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## Antioxidant nutrients: a systematic review of trace elements and vitamins in the critically ill patient

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**Abstract** *Objective:* Critical illness is associated with the generation of oxygen free radicals and low endogenous antioxidant capacity leading to a condition of oxidative stress. We investigated whether supplementing critically ill patients with antioxidants, trace elements, and vitamins improves their survival. *Methods:* We searched four bibliographic databases from 1980 to 2003 and included studies that were randomized, reported clinically important endpoints in critically ill patients, and compared various trace elements and vitamins to placebo. *Results:* Eleven articles met the inclusion criteria. When the results of all the trials were aggregated, overall antioxidants were associated with a significant reduction in mortality [Risk Ratio (RR) 0.65, 95% confidence intervals (CI) 0.44–0.97,  $p=0.03$ ] but had no effect on infectious complications. Studies that utilized a single trace element were associated with a significant reduction in mortality [RR 0.52, 95% CI

0.27–0.98,  $p=0.04$ ] whereas combined antioxidants had no effect. Studies using parenteral antioxidants were associated with a significant reduction in mortality [RR 0.56, 95% CI 0.34–0.92,  $p=0.02$ ] whereas studies of enteral antioxidants were not. Selenium supplementation (alone and in combination with other antioxidants) may be associated with a reduction in mortality [RR 0.59, 95% CI 0.32–1.08,  $p=0.09$ ] while non-selenium antioxidants had no effect on mortality. *Conclusions:* Trace elements and vitamins that support antioxidant function, particularly high-dose parenteral selenium either alone or in combination with other antioxidants, are safe and may be associated with a reduction in mortality in critically ill patients.

**Keywords** Enteral nutrition · Parenteral nutrition · Antioxidants · Oxidative stress · Trace elements · Meta-analyses

### Introduction

Oxidative stress is increasingly being recognized as central to the underlying pathophysiology of critical illness, especially the development of organ failure. Reactive oxygen species (ROS) and reactive nitrogen-oxygen species (RNOS) have clearly identified roles in modulating cell signaling, proliferation, apoptosis, and cell protection. However, ROS and RNOS are also capable of attacking proteins, polysaccharides, nucleic acids, and

polyunsaturated fatty acids resulting in cellular damage and tissue dysfunction [1]. In critical illness ROS can be produced from mitochondrial dysfunction, as is classically observed in septic shock [2], the NADPH oxidase enzyme of stimulated neutrophils and macrophages, and the conversion of xanthine dehydrogenase to xanthine oxidase which is activated during ischemia/reperfusion injury [1]. Moreover, ROS/RNOS may trigger the release of cytokines from immune cells, activate inflammatory cascades, and increase the expression of adhesion mole-

cules, probably mediated through inducing the expression of nuclear factor  $\kappa$ B [3]. Thus inflammation and tissue injury result in the accumulation of granulocytes in organs that lead to increased generation of ROS, which further perpetuates or amplifies the inflammatory response and subsequent tissue injury [4]. These pathways and cycles are central to the underlying pathophysiology in critically ill patients with a systemic inflammatory response and multiorgan dysfunction.

In humans there is a complex endogenous defense system designed to protect tissues from ROS/RNOS induced cell injury. Special enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (including their cofactors selenium, zinc, manganese, and iron), sulfhydryl group donors (i.e., glutathione), and vitamins (i.e., vitamins E, C, and  $\beta$ -carotene) form a network of functionally overlapping defense mechanisms. In critically ill patients there are reduced stores of antioxidants, reduced plasma or intracellular concentrations of free electron scavengers or cofactors, and decreased activities of enzymatic systems involved in the detoxification of ROS in critically ill patients [5]. The circulating antioxidant levels decrease rapidly after insult, trauma, or surgery and remain below normal levels for several days or even weeks [6]. The more severe the trauma, systemic inflammatory response syndrome (SIRS), or sepsis, the larger the depletion of antioxidants appears to be [7, 8]. These described observations are not mere epiphenomenon as low endogenous stores of antioxidants are associated with an increase in free radical generation, an augmentation of the systemic inflammatory response, subsequent cell injury, increased morbidity, and even higher mortality in the critically ill [6, 9].

It is hypothesized that the exogenous supply of defined trace elements and vitamins would be helpful to regain the balance between oxidants and antioxidants in critical illness. However, most of these studies were performed in relatively small patient populations presenting with trauma, burns, sepsis, or acute respiratory distress syndrome (ARDS) and thus are underpowered to detect a treatment effect on clinically important outcomes. The purpose of this study was to systematically review, critically appraise, and statistically aggregate randomized trials of antioxidant strategies (i.e., trace elements and vitamins) to determine their effect on mortality and infectious complications. We have previously presented these results in abstract form at the Society for Critical Care Medicine's annual scientific symposium [10].

## Methods

### Search strategy

To locate articles to be included in this review, four bibliographic databases (MEDLINE, EMBASE, CINAHL the Cochrane Con-

trolled Trials Register, and the Cochrane Database of Systematic Reviews) were searched from 1980 to December 2003. Search terms included: "nutritional support" or "dietary supplementation" or "trace elements" or "vitamins" or "enteral nutrition" or "parenteral nutrition", and "critical care" or "critical illness" or "intensive care units." We accepted abstracts from scientific meetings for inclusion into this review if we could obtain a copy of a manuscript or reports that provided the data necessary for abstraction. Manufacturers were contacted for abstracts or articles. In addition, personal files and relevant review articles were searched for additional studies.

### Study selection criteria

Studies were selected for inclusion in the review if they met the following criteria: (a) study design: randomized clinical trials (pseudorandomized trials were excluded); (b) population: critically ill adult patients receiving treatment with micronutrients (as opposed to prophylaxis); (c) intervention: trace elements and/or vitamins vs. placebo (either via enteral, parenteral, or both). We defined critically ill patients as those care for in an intensive care unit (ICU). Included studies were not limited to those in English. We excluded studies that did not report clinically important endpoints such as mortality, complications, and length of stay. We also excluded studies of multiple nutrients studied in addition to vitamins and trace elements. We assessed the methodological quality of all selected articles in duplicate, independently, using a scoring system that we have used previously [11] (see Table 1). Disagreement was resolved by consensus. If necessary information was missing from published studies, we attempted to contact the authors of included studies and requested this information from them. Data abstraction was carried out in duplicate, independently, and differences were resolved by consensus.

As a hypothesis-generating exercise, a priori, we developed several subgroup analyses to explore the optimal composition, route of delivery, and dose of antioxidants. We compared the results of studies of single antioxidants to combined antioxidants and the results of studies in which antioxidants were administered via the parenteral route to enteral. Given our awareness of the existing data and the several positive studies that evaluated selenium supplementation, we compared the studies of selenium to those that used nonselenium antioxidants. Finally, we compared high-dose to low-dose selenium using the median dose as the cutoff (500  $\mu$ g/day). For the analysis of single antioxidants only data pertaining to the group receiving selenium alone vs. placebo from the Berger 2001 study [12, 13] was included (reported as Berger 2001a in the figures) whereas the data from the combined selenium vs. placebo intervention arm (reported as Berger 2001b in the figures) was included in the analysis for combined antioxidants. For the analyses for overall effect, parenteral route, and selenium both the intervention arms of the study were included and reported separately (reported as Berger 2001a and 2001b in the figures). Studies that used both routes of enteral and parenteral administration were excluded from the subgroup analyses of parenteral vs. enteral administration [14, 15].

### Analysis

The primary outcomes of interest were mortality (ICU and hospital) and number of patients who developed infectious complications (pneumonia, line-related sepsis, and others as defined by the primary authors). The secondary outcome was length of stay in hospital or ICU. We combined data from all studies to estimate the common risk ratio (RR) and associated 95% confidence interval (CI) for death and infectious complications. The common RRs and their CIs were estimated using the random effects model of Der-

**Table 1.** Study designs of randomized trials evaluating antioxidant strategies in critically ill patients (*concealed* concealed randomization, *EN* enteral nutrition, *PN* parenteral nutrition, *ITT* intent to treat, *NA* not available, *TBSA* total body surface area, *SIRS* systemic inflammatory response syndrome, *APACHE* Acute Physiology and Chronic Health Evaluation)

	Population	Methods score	Route	Intervention
Berger et al. [12, 13]	Trauma patients, surgical ICU ( <i>n</i> =32)	Concealed, yes; ITT, yes; blinding, double blind; score 9	Intravenous	Intravenous selenium supplementation (500 µg/day) vs. placebo (selenium group randomized further to two groups: (a) 500 µg selenium alone vs. (b) 500 µg selenium + 150 mg α tocopherol + 13 mg zinc) given slowly from day 1–5 after injury; all groups received EN
Porter et al. [14]	Surgical ICU, penetrating trauma patients with injury severity score ≥25 ( <i>n</i> =18)	Concealed, yes; ITT, yes; blinding, no; score 9	Intravenous and EN	50 µg selenium intravenous q 6 h + 400 IU vitamin E, 100 mg vitamin C q 8 h, and 8 g <i>N</i> -acetylcysteine q 6 h; from day 0–7 via nasogastric or oral route vs. none
Nathens et al. [15]	General surgical, trauma ICU ( <i>n</i> =770)	Concealed, not sure; ITT, no; blinding, no; score 7	Intravenous and EN	α Tocopherol 1000 IU q 8 h via naso- or orogastric tube and ascorbic acid 1000 mg q 8 h via intravenous vs. standard care <sup>a</sup>
Berger et al. [19]	Burns >30% TBSA ( <i>n</i> =20)	Concealed, yes; ITT, yes; blinding, double blind; score 12	Intravenous	Intravenous copper (40.4 µmol), selenium (159 µg), zinc (406 µmol) + standard trace elements vs. standard trace elements (copper 20 µmol, selenium 32 µg, zinc 100 µmol); from day 0–8; all received early EN
Kuklinski et al. [18]	Patients with acute pancreatic necrosis ( <i>n</i> =17)	Concealed, not sure; ITT, yes; blinding, no; score 8	Intravenous	Intravenous selenium supplementation (500 µg/d) vs. PN without selenium supplementation <sup>a</sup>
Zimmerman et al. [20]	Patients with SIRS, APACHE >15 and multiorgan failure score >6 ( <i>n</i> =40)	Concealed, not sure; ITT, yes; blinding, no; score 5	Intravenous	1000 µg Na-selenite as a bolus intravenous then 1000 µg Na-selenite/24 h as a continuous infusion over 28 days vs. standard <sup>a</sup>
Angstwurm et al. [21]	Patients with SIRS ( <i>n</i> =42)	Concealed, not sure; ITT, yes; blinding, no; score 10	Intravenous	PN with high dose selenium from 24 h from admission for 9 days (535 µg x3 days, 285 µg x3 days and 155 µg x3 days and 35 µg thereafter) vs. low-dose selenium, 35 µg/day for duration of study
Preiser et al. [22]	Mixed ICU ( <i>n</i> =51)	Concealed, not sure; ITT, no; blinding, single; score 7	EN	Antioxidant rich formula via EN (133 µg/100 ml vitamin A, 13 mg/100 ml vitamin C, and 4.9 mg/100 ml vitamin E) vs. isonitrogenous, isocaloric standard formula (67 µg/100 ml vitamin A, 5 mg/100 ml vitamin C and 0.81 mg/100 ml vitamin E) from day 0–7
Young et al. [23]	Severely head-injured patients, ventilated ( <i>n</i> =68)	Concealed, not sure; ITT, yes; blinding, double; score 12	Intravenous then oral	12 mg elemental zinc via PN, then progressing to oral zinc from 0–15 days vs. 2.5 mg elemental zinc, then progressing to oral placebo
Maderazo et al. [24] <sup>b</sup>	Blunt trauma ( <i>n</i> =46)	Concealed, yes; ITT, yes; blinding, double; score 8	Intravenous	200 mg ascorbic acid, then ↑ 500 mg + 50 mg α tocopherol in 100 ml D5W vs. 100 ml of D5W (experimental group divided into two groups, 200 mg ascorbic acid vs. 50 mg α tocopherol), given as 2 h infusions from day 0–7; all groups received enteral nutrition or oral intake
Berger et al. [25]	Burns >20% TBSA ( <i>n</i> =17)	Concealed, yes; ITT, yes; blinding, double; score NA	Intravenous	100 ml copper (59 µmol) + selenium (380 µg) + zinc (574 µmol) vs. NaCl (0.9%) from admission for 14–21 days

<sup>a</sup> Unable to determine precise timing of intervention

<sup>b</sup> Only data pertaining to the group receiving ascorbic acid + α tocopherol vs. placebo presented here  
Conversion: Selenium 1 µg=0.0126 µmol

Simonian and Laird [16] as implemented in RevMan 4.2.7 [17]. We also assessed publication bias with a funnel plot using the fixed effects methods in RevMan 4.2.7. Where possible, studies were aggregated on an intention-to-treat basis (see Table 1 for those studies reporting an intention-to-treat analysis). Post hoc we noted that one small study with poor methodological quality [18] had a large treatment effect, and we conducted a sensitivity analysis removing this study from the aggregated results to evaluate the influence of this study on the overall conclusions. We considered differences at the level of  $p < 0.05$  to be statistically significant and those at  $p < 0.20$  as indicating a trend.

## Results

### Study identification and selection

A total of 44 relevant citations were identified from a search of computerized bibliographic databases, our personal files, and a review of reference lists from related articles. Of these potentially eligible papers 11 met the inclusion criteria [12, 14, 15, 18, 19, 20, 21, 22, 23, 24, 25.] One trial of trauma patients was published in two parts, focusing on thyroid hormones, which excluded a patient with prior hypothyroidism [12] and on outcomes and antioxidant variables by intent-to-treat [13]. The details of study design and methodological quality are presented in Table 1 and the study outcomes in Table 2. Of the 11 studies 6 had inadequate allocation concealment [15, 18, 20, 21, 22, 23]. Several authors examined the effects of single antioxidants [12, 18, 20, 21, 23]. Of these most looked at selenium alone [12, 18, 20, 21] whereas one studied the effect of zinc supplementation in ventilated head-injured patients [23]. The effects of selenium combined with other antioxidants were studied in four studies [12, 14, 19, 25], resulting in a total of seven studies including selenium with or without other antioxidants [12, 14, 18, 19, 20, 21, 25]. Three studies focused on the effects of vitamins A, C, and E [15, 22, 24]. Reasons for excluding relevant studies included lack of randomization [26, 27, 28, 29, 30, 31, 32, 33], studies of non-critically ill patients [34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44] no clinical outcomes reported [45, 46, 47, 48, 49], study was in abstract form only without access to manuscript [50], and short duration of intervention (6 h) [51]. We also excluded studies of nutrients other than trace elements or vitamins such as *N*-acetylcysteine alone [52, 53, 54, 55, 56] and those with multiple nutrients studied in addition to other vitamins and trace elements [57, 58].

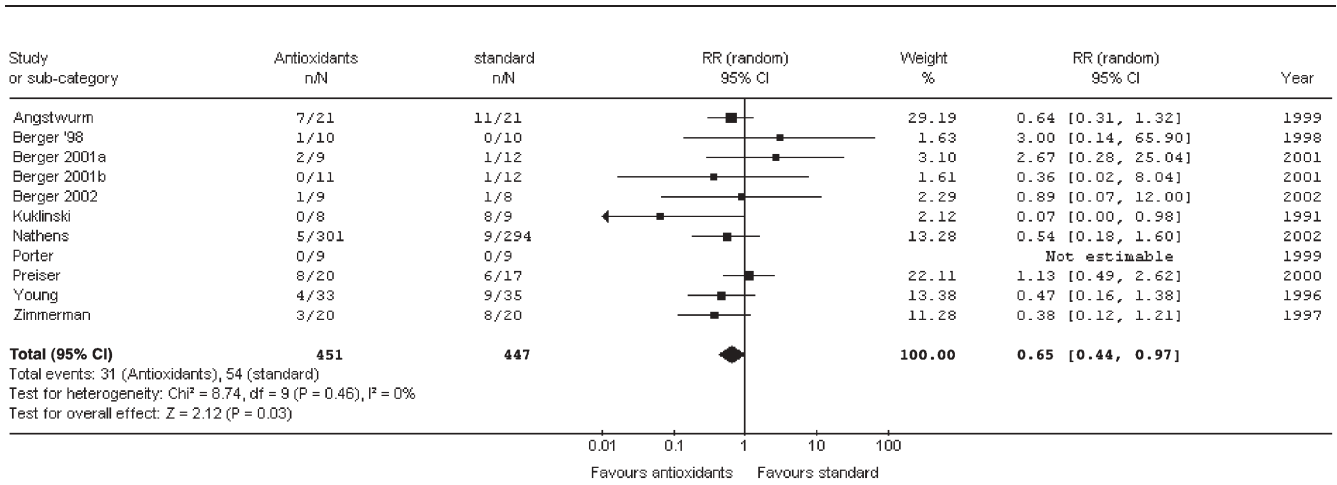
### Overall effect on mortality and infectious complications

When the results of all 11 randomized controlled trials were aggregated ( $n=886$ ), overall antioxidants were associated with a significant reduction in mortality (RR 0.65, 95% CI 0.44–0.97,  $p=0.03$ ; see Fig. 1). There is no

**Table 2** Results of randomized trials evaluating antioxidant strategies in critically ill patients (NA not available)

	Mortality <sup>a</sup>		Infections <sup>b</sup>		Length of stay (days)		<i>p</i>	
	Experimental	Control	Experimental	Control	Experimental	Control		
Berger et al. [12, 13]	(a) 2/9 (22%); (b) 0/11 (0%)	1/12 (8%)	NS	5/12 (42%)	NS	(a) ICU 8.0±4.0, hospital 67±74; (b) ICU 5.8±4.4, hospital 60±48	ICU 8.6±8.1, hospital 60±39	NS
Porter et al. [14]	0/9 (%)	0/9 (%)	NA	8/9 (89%)	—	ICU 22±25.2, hospital 31.3±23.4	ICU 35.8±21.9, hospital 49±30	<0.05
Nathens et al. [15]	ICU 3/301 (1%), hospital 5/301 (2%), 28-day 4/301 (1%)	ICU 9/294 (3%), hospital 9/294 (3%) 28-day 7/294 (2%)	NA	36/301 (12%)	NA	ICU 5.3, hospital 1 4.6 (means)	ICU 6.4, hospital 15.1 (means)	NA
Berger et al. [19]	1/10 (10%)	0/10 (0%)	NA	1.9±0.9 (1–4) per patient	0.013	ICU 30±12, hospital 54±27	ICU 39±13, hospital 66±31	NS
Kuklinski et al. [18]	ICU 0/8 (%)	ICU 8/9 (89%)	NA	NA	—	NA	NA	—
Zimmerman et al. [20]	3/20 (15%)	8/20 (40%)	NA	NA	—	NA	NA	—
Angstwuerm et al. [21]	Hospital 7/21 (33%)	Hospital 11/21 (52%)	0.135	NA	—	NA	NA	—
Preiser et al. [22]	ICU 3/20 (15%), hospital 8/20 (40%)	ICU 3/17 (18%), hospital 6/17 (35%)	NS	3/20 (15%)	NS	ICU 5 (3–26)	ICU5 (3–18)	NS
Young et al. [23]	4/33 (12%)	9/35 (26%)	0.09	NA	—	NA	NA	—
Madrero et al. [24] <sup>b</sup>	NA	NA	—	5/18 (28%)	NA	NA	NA	—
Berger et al. [25]	1/9 (11%)	1/8 (13%)	NS	NA	—	ICU 39±7	ICU 38±12	NS

<sup>a</sup> Presumed hospital mortality unless otherwise specified <sup>b</sup> Refers to the no. of patients with infections unless specified

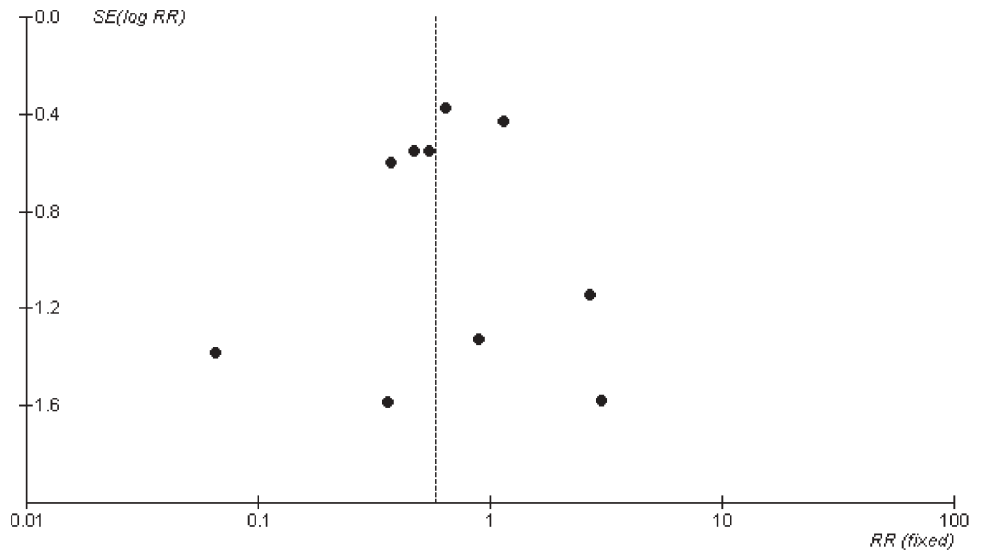


**Legend:**

n: number of patients that died  
 N: total number of patients in group  
 RR: Relative Risk  
 95% CI: 95% confidence intervals

**Fig. 1** Effect of antioxidants on mortality in critically ill patients

**Fig. 2** Funnel plot For studies using antioxidants (mortality)

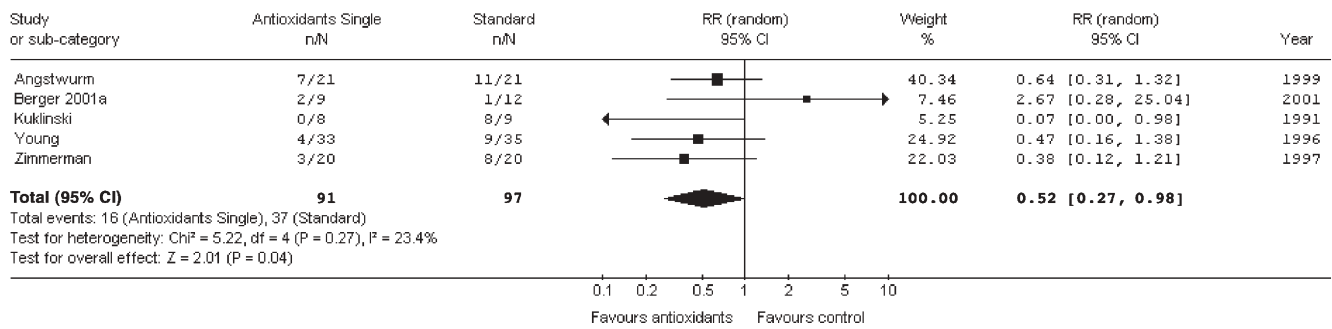


**Legend:**

SE: standard error  
 RR: relative risk

indication that a publication bias accounts for the apparent benefit observed with respect to mortality in the studies using antioxidants (see Fig. 2). Only five studies ( $n=728$ ) reported infectious complications as number of patients with infections [12, 14, 15, 22, 24] and when these results were aggregated, antioxidants showed no effect on infectious complications (RR 0.90, 95% CI

0.65–1.24,  $p=0.51$ ). In our sensitivity analysis we excluded one study, and the overall estimate of treatment effect did not change appreciably but statistical significance was borderline (adjusted RR without [18] 0.69, 95% CI 0.46, 1.02,  $p=0.06$ ). Length of stay data were sparse and variably recorded such that we could not aggregate these data.



### Legend:

n: number of patients that died

N: total number of patients in group

RR: Relative Risk

95% CI: 95% confidence intervals

**Fig. 3** Effect of single antioxidants on mortality

### Subgroup analysis

In the 11 trials of antioxidant intervention there was great variation in the type of antioxidants studied, the route of administration, the dosage, and the effect of other combination nutrients, thereby making it difficult to attribute the effects to one single or combination of antioxidants. Several subgroup analyses were carried out in an attempt to determine which antioxidant strategies were more likely to affect clinical outcomes (i.e., mortality).

#### Single nutrients vs. combined antioxidants

When the results of all the studies using single antioxidants were aggregated ( $n=188$ ) [12, 18, 20, 21, 23] antioxidants were associated with a significant reduction in mortality (RR 0.52, 95% CI 0.27–0.98,  $p=0.04$ ) (see Fig. 3). The number of patients with infectious complications was reported in only one of the five studies using single antioxidants [12]. When the results of the six studies of combined antioxidants were aggregated [12, 14, 15, 19, 22, 25] there was no effect on mortality ( $n=710$ , RR 0.87, 95% CI 0.47–1.62,  $p=0.67$ ) or infectious complications ( $n=719$ ) (RR 0.85, 95% CI 0.60–1.23,  $p=0.39$ ).

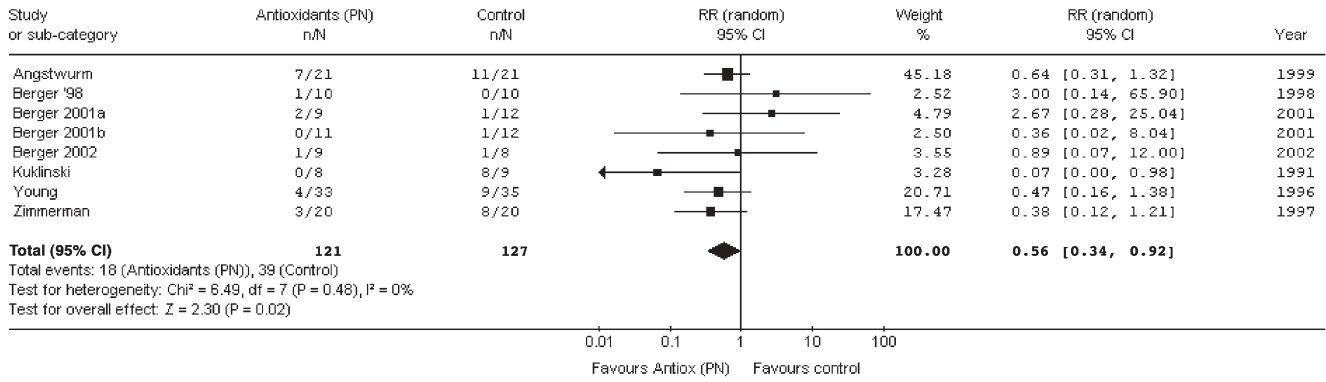
#### Parenteral/intravenous vs. enteral route

Antioxidants were supplied via the parenteral/intravenous route in 8 of the 11 studies [12, 18, 19, 20, 21, 23, 24, 25], via an enteral formula in one study [22] and via combined routes of parenteral/intravenous and enteral in two studies [14, 15]. When the results of the studies using parenteral antioxidants were aggregated ( $n=236$ ), antioxidants were

associated with a significant reduction in mortality (RR 0.56, 95% CI 0.34–0.92,  $p=0.02$ ; see Fig. 4) but had no effect on infectious complications ( $n=78$ , RR 1.26, 95% CI 0.73–2.16,  $p=0.41$ ). There was only one study in which antioxidants were administered via the enteral route alone [22] (RR 1.13, 95% CI, 0.49–2.62,  $p=0.77$ ), and there were no significant differences in mortality or infectious complications between groups.

#### Selenium vs. nonselenium

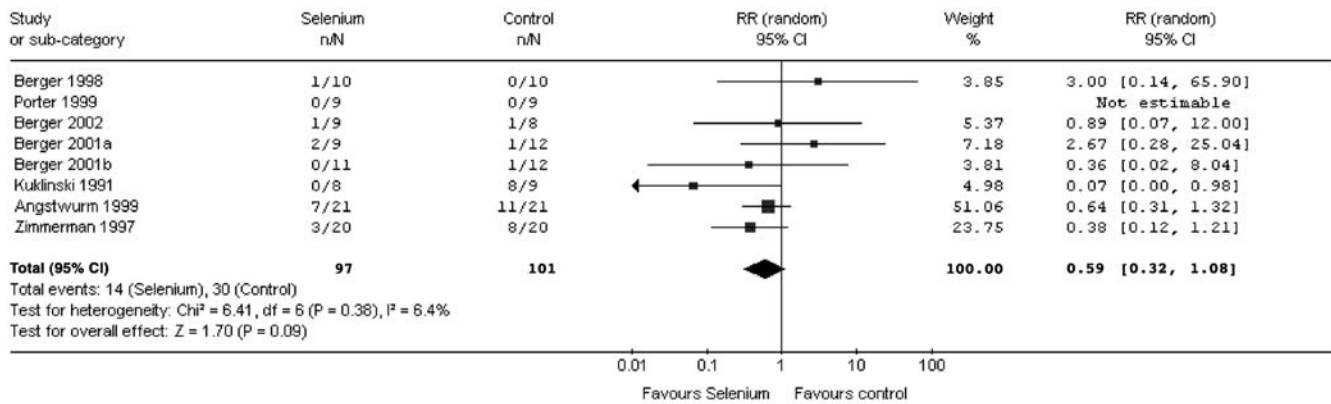
There were seven studies that included selenium as a component of the antioxidant strategy (alone or in combination with other antioxidants) [12, 14, 18, 19, 20, 21, 25]. When aggregated across seven studies ( $n=186$ ), selenium supplementation (alone and in combination with other antioxidants) was associated with a trend towards a lower mortality (RR 0.59, 95% CI 0.32, 1.08  $p=0.09$ ; see Fig. 5), while nonselenium antioxidants (i.e., vitamins A, C, E and zinc;  $n=700$ ) were found to have no effect on mortality (RR 0.73, 95% CI 0.41, 1.29,  $p=0.3$ ). The effects of selenium supplementation (combined and alone) on infectious complications were reported in only two studies ( $n=50$ ) [12, 14], and when these were aggregated, selenium supplementation showed no effect on infectious complications (RR 0.78, 95% CI 0.49–1.26,  $p=0.3$ ). Three studies on nonselenium antioxidants [15, 23, 24] reported infectious complications, and when the results of these were aggregated ( $n=678$ ), nonselenium antioxidants had no effect on infectious complications (RR 1.10, 95% CI 0.60–2.04,  $p=0.8$ ). Studies using higher than the median dose of selenium (500–1000  $\mu\text{g}/\text{day}$ ,  $n=131$ ) [12, 18, 20, 21] were associated with a trend towards a lower mortality (RR 0.52, 95% CI 0.24–1.14,  $p=0.10$ ), whereas



**Legend:**

n: number of patients that died  
 N: total number of patients in group  
 RR: Relative Risk  
 95% CI: 95% confidence intervals

**Fig. 4** Effect of parenteral antioxidants on mortality



Studies are listed in ascending order of dose of selenium

**Legend:**

n: number of patients that died  
 N: total number of patients in group  
 RR: Relative Risk  
 95% CI: 95% confidence intervals

**Fig. 5** Effect of selenium on mortality: dose response curve

studies using a selenium dose lower than the median (<500 µg/day, n=55) [14, 19, 25] were found to have no effect on mortality (RR 1.47, 95% CI 0.20–10.78, p=0.7). Figure 5 shows the dose response curve for the effect of selenium on mortality.

**Discussion**

The association between increased oxidative stress and poor outcomes in the critically ill is well documented [59, 60, 61, 62, 63]. Supplementation with trace elements and vitamins has been shown to improve antioxidant capacity in critically ill patients (as demonstrated by increased activity of glutathione peroxidase) [12, 22]. Nevertheless, it is still not clear whether antioxidant supplementation is beneficial, and the trials until now have not enabled de-

finitive conclusions as they generally included small patient populations.

The present meta-analysis is the first dedicated to using antioxidant micronutrients to treat oxidative stress in critically ill patients and aimed to summarize existing knowledge, to provide an estimate of the overall treatment effect, and to suggest improvements in future trials in the area. In this systematic review we comprehensively searched the available literature and found 44 relevant citations, but only 11 studies of antioxidant therapy met the technical inclusion criteria (randomization, critical illness, clinically important endpoints). With one exception [15] the majority of these trials were small ( $n < 70$ ) and inadequate to detect clinically important treatment effects on mortality. The 11 trials were characterized by the inclusion of well defined patient populations (brain injury, burns, pancreatitis, SIRS, trauma), which contributes to explaining why the investigators achieved significant results. The common characteristics of these groups were a high oxidative stress and micronutrient depletion. When the results of the 11 trials of 886 patients were aggregated, we found a statistically significant reduction in mortality associated with the provision of antioxidants and particularly with selenium. These trace elements were not associated with a reduction in infectious complications, suggesting that the mortality effect was mediated by some other mechanisms, perhaps related to improved organ function. Importantly, none of the trials using micronutrients reported deleterious effects of their administration. Furthermore, as the 95% CIs exclude 1.0, our overall estimate of treatment effects excludes the possibility of excess mortality associated with these micronutrients. Thus these micronutrients can be considered as safe. The administration of antioxidant therapy in the studies was generally initiated upon admission to the ICU (i.e., the acute phase of injury), suggesting that timing of the intervention is important. Given the heterogeneous nature of the populations included in the studies (trauma, surgical, burns, pancreatitis, SIRS, head injury), the results of the meta-analysis may be applied to all ICU populations.

The meta-analysis included studies that focused on essential antioxidant trace elements and vitamins and not other nutrients or pharmacological compounds. The positive results of some of the studies that evaluated other nutrients or pharmacological compounds in combination with trace elements and vitamins [50, 51, 56, 60] further extend our position that antioxidant strategies in critically ill patients are safe and possibly beneficial.

This review included only randomized controlled trials, as nonrandomized trials tend to show larger (and frequently "false-positive") treatment effects than do randomized trials [63]. We included studies that evaluated clinically important endpoints although the choice of clinically relevant endpoints for these trials and hence for the meta-analysis is difficult. Although plasma levels of

circulating antioxidants are repeatedly demonstrated to be low, and markers of oxidative stress are high in critical illness, we cannot assume that favorable changes to these surrogate markers will translate into meaningful changes to clinical outcomes.

Selenium, glutathione, vitamin E, and vitamin C function synergistically to regenerate both water and fat-soluble antioxidants [64]. Thus when the present study was initiated, the hypothesis was that the provision of a combination of endogenous antioxidant micronutrients (i.e., a multimodal approach) early during the course of acute disease improves clinical outcome and is superior to single micronutrients. Moreover, the administration of single antioxidants may introduce disturbances in the entire system of overlapping antioxidant defenses as antioxidants may turn into pro-oxidants if auxiliary systems for radical scavenging are missing [62]. For example, ascorbate does recycle the tocopheryl radical to tocopherol. Thus ascorbate serves as a biochemical link between the selenium/glutathione peroxidase system and vitamin E [63]. Moreover, to generate sufficient intracellular glutamate to ensure adequate amounts of glutathione, sufficient amounts of glutamine need to be provided as well [64, 65, 66, 67]. Considering all these implications, we suspected that combinations of antioxidants would provide a larger treatment effect than single micronutrient strategies. However, the results of the meta-analysis do not confirm this hypothesis as it appears that single micronutrients (selenium and zinc) were responsible for the observed reduction in mortality. Furthermore, there appears to be a stronger signal that selenium supplementation (alone or in combination with other micronutrients) is associated with a mortality reduction in acute ICU conditions than zinc or other antioxidant strategies. Multivitamin supplementation alone [15] was not associated with such benefits even when tested in a trial with sufficient numbers, although the patient population may not have been sick enough to demonstrate a clinically relevant difference in mortality (baseline mortality 3.1%).

Why do our results not confirm the hypothesis that a combination strategy of antioxidant supplementation is better than single selenium delivery? Perhaps selenium is the cornerstone of the antioxidant defense in acute conditions. Nearly all patients with sepsis or shock have low plasma selenium levels that are inversely correlated with the severity of SIRS and subsequent outcome [68]. Patients with a low plasma selenium level were three times more likely to die than those with a higher plasma level [68]. Supplementing with selenium may improve clinical outcomes as selenium is an essential cofactor in glutathione enzymatic function and has favorable effects on cellular immune function [60]. Selenium is also a component of selenoproteins other than glutathione peroxidase, some of which have important functions such as activation and regulation of thyroid hormones, reduction

in nucleotides in DNA synthesis, regeneration of antioxidant systems, and cell viability and proliferation [60]. Although selenium may be an essential component required to achieve a beneficial effect on patient outcomes, our results cannot be extrapolated to say it is selenium only. Other trace elements and vitamins still may be of value and this requires further study.

Further research needs to be carried out to define not only the optimal combination of trace elements but also the optimal dose of each micronutrient. The selenium doses used in the trials with beneficial mortality effects were 5–20 times the recommended parenteral nutrition intakes (300–1000 µg per day). From our subgroup analysis it appears that studies that utilized a higher dose are associated with a greater treatment effect than those with a lower dose of selenium. With respect to the optimal dose of vitamins, the study by Nathens et al. [15] used the highest doses of vitamins (3 g vitamins C and E per day) compared to standard intakes, and yet they failed to demonstrate a significant effect on mortality. Recommended or standard doses of these micronutrients are based on requirements and metabolism in healthy subjects and have little meaning in critically ill patients. At high doses vitamin C, vitamin E, and selenium have been shown to have some pro-oxidant properties [69, 70]. Therefore, more is not necessarily better. If correction of an altered circulating antioxidant status is the target, this is probably achieved with moderately elevated doses but more research is needed to determine the optimal dose.

Due to the low number of studies, enteral and intravenous trials were combined. Most of the trials delivered the micronutrients intravenously (8/11), two using a combined enteral and intravenous approach, and only one using purely the enteral route. Nevertheless, the reduction in mortality was observed only by the intravenous route. The limited number of trials did not illuminate whether there was an advantage of either route of delivery. In those trials determining plasma concentrations, intravenous supplements significantly increased the plasma

concentrations; this is likely to produce whole-body effects faster than enteral delivery. Indeed during acute diseases the gut function is frequently altered, which may delay absorption of various nutrients. On the other hand, delivering antioxidants to the gut may prove beneficial through prevention of the local gut inflammatory response [71]. Theoretically at least, the target of both routes appears complementary, which deserves further investigation.

There are limitations to the meta-analysis approach. First, the meta-analysis cannot be better than its components. Despite only including randomized trials, the methodological quality of the individual studies ranged from 4 to 12 (of a possible 14). Only 5 of the 11 randomized studies used blinded allocation, 6 were blinded, and 9 reported an intention-to-treat analysis. The small number of controlled trials ( $n=11$ ) and of patients ( $n=886$ ) in total (and only 186 for the trials using selenium), further limits the stability of our estimates. Although we observed no statistical heterogeneity, since enteral and parenteral modes of antioxidant application as well as single and multimodal treatments were combined, we refrain from making strong inferences of the pooled overall results. As previously stated, given the limited number of endpoints and limited number of studies, data presented in the meta-analysis could be viewed more as hypothesis generating rather than hypothesis confirming.

In conclusion, the meta-analysis shows that trace elements and vitamins that support antioxidant function, particularly selenium, either alone or in combination with other antioxidants are safe and may be associated with a reduction in mortality in critically ill patients. The high-dose parenteral route appears to have a stronger impact on outcome than the enteral route, but the number of trials is small and the inferences are weak. Clearly, a large multicenter randomized trial confirming the benefits of antioxidant supplementation is warranted given the potential for a large mortality reduction.

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