The impact of antioxidant supplementation on clinical outcomes in the critically ill: A meta-analysis

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Background. Critical illness is associated with increased oxidative stress that can influence outcome. Many studies have investigated the effects of exogenous antioxidant supplementation, without showing significance owing to the small patient populations.
Methodology. A systematic review and meta-analysis of the English literature was performed to determine the effect of antioxidant micronutrient supplementation on clinically important outcomes in the critically ill. Pubmed, Google Scholar and Science Direct electronic databases were searched for papers published between January 1990 and June 2010.
Selection criteria. Randomised controlled trials were selected for inclusion if they investigated the effects of antioxidant supplementation in the critically ill and reported on clinically significant endpoints.
Data collection and analysis. The data were analysed using a random effects model in Comprehensive Meta-analysis Version 2 (Biostat, USA) to obtain the odds ratio (OR) with a 95% confidence interval (CI) and statistical significance of $p<0.05$.
Results. Twelve studies met the inclusion criteria. Selenium supplementation was associated with a trend towards decreased mortality (OR=0.717, p=0.106, CI 0.48 - 1.07). Mixed antioxidant supplementation was associated with reduced hospital length of stay (OR= 0.710, p=0.002, CI 0.57 - 0.83), reduced infectious complications (OR=0.494, p=0.024, CI 0.28 - 0.98) and reduced mechanical ventilation (OR=0.259, p=0.023, CI 0.08 - 0.83).
Conclusion. A combination of antioxidant micronutrients might be associated with improved clinical outcome in the critically ill.

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In the healthy, stable state, a balance exists between free radical production and the physiological defence mechanisms that protect cells from the damaging effects of excess free radical build-up. The endogenous antioxidant defence system in humans consists of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase and their cofactors; zinc, copper, manganese and selenium; and vitamins such as vitamin C, E and β-carotene. When the balance between free radicals and antioxidant nutrients is maintained, free radicals have beneficial cellular effects, including an important role in the inflammatory response to bacterial infection. When the balance is disturbed, either by an increased production of free radicals or by the ineffective removal of these molecules by antioxidants, the result is a state of oxidative stress, which is characterised by damage to cell membranes, nucleic material and mitochondrial dysfunction.[1]

The above factors are of increased significance in the critically ill population, as critical illness is associated with an increase in free radical production as well as low endogenous antioxidant capacity.[2,3] 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 reactive oxygen species.[1,4,5] Metnitz *et al.*[6] observed that circulating antioxidant concentrations decrease rapidly at the onset of an insult, trauma or surgery, and remain low during the course of illness. Low antioxidant capacity is strongly associated with poor survival and outcome.[7] Oxidative stress is implicated in cell damage and death, and may contribute to the development of organ failure. Moreover, free radicals, such as reactive oxygen and nitrogen molecules, are implicated in the release of cytokines from immune cells, the activation inflammatory cascades, and increase in the expression of adhesion molecules, resulting in the augmentation of the inflammatory response that may lead to increased morbidity and mortality in the critically ill.[1]

Consequently, there is interest in whether restoring levels of antioxidant nutrients in ICU patients may blunt the oxidative stress and have beneficial effects on clinical outcome. Many small studies investigating the effects of antioxidant supplementation on clinically important outcomes such as mortality, infection rate, length of stay and duration of mechanical ventilation were meta-analysed by Heyland *et al.*[1] The findings were that there was a significant reduction in mortality associated with antioxidant supplementation and that selenium in particular was associated with this improved outcome. The provision of other trace elements, however, had no significant effect on other clinical outcomes. Since this meta-analysis, many other studies investigating the effects of antioxidant supplementation on clinical outcomes have been conducted. The purpose of the present study was to systematically review and statistically aggregate randomised controlled trials of antioxidant supplementation in the critically ill to determine their effect on mortality, length of hospital and ICU stay, infection and length of mechanical ventilation.

Criteria for study selection

Types of studies

Studies were included if they were randomised controlled clinical trials published in English. Studies were considered if they used comparative groups to investigate the effects of antioxidant supplementation v. a control on clinically important outcomes in critically ill patients.

Types of patient

Studies had to include critically ill adult patients.

Types of intervention

Interventions that met inclusion criteria were antioxidant supplementation of vitamins and trace elements, particularly vitamin A, C, E, beta-carotene, zinc, copper and selenium. The mode of administration of the antioxidants could be oral, intramuscular or intravenous, of any dose and dosing schedule.

Types of outcome

Studies were included if they investigated clinically important outcomes such as mortality, duration of stay in ICU, duration of mechanical ventilation, duration of stay in hospital, the number of patients who developed infectious complications, safety and costs.

Search methods for identification of studies

A systematic review of the literature was undertaken using electronic databases (such as Pubmed, Science Direct and Google Scholar) to search for relevant papers published between January 1990 and June 2010. The primary Medical Subject Heading (MeSH) search terms included critically ill, ICU patients and oxidative stress in the critically ill, which were combined with terms such as antioxidant supplementation, or with the names of individual antioxidants such as selenium supplementation. The filter in each electronic database was used to select randomised controlled trials only: Additional articles were found using manual searching, such as reviewing the reference lists of other review articles.

Data collection and analysis

Selection of studies

Full-text articles were used for all the identified studies that met the inclusion criteria. The relevant data were extracted and collected by one reviewer, and separately extracted by a second reviewer (supervisor).

Assessment of bias

Methodological quality of the studies was assessed using a scoring system adapted from McClave *et al*.,[8] Dellinger *et al*.[9] and Heyland *et al*.[10] Studies for inclusion were limited to large randomised trials with clear-cut results; low risk of false positive (alpha) error or false negative (beta) error; or small randomised trials with uncertain results: moderate to high risk of false positive (alpha) and/or false negative (beta) error. The validity, effect size, associated confidence interval, homogeneity, safety, feasibility and cost were also considered for each intervention.

Treatment effect

The treatment effect was measured using the odds ratio (OR) with a 95% confidence interval (CI). The *p*-value was set at 0.05.

Unit of analysis

All included studies randomised participants to a treatment or a control group. Treatment groups received varying doses of antioxidants in the form of vitamins and minerals, whereas controls were given a placebo, vehicle or no additional vitamins.

Dealing with missing data

No missing data were identified in any of the studies.

Assessment of heterogeneity

Heterogeneity was calculated using the chi-square statistic (χ^2= 100% x (Q-df)/Q). This test describes the percentage of variation across studies due to heterogeneity, rather than chance and is not dependant on the number of studies considered.

Data synthesis

Data were entered into the statistical analysis software Comprehensive Meta-analysis Version 2 (Biostat, USA) for analysis. Data were combined from all studies to estimate the common OR with a 95% CI using the random effects model. Differences at the level of $p<0.05$ were considered to be statistically significant. Forest plots were plotted to depict the intervention effect.

Subgroup analysis

The separation of the literature guided the analysis into two arms: studies supplementing selenium alone, and studies supplementing mixed antioxidants. These were discussed and analysed separately.

Reliability, validity and quality assessment

Reliability, validity and quality assessment of study data were ensured by using the scoring system for assessing the methodological quality of the studies as well as by strictly adhering to the inclusion and exclusion criteria.

Results

Search results

Fifteen publications were identified using the search criteria. Two studies were excluded as they were not published in English,[11,12] and one was excluded as it did not meet the inclusion criteria.[13] Of the 13 published studies included, one[11] investigated the effects of both selenium v. placebo (Intervention 1) and mixed antioxidants v. placebo (Intervention 2). These two separate interventions were meta-analysed separately, giving a total of 13 interventions from 12 published papers. Five involved the use of selenium alone as a therapeutic intervention, and 7 studies involved supplementation with copper, zinc, selenium, manganese, vitamin E, vitamin C and N-acetylcysteine in various combinations and doses. Many of the studies failed to explain techniques of randomisation and blinding in adequate detail (Table 3).

Selected studies

All of the studies identified were randomised controlled trials. The studies combined included 1 737 patients, of whom 401 were supplemented with selenium only, and 1 336 with mixed antioxidants. The majority of interventions were delivered intravenously, with the exception of alpha-tocopherol which was delivered nasogastrically in 3 studies, and N-acetylcysteine which was delivered nasogastrically in 1 study. We did not separately

Table 1. Characteristics of studies involving selenium only

Study	Population	Sample size	Intervention	Control	Outcome	Outcome	
						Intervention	Control
Angstwurm 1999[16]	ICU patients with an APACHE II score >15 and SIRS	*N*=42 Control=21 Intervention=21	IV 535 µg Se x 3 days + 285 µg x 3 days + 155 µg x 3 days and 35 µg daily thereafter for remainder of the study	IV 35 µg Se throughout total study	Mortality Hospital LOS ICU LOS Ventilator days	7/21 28 (8 - 90) 14 (3 - 75) 9 (3 - 23)	11/21 36 (16 - 70) 11 (2 - 67) 10 (1 - 43)
Berger *et al*. 2001[14]	Critically ill trauma patients ISS>15 points	*N*=31 Control=11 Intervention 1 (Se alone)=9 Intervention 2 (Se + Zn + Cu + α tocopherol)=12	Intervention 1: IV 500 µg Se per day Intervention 2: 500 µg Se + 150 mg α tocopherol + 13 mg Zn + 2.6 mg Cu	Vehicle	Mortality Infection Hospital LOS ICU LOS Ventilator days	2/9 5/9 82±78 8±4 6.2±3.5	1/12 5/12 64±39 8.6±8.1 5.4±6.5
Forceville 2007[15]	Severe septic shock or documented infection, mechanically ventilated ICU patients	*N*=60 Control=29 Intervention=31	IV 4 000 µg Se x 1 day + 1 000 µg Se x 9 days	Matching placebo	Mortality Hospital LOS ICU LOS Ventilator days Infection	14/31 25 (7 - 68) 21 (7 - 40) 19 (7 - 34) 17/31	13/29 33 (11 - 51) 18 (10 - 31) 14 (8 - 23) 13/29
Angstwurm 2007[17]	Mixed ICU patients (SIRS, sepsis, septic shock, APACHE score >70)	*N*=238 Control=122 Intervention=116	IV 1 000 µg Se stat dose + 1 000 µg Se continuous infusion for 14 days	Vehicle	Mortality ICU LOS Infection	46/116 15.1±10 10/116	61/122 12.7±9 10/122
Mishra 2007[21]	Septic ICU patients with APACHE II score >15 and more than 1 organ dysfunction	*N*=40 Control=22 Intervention=18	IV 474 µg Se x 3 days + 316 µg x 3 days + 158 µg x 3 days + 31.6 µg daily thereafter for the remainder of the study	50 ml saline. Daily standard dose of 31.6 µg Se in EN or PN nutrition	Mortality Infection ICU LOS	8/18 1.5±1.9 21.3±16.2	11/22 1.8±1.6 20.8±21.8

APACHE = acute physiology and chronic health evaluation; SIRS = systemic inflammatory response syndrome; ISS = injury severity score; IV = intravenous; Hospital LOS = hospital length of stay; ICU LOS = ICU length of stay; Se = selenium; Zn = zinc; Cu = copper; EN = enteral nutrition; PN = parenteral nutrition.
Mean ± standard deviation
Median (interquartile range)

analyse for differing routes of supplement administration, especially since the studies which included nasogastric delivery also delivered other mixed antioxidant supplements concurrently via the intravenous route, making a route of delivery effect difficult to discern.

The outcomes of the studies are shown in Table 1 for selenium-only supplementation. Studies involving mixed antioxidants are shown in Table 2. The methodological qualities of the studies are compared in Table 3. For selenium-only supplementation, the experimental and control groups were comparable at baseline in most of the studies, in terms of patient characteristics and clinical scores. However, Berger *et al.*[14] reported to have found more brain injuries in the experimental group (non-significant) and Forceville[15] reported to have found significantly more medical patients in the experimental group ($p<0.01$) as well as significantly lower haemologbin in the control group ($p<0.01$) at randomisation. Only 2 of the studies[16,17] investigated clinical outcomes as their primary endpoint.

For mixed antioxidant supplementation, the experimental and control groups were comparable at baseline in all measured parameters in all of the studies, except for Berger *et al*.,[18] where there were significantly more severe head injuries in the treatment group. Only 2 studies[19,20] investigated the specified clinical outcomes as their primary endpoint, while in the remaining studies it was considered to be a secondary endpoint.

Hospital length of stay was reported on in three of the studies involving selenium alone as an antioxidant supplementation strategy. When aggregated, these results indicated that selenium supplementation had no effect on hospital length of stay (*N*=123, OR 0.700, 95% CI 0.29 - 1.64, $p=0.413$). Selenium was also shown to have no effect on mechanical ventilation (*N*=123, OR 0.762, 95% CI 0.31 - 1.85, $p=0.549$). Selenium supplementation was, however, associated with a significant increase in ICU length of stay (Fig. 1) but a non-significant decrease in infection complications in the placebo groups (Fig. 2). Mortality was reported in all 5 of the included studies; when the studies were aggregated, selenium supplementation alone was associated with a non-significant trend towards decreased mortality, favouring the antioxidant group (Fig. 3).

When the results of the studies of combined antioxidants were aggregated, there was a significant reduction in hospital

Table 2. Characteristics of studies involving mixed vitamins and trace elements

Study	Population	Sample size	Intervention	Control	Outcome		
					Outcome	Intervention	Control
Berger *et al.* 1998[22]	Thermal burns covering ≥30% of TBSA	*N*=20 Control=10 Intervention=10	IV 40.4 µmol Cu+159 µg Se + 406 µmol Zn	Vehicle	Mortality Infection ICU LOS Hospital LOS	1/10 1.9±0.9 30±12 54±27	0/10 3.1±1.1 39±13 66±31
Porter *et al.* 1999[23]	Surgical ICU patients with penetrating injury involving several systems, with an injury severity score ≤25	*N*=18 Control=9 Intervention=9	IV 50 mg Se 6-hourly + 400 IU α tocopherol + 100 mg vit C + 8 g N-acetylcysteine 8-hourly per NGT	Control not specified	Mortality ICU LOS Hospital LOS Infection	0/9 22.0±8.4 31.3±7.8 5/9	0/9 35.8±8.3 49.0±10 8/9
Berger *et al.* 2001[14]	Critically ill trauma patients ISS >15 points	*N*=31 Control=11 Intervention 1 (Se alone)=9 Intervention 2 (Se + Zn + Cu + vit E)=20	Intervention 1: IV 500 µg Se Intervention 2: 500 µg Se + 150 mg α tocopherol + 13 mg Zn + 2.6 mg Cu	Vehicle	Mortality Infection ICU LOS Hospital LOS Ventilator days	0/11 3/11 5.8±4.4 60±48 4.1 ± 3.6	1/12 3/12 8.6±8.1 64±39 5.4±6.5
Nathens 2003[24]	General surgical/trauma ICU patients	*N*=770 Control=294 Intervention=301	1 000 IU α tocopherol per OGT/NGT 8-hourly + IV 1 000 mg vit C 8-hourly	Control not specified	Mortality Infection ICU LOS (mean) Hospital LOS (mean) Ventilator days (mean)	5/301 36/301 5.3 14.6 3.7	9/294 44/294 6.4 15.1 4.6
Crimi *et al.* 2004[25]	Coronary care unit patients and medico-surgical ICU patients	*N*=224 Control=112 Intervention=112	500 mg vit C + 400 IU α tocopherol per OGT/NGT	Placebo	Mortality Hospital LOS Ventilator days	49/112 26.5 6.2±2.3	76/112 27.5 8.9±1.8
Berger *et al.* 2007[19]	Burns ≥20% of BSA	*N*=21 Control=10 Intervention=11	IV 59 µmol Cu + 4.8 µmol Se + 574 µmol Zn	Vehicle	Mortality Infection (mean per patient) Ventilator days ICU LOS	1/11 2.1±1.0 7.6±6 35±27	1/10 3.6±1.3 12.6±6 47±37
Berger *et al.* 2008[18]	Mixed ICU patients	*N*=200 Control=98 Intervention=102	IV 60 mg Zn + 540.4 µg Se + 2 700 mg vit C + 305 mg thiamine + 12.8 mg α tocopherol (IV) + 600 mg α tocopherol (per OGT/NGT) days 1 - 2. Half of the above-mentioned doses for days 3 - 5	Control not specified	Mortality Infection ICU LOS Hospital LOS	14/102 36/102 5.8±5.4 23±20	9/98 34/98 5.4±5.7 26±20
El-Attar 2009[20]	Chronic obstructive pulmonary disease (COPD) ICU patients	*N*=120 Control COPD=40 Control non-COPD=40 Intervention: 40	IV 100 µg Se, 2 mg Zn + 0.4 mg Mn	Control not specified	Mortality Infection Ventilator days	2/40 5/36 9.4±7.3	1/40 7/34 17.8±7.6

TBSA = total burn surface area; BSA = burn surface area; ISS = injury severity score; IV = intravenous; ICU = intensive care unit; IU = international units; Hospital LOS = hospital length of stay; ICU LOS = ICU length of stay; Se = selenium; Zn = zinc; Mn = manganese; Vit C = vitamin C; Cu = copper; OGT/NGT = orogastric tube/nasogastric tube.
Mean±standard deviation.

Table 3. Methodological quality of studies

Study	Blinding	Random allocation	ITT analysis	Level of evidence
Angstwurm 1999[16]	Not blinded (a resident blinded to the study assessed APACHE III score)	Yes Patients stratified randomly into 2 groups	Yes	II
Berger *et al*. 2001[14]	Yes Methods not stated	Yes Patients randomised on a 2:1 treatment/placebo basis	No	III
Forceville 2007[15]	Yes All patients, medical and nursing staff, and pharmacists were blinded throughout the study period	Yes Patients randomly assigned in a 1:1 manner to receive either intervention or placebo	No	II
Angstwurm 2007[17]	Yes Methods not stated	Yes Methods not stated	No	I
Mishra 2007[21]	Yes Methods not stated	Yes Patients randomly allocated to receive either intervention or placebo	Yes	I
Berger *et al*. 1998[22]	Yes Colourless, transparent prepared solutions were used and identified by numeric code	Yes Methods not stated	Yes	II
Porter *et al*. 1999[23]	No	Yes Prospectively randomised by sealed manila envelopes	Yes	II
Nathens 2003[24]	No	Yes Methods not stated	No	I
Crimi *et al.* 2004[25]	Yes Methods not stated	Yes Patients randomised by using computer-generated random numbers. Blockwise randomisation in a 1:1 ratio was used to obtain balanced sample sizes	Yes, both ITT and per protocol analysis done	I
Berger *et al*. 2007[19]	Yes Methods not adequately stated	No	Yes	II
Berger *et al*. 2008[18]	Yes Patients, clinicians and investigators blinded to the treatment. Black plastic bags covered the solutions and coloured tubing was used for infusion	Yes Patients randomly assigned to receive intervention or placebo by a pharmacist	Yes	I
El-Attar 2009[20]	Yes All clinicians and other health care providers were totally blinded to randomisation allocation	Yes A randomisation schedule was provided using random allocation software	Yes	II

APACHE = acute physiology and chronic health evaluation; ITT = intention to treat.

length of stay (Fig. 4), and a trend towards a decreased ICU length of stay (*N*=1 050, OR 0.702, 95% CI 0.46 - 1.07, *p*=0.098). Combined antioxidant use was associated with no improvement in mortality (*N*=1 180, OR 0.654, 95% CI 0.36 - 1.18, *p*=0.16). Mixed antioxidants were associated with a significant reduction in the occurrence of infectious complications (Fig. 5), as well as a significant reduction in the duration of mechanical ventilation (Fig. 6).

Discussion

The results of the present meta-analysis indicate that the supplementation of mixed antioxidants is associated with a significant decrease in the duration of mechanical ventilation, infectious complications and length of hospital stay.

Many antioxidants have a dual role in both antioxidant functioning and the immune system. For example, zinc has a well-documented

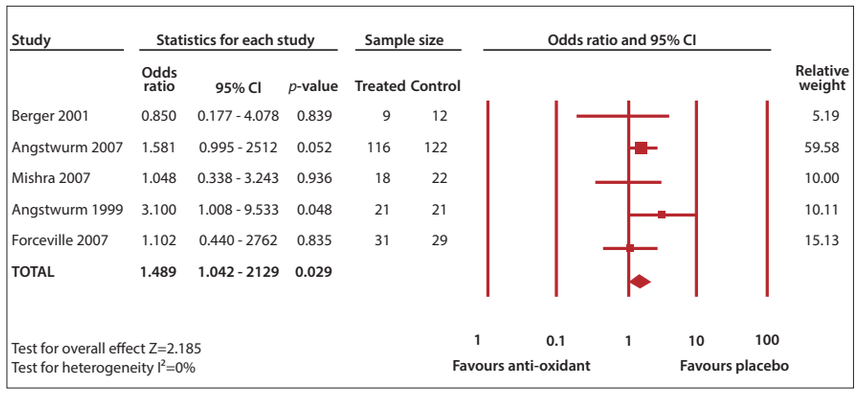

Fig. 1. Random effects analysis: impact of selenium supplementation on ICU length of stay.

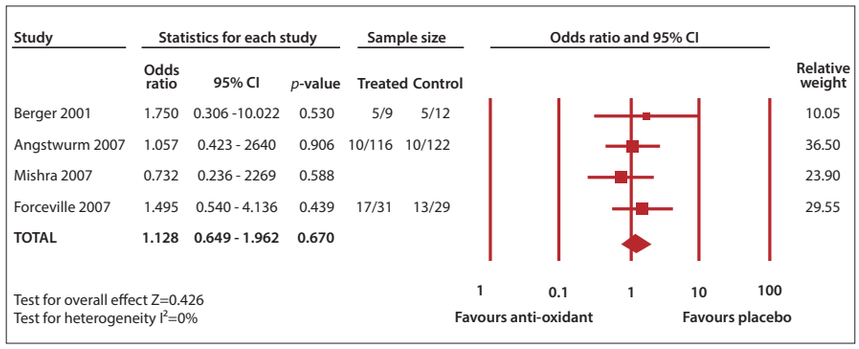

Fig. 2. Random effects analysis: impact of selenium supplementation on infectious complications.

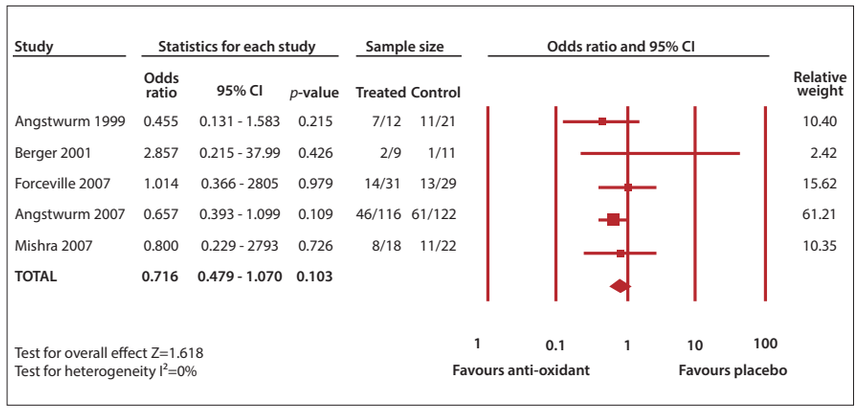

Fig. 3. Random effects analysis: impact of selenium supplementation on mortality.

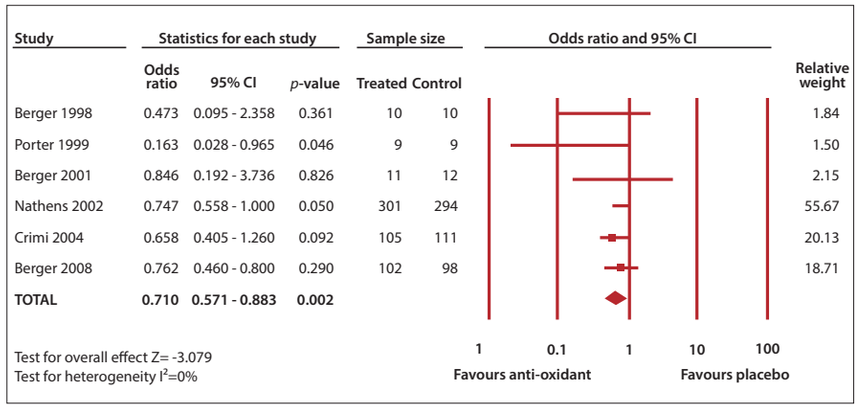

Fig. 4. Random effects analysis: impact of mixed antioxidant supplementation on hospital length of stay.

role in both the innate and adaptive immune system, and selenium may have a role in limiting the extent of the inflammatory response by decreasing the expression of pro-inflammatory genes.[13] This ability of vitamins and trace elements to act as antioxidants, immune regulators and anti-inflammatory mediators could be of benefit to critically ill patients, resulting in improved patient outcomes such as decreased infection rates and decreased length of stay as the results of this study have indicated, and have been shown in similar current analyses.[26]

The duration of mechanical ventilation was chosen as a clinically important outcome in this review as muscle atrophy and difficulty weaning from the ventilator is strongly associated with damage caused by oxidative stress. This is clinically significant in the ICU patient, as ventilator-induced diaphragmatic weakness contributes to difficulty weaning from mechanical ventilation[27,28] and may lead to complications such as ventilator-associated pneumonia, impaired swallowing and tracheal injury.[29] Results from this and another[26] recent meta-analysis indicate that mixed antioxidant supplementation was associated with a significant reduction in mechanical ventilation, which suggests that providing exogenous sources of antioxidants may help to restore antioxidant balance in mechanically ventilated patients and, in doing so, make it easier for patients to be weaned from ventilation.

Our results also indicate that mixed antioxidant supplementation was associated with no effect on mortality, as was also shown in a prior meta-analysis,[30] which is in contrast to a recent review that suggests micronutrient supplementation has an overall mortality benefit.[26] From these studies, what appears important in relation to the mortality benefit of micronutrient supplementation is the route, the duration of supplementation, and the overall risk of death in the study population. Patients with high risk of death who are enterally supplemented may experience a mortality benefit from mixed antioxidants. Therefore, sub-group selection may determine the effect. In our analysis, there was a high level of heterogeneity, and two of the largest studies produced conflicting results. Crimi *et al.*[25] observed a significant reduction in mortality associated with the use of combined antioxidants, in a highly controversial result highly criticised for the excessively high mortality in the patient collective.

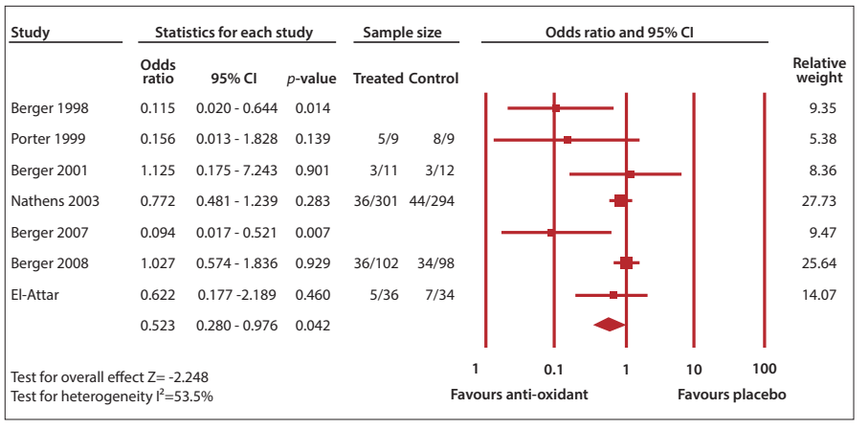

Fig. 5. Random effects analysis: impact of mixed antioxidant supplementation on infectious complications.

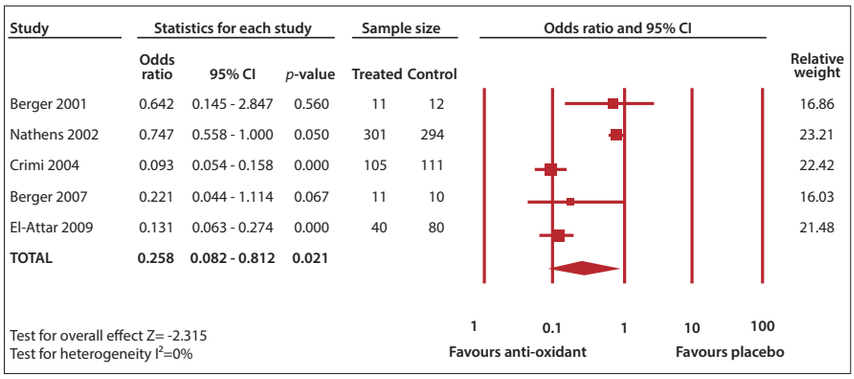

Fig. 6. Random effects analysis: impact of mixed antioxidant supplementation on duration of mechanical ventilation.

This particular study heavily weighted the meta-analysis findings of Mazanares *et al*.,[26] which showed a mortality benefit probably because the risk of death was so high. Berger *et al*.,[18] on the other hand, showed that mortality tended to be higher in the treated group, which they explained as due to a large number of deaths owing to severe brain injury. The high amount of heterogeneity in this analysis may be responsible for the lack of a significant finding on mortality.

In contrast to findings regarding mixed antioxidant supplementation, selenium alone was associated with no distinct clinical benefit. Studies such as those by Heyland *et al*.[1] and Angstwurm[17] previously showed that selenium supplementation was strongly associated with a significant reduction in mortality. Interestingly, our study failed to show this, but a trend towards a decrease in mortality was observed. This result is consistent with the recent meta-analysis of Heyland's group,[26] which showed that selenium monotherapy only showed a trend toward reduced mortality particularly where the intravenous dose of selenium was high. As mortality is a highly robust measure, a large sample size is needed to obtain statistical significance, and the relatively small sample size of our aggregated analysis lacked the necessary statistical power.

In fact, supplementation of selenium alone was associated with a significantly longer ICU stay in the treated group. This result was particularly interesting as there was no heterogeneity among the studies included in this analysis, suggesting that selenium supplementation might be implicated in this increase in ICU length of stay. Selenium supplementation was also associated with a non-significant increase in infectious complications in the treated group, which may offer an explanation for the increased length of ICU stay in this group. These results suggest that supplementation with megadoses of a single micronutrient might bring about disturbances in the balance that exists between interrelated antioxidant systems, possibly even resulting in a pro-oxidant state and poor immune function, which may result in increased morbidity and infection, resulting in a longer stay. The lack of benefit of single nutrients in supplemental amounts is suggested by the meta-analysis of Visser *et al*.,[30] which showed that single nutrient supplementation did not improve clinical outcomes.

Previous studies have indicated that selenium as a single micronutrient played a key role in improving antioxidant status and improving clinical outcomes such as mortality in the critically ill.[1,17] Our results have indicated that this is not the case. A combination of micronutrients was effective in significantly reducing hospital length of stay, the occurrence of infectious complications, and the duration of mechanical ventilation. These findings are supported by Level I evidence consisting of large randomised trials that have produced clear results. Selenium supplementation alone was associated with no apparent benefit, which may be because micronutrients work together in an overlapping manner to maintain a balance between reduction and oxidation reactions. For example, vitamin E is responsible for inhibiting lipid peroxidation by scavenging peroxyl fatty acid radicals in cell membranes. Vitamin C functions primarily as an electron donor that can directly detoxify superoxide, hydrogen peroxide, hydroxide radicals, peroxyl radicals and singlet oxygen radicals, but it also plays a role in the regeneration of tocopherol from the alpha-tocopherol radical, therefore it is also important in providing membrane protection.[31] Providing megadoses of only one of these micronutrients may therefore cause a short circuit in this carefully orchestrated antioxidant system. The same scenario can be applied to superoxide dismutase, catalase and glutathione peroxidase, which have to work closely with each other to neutralise the superoxide radical. This may explain why providing numerous micronutrients may lead to a larger treatment effect and a more favourable outcome than providing a single micronutrient.

Our results were further substantiated by a set of recently conducted retrospective studies[32,33] that examined the effect of high-dose antioxidant supplementation on clinical outcome in acutely injured patients (both ICU and non-ICU patients). Although not randomised clinical trials, these studies are significant because they are the only large trials conducted on antioxidant supplementation to date. The major findings of these studies were that

antioxidant supplementation was associated with a 28% relative risk reduction, and a significant reduction in ICU length of stay, hospital stay and duration of mechanical ventilation. Using the same data, Giladi *et al*.[33] conducted a subset analysis on those patients who spent 24 hours or more in the ICU. Length of hospital and ICU stay and overall infectious complications remained significantly lower in the supplemented group than the control group. The study design does, however, infer some weakness. Owing to the study having historical controls, the associated benefits cannot specifically be attributed to the antioxidant intervention. There was, however, no significant critical care change reported from the time of antioxidant supplementation. Consequently, these studies may be considered particularly noteworthy as they provide an indication of the possible effects that antioxidant supplementation could have on larger sample sizes of critically ill patients. These results, together with the results from our meta-analysis, indicate that supplementation with several micronutrients could be effective in improving clinical outcomes in the critically ill.

Our review offers a number of strengths. It had a larger sample size than previous meta-analyses[1] reporting on this topic, which increases its precision and power. Our choice of statistical model for performing this meta-analysis was an OR. Although no statistical method gives completely unbiased estimates, the OR method at events rates up to 10% appears to be the least biased and most powerful when there is no substantial imbalance in treatment and control group sizes within trials, and treatment effects are not exceptionally large.[34]

Our study has several limitations. One of the flaws of conducting a meta-analysis is that of publication bias as this meta-analysis did not include results from unpublished data. There were a large number of inconsistencies within the trials included for review, which could be attributed to both clinical as well as methodological heterogeneity. One of the weaknesses associated with these trials were confounding factors that could have an effect on clinical outcome, such as inotropic support, mode of nutritional support, amount of energy and protein provided, provision of blood products, blood glucose control, and renal replacement therapy and antibiotic administration that were not adequately considered and compared between groups in any of the trials. It is therefore hard to assess if the effects observed in these trials could be attributed to antioxidant supplementation alone. The varying quality of the different studies may also affect the outcome of a meta-analysis. Despite only including randomised control trials, generally the studies failed to explain techniques of randomisation and blinding in sufficient detail (Table 3), indicating poor methodology. These studies were, however, still included in this review as they met the specified inclusion criteria for this meta-analysis, as they were randomised clinical trials investigating the effect of antioxidant supplementation on clinically significant outcomes in the critically ill population. We recognise that the meta-analysis done on the effect of mixed antioxidant supplementation on infectious complications has a moderate heterogeneity (Fig. 5). Berger *et al*.,[22] Porter *et al*.[23] and Berger *et al*.[19] strongly favoured antioxidant supplementation in this sub-analysis. Individually, these studies reported a significant reduction in infectious complications with mixed antioxidant supplementation, with both groups being comparable at baseline. The other studies failed to show significance, resulting in the higher heterogeneity seen in this analysis. With this said, studies such as those done by the Collier-Gilardi[32,33] group may show more insight on the effects of antioxidants on clinical outcomes than they are given merit for.

Heyland *et al*.[35] have recently published the results of a multi-centred randomised controlled trial on the effects of antioxidant supplementation on 28-day mortality in critically ill patients with severe organ dysfunction. The results have been eagerly anticipated owing to the very large sample size powered to detect a true mortality effect. The results indicate that supplemental antioxidants alone in 307 patients did not confer a mortality benefit, which supports the findings of the present meta-analysis. Heyland's study, however, did not demonstrate a positive effect on any other clinical outcome, which is a departure from our results. Therefore, questions remain regarding the clinical utility of mixed antioxidant supplementation.

Conclusion

The results of this meta-analysis indicate that supplementation of mixed antioxidants may have the potential to be beneficial in improving clinical outcomes in the critically ill, such as length of stay, infectious complications and duration of mechanical ventilation. Although no benefit on mortality was observed, a reduction in the aforementioned outcomes might reduce costs associated with critical care. More studies on the effects of antioxidant supplementation are warranted to gain further insight on appropriate dosing schedules for clinical use.

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