulted in worsening of the haemodynamic status and increased mortality,<sup>8,9,37,38</sup> probably because of an imbalance between the protective and pathological actions of NO.<sup>38</sup>

A relatively new approach to the treatment of VS, although using a quite old compound, might be the administration of MB. It was proven to be effective in reversing hypotension and restoring the physiological response to vasoactive drug administration in the setting of VS related to cardiac surgery, sepsis, anaphylaxis, ischaemia–reperfusion injury, liver failure and haemodialysis,<sup>12,13,23-27</sup> although the evidence in favour of such a use derived mainly from case series or small RCTs.

The efficacy of MB in raising MAP without adverse effects on survival could be explained by the peculiar ability of MB to modulate the NO system: several modes of action have been postulated, with different mechanisms that may predominate in different clinical situations. Although it was reported to have NO-scavenging properties<sup>39</sup> and, possibly, to directly inhibit its synthesis by NO synthase,<sup>40</sup> the main action is by inhibition of soluble guanylate cyclase.<sup>10,11,41,42</sup> It also acts as an antioxidant and a pro-oxidant, inhibits prostacyclin synthesis, and accelerates reductive processes in the cell.<sup>43</sup>

### Conclusions

In vasoplegic patients, MB significantly increases MAP without a detrimental effect on survival. Considering the heterogeneity and small size of the populations included in our study, large randomised trials to investigate the possible beneficial effects of this intervention are needed.

### Acknowledgement

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### Competing interests

None declared.

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### Appendix (search strategy)

(("methylene blue"[mh] OR "methylthionine chloride"[mh]) AND ("sepsis"[mh] OR "critical care"[mh] OR "critical illness"[mh] OR "cardiovascular diseases"[mh])) AND (randomized controlled trial[pt] OR controlled clinical trial[pt] OR randomized controlled trials[mh] OR random allocation[mh] OR double-blind method[mh] OR single-blind method[mh] OR clinical trial[pt] OR clinical trials[mh] OR ("clinical trial"[tw]) OR ((singl\*[tw] OR doubl\*[tw] OR trebl\*[tw] OR tripl\*[tw]) AND (mask\*[tw] OR blind\*[tw])) OR ("latin square"[tw]) OR placebos[mh] OR placebo\*[tw] OR random\*[tw] OR research design[mh:noexp] OR comparative study[mh] OR evaluation studies[mh] OR follow-up studies[mh] OR prospective studies[mh] OR cross-over studies[mh] OR control\*[tw] OR prospectiv\*[tw] OR volunteer\*[tw]) NOT (animal[mh] NOT human[mh])) □