Antioxidant supplementation in sepsis and systemic inflammatory response syndrome

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Objective: **Summarize the current knowledge about oxidative stress-related organ dysfunction in inflammatory and septic conditions, and its potential prevention and treatment by antioxidants in critically ill patients, focusing on naturally occurring antioxidants and clinical trials.**

Study Selection: **PubMed, MEDLINE, and personal database search.**

Synthesis: **Plasma concentrations of antioxidant micronutrients are depressed during critical illness and especially during sepsis. The causes of these low levels include losses with biological fluids, low intakes, dilution by resuscitation fluids, as well as systemic inflammatory response syndrome-mediated redistribution of micronutrients from plasma to tissues. Numerous clinical trials have been conducted, many of which have shown beneficial effects of supplementation. Interestingly, among the candidates, glutamine, glutathione, and selenium are linked with the potent glutathione peroxidase enzyme family at some stage of their synthesis and metabolism.**

Conclusions: **Three antioxidant nutrients have demonstrated clinical benefits and reached level A evidence: a) selenium improves clinical outcome (infections, organ failure); b) glutamine reduces infectious complication in large-sized trials; and c) the association of eicosapentaenoic acid and micronutrients has significant anti-inflammatory effects. Other antioxidants are still on the clinical benchmark level, awaiting well-designed clinical trials. (Crit Care Med 2007; 35[Suppl.]:S584–S590)**

KEY WORDS: selenium; glutathione; critically ill; glutathione peroxidase; outcome; infection; renal failure; burns; trauma

Sepsis remains an important cause of mortality in intensive care unit (ICU) patients (1). Infection and endotoxemia provoke a cascade of local and systemic responses, including increased free radical production, cytokines and multiple mediators release, and lipid peroxidation (2), the combination of which finally results in multiple organ failure. This stereotyped response is called systemic inflammatory response syndrome (SIRS). Different acute conditions may release this response: sepsis, respiratory failure, pancreatitis, trauma and burns, or ischemia/reperfusion. Free radical production is implicated in this process, both as a mechanism for direct cellular injury and in activation of intracellular signaling cascades within inflammatory cells resulting in progression of the inflammatory response.

Free radical atoms and molecules are characterized by the presence of one or more unpaired electrons in their outermost orbit; they are unstable and strive to restore parity (3). This may be either oxygen centered (called *reactive oxygen species*) or nitrogen centered, mainly derived from nitric oxide metabolism. The first are produced under normal conditions by leukocytes and by the respiratory mitochondrial chain for cell signaling, as well as for bacterial defense (4), while the latter are the normal byproduct of endothelial metabolism, with an increased production during sepsis. Free radicals cause a cascade of intracellular events resulting in liberation in the cytoplasm of nuclear transcription factor-κB (NFκB) from its inhibitory protein IκB. This permits its translocation into the nucleus, where NFκB binds to DNA, enabling the initiation of the transcription process of the genes involved in inflammation. NFκB controls the production of the acute phase mediators such as tumor necrosis factor-α, interleukin-2, and interleukin-2 receptors, which in turn activate NFκB, amplifying the inflammatory cascade.

Liver dysfunction is considered an early event during sepsis. In an experimental model, endotoxin injection results in lipid peroxidation and membrane damage in the liver, decreasing levels of free radical scavengers (5). Sepsis induces an imbalance in hepatic vasoregulatory gene expression, which includes reduced concentrations of glutathione (GSH) simultaneously with increased messenger RNA expression of a series of endothelins, nitric oxide (NO) synthase, and heme oxygenase (6).

The body is equipped with numerous potent endogenous antioxidant (AOX) agents, collectively referred to as the *AOX defense system*. They aim at containing the free radicals in limited amounts and defined compartments and act through a series of complementary mechanisms, most being free radical scavengers, chain-breaking agents, or catalytic agents (Table 1). All the classic AOXs are potential electron donors, and exist in both reduced and oxidized forms; they are therefore both antioxidant and pro-oxidant. A series of micronutrients (trace elements and vitamins) have been investigated for their AOX properties over the last 20 yrs (4). Experimental data are very convincing. In a model of sepsis, the administration of α-tocopherol completely prevents endotoxin-induced injury to the liver membranes, suggesting that lipid peroxidation by free radicals might occur in

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Table 1. Antioxidants investigated in sepsis and used in animal and clinical supplementation trials

Antioxidant	Supplementation Trials ICU Patients	Comment
Selenium	Septic ICU patients (7, 8); major burns in combination with Cu and Zn (9, 10); trauma patients (11)	Ceiling effect >750 μg/day?
Zinc	Pneumonia in children: clinical course significantly shortened (12)	Immune depression if doses >50 mg/day are provided
Cu-Se-Zn	Burns: trials showing reduction of infectious complications (pneumonia) and improved wound healing (9, 10)	Doses were calculated to compensate for the exudative losses
Vitamin E (α-tocopherol)	SIRS enteral supplementation (13)	Convincing animal data (6, 14)
Vitamin C (ascorbic acid)	Burns, megadose during the first 24 hrs after injury (15); trauma, combined with vitamin E (13)	Possibly an endothelial mechanism
β-carotene	In combination with vitamins C and E	
EPA/DHA	ARDS (16); sepsis: enteral route (17)	The diets used in both trials contain combinations of many nutritional antioxidants
Glutamine	Glutamine-enriched enteral and parenteral nutrition with reduction of infectious complications (18–21)	Multimodal impact on the immune system
Glutathione	Combined with NAC (22)	
R-α-Lipoic acid	No	Suggestive animal data (23)
Melatonin	No	Many studies in non-ICU patients (24)
NAC	ARDS, sepsis, septic shock (22, 25)	Disappointing despite many promising preliminary trials

ICU, intensive care unit; *Cu,* copper; *Zn,* zinc; *Se,* selenium; *SIRS,* systemic inflammatory response syndrome; *EPA,* eicosapentaenoic acid; *DHA,* docosahexaenoic acid; *NAC,* N-acetylcysteine; *ARDS,* acute respiratory distress syndrome.

Table 2. Summary of oxidative stress signals observed in sepsis and systemic inflammatory response syndrome (2, 25)

	Plasma Variable
↑ Lipid peroxidation	↑ MDA (TBAR), F2-isoprostane
↑ NO synthesis	↑ Nitrite, nitrate, nitrotyrosine
↓ Circulating antioxidants	↓ Uric acid, protein SH groups, bilirubin (unconjugated)
	↓ Ascorbic acid, α-tocopherol, β-carotene, lycopene
	↓ Antioxidant enzymes (GSHPx)
	↓ Selenium, zinc
	↓ GSH
Xanthine oxidase activation	↑ Plasma xanthine oxidase

MDA, malondialdehyde acid; *TBAR,* thiobarbituric acid reacting; *NO,* nitric oxide; *SH,* sulfhydryl; *GSHPx,* selenoenzyme glutathione peroxidase; *GSH,* glutathione.

ischemic tissue, probably by disseminated intravascular coagulation. It has been further suggested that the oxidative stress caused by endotoxin may be partly due to the changes in endogenous zinc or selenium regulation during endotoxemia (5) and inflammation (26). Alterations of hepatic vasodilator gene expression also can be improved by α-tocopherol supplementation (6).

Via cytokine-mediated mechanisms, SIRS causes a reprioritization of protein synthesis and a redistribution of micronutrients (26) from the circulating compartment to tissues and organs that are involved in immune defense and synthesis (27, 28), causing a depletion of the plasma AOX capacity (Table 2). The interpretation of the low plasma levels of micronutrients is, therefore, complex; although SIRS-mediated redistribution is an important cause, also contributing are acute losses of micronutrients through biological fluids, dilution due to resuscitation fluids, and insufficient intakes. In addition, the European and Australasian patients admitted to the ICU often have low selenium status prior to illness (29).

Immune and AOX defense are closely linked. The demonstration that selenium deficiency favors increases virus virulence by DNA mutation, through the reduction of the activity of the selenoenzyme glutathione peroxidase (GSHPx) (30), suggests that so-called antioxidation may be an essential step in defenses against infection.

We intend to summarize the clinical results of supplementation with naturally occurring AOXs in inflammatory and septic conditions. We will focus on clinically relevant evidence in the critically ill patient.

Laboratory Markers of Oxidative Stress

Reactive oxygen species have a very short half-life; however, increased oxidative stress can be measured by the presence of byproducts of lipid, DNA, or protein oxidation. Chemical markers of AOX status and oxidative stress recently have been reviewed (25, 31, 32). There are limitations to each of the elaborate assays used for *in vivo* determination of AOX defenses, oxidative stress, and related damage, assays such as total antioxidant capacity, antioxidant gap, electron spin resonance, and comet assay. In addition, many assays are expensive and not available for clinical purposes. Recently, the mitochondrial DNA/nuclear DNA ratio has been proposed as an indirect marker of mitochondrial function and oxidative stress (normal ratio = 1.13; an increase is consistent with mitochondrial recovery) (33). Promising, it has mainly been used in human immunodeficiency virus infections; its use in other conditions must be validated (34). For the moment, the most realistic approach is the determination of a combination of oxidized biomolecules and endogenous AOXs. Among the various markers, malondialdehyde, a very global and crude test of lipid peroxidation, remains the most useful in clinical settings. It should be used in association with plasma micronutrient levels and plasma GSHPx (31), the latter being a very sensitive marker of response to supplementation.

Antioxidant Supplementation Candidates

Depressed circulating and tissue AOX concentrations during SIRS and sepsis

have been shown repeatedly in animal and human studies (4, 25, 35). The circulating concentrations of most trace elements (iron, selenium, zinc) and of their carrier proteins decrease, as do the water-soluble vitamins (28, 36, 37). When depleted, the endogenous AOX defenses will be overwhelmed by the free radical production caused by sepsis, resulting in oxidative stress, which was defined in 1985 by Sies as "a disturbance in the prooxidant–antioxidant balance in favor of the former resulting in cell damage and disease" (38).

The inflammation-induced oxidative stress process rapidly impacts the intracellular and intranuclear compartments; DNA replication and subsequent cytokine and mediator production can be amplified or contained depending on intracellular AOX status. A series of micronutrients, including selenium, limits the release of NFκB caused by increased reactive oxygen species. Indeed, selenium has been shown to down-regulate NFκB and thereby to limit the extension of the inflammatory response (39, 40). It has been hypothesized that the redistribution of trace elements occurring during the acute phase response may therefore be deleterious if prolonged, due to the depletion of the circulating compartment's AOXs (35).

Among micronutrients, selenium appears to be the most potent AOX agent in clinical settings (41), followed by zinc and vitamins C, E, and β-carotene. AOX properties are not exclusive to micronutrients, however. Many other compounds generally present in food have AOX properties. Among the n-3 polyunsaturated fatty acids, eicosapentaenoic and docosahexaenoic acids from fish oil are known to exert significant anti-inflammatory properties (42) and to down-regulate the response to endotoxin (43). Glutamine repeatedly has been shown to mitigate infection complications; the mechanism appears to be multifactorial via heat shock proteins (44), but probably also via an AOX mechanism. GSH is quantitatively the most important scavenger (45), and evidence increases that its depletion is directly associated with the severity of diseases. The thiol compound lipoic acid, and its reduced form dihydrolipoic acid, have the ability to scavenge various free radicals; in addition, they are involved in recycling other AOXs such as vitamins E and C and GSH (23). Melatonin is also a potent endogenous free radical scavenger, exerting an AOX action that is independent of its many receptor-mediated effects—hundreds of confirmatory animal and human investigations have been carried out over recent years (24). Finally, N-acetylcysteine has long been considered a good AOX candidate for various conditions such as acute respiratory distress syndrome and sepsis, but has proven disappointing.

Intervention Studies in SIRS and Sepsis

Because the AOX status is depleted in sepsis while the patient faces high oxidative stress, the rationale for intervention appears solid. It consists of either restoring endogenous AOX status by intravenous or enteral supplementation of pharmacologic doses of nutrients with AOX properties, or providing exogenous compounds with AOX properties whether naturally occurring or produced by the pharmaceutical industry (e.g., desferoxamine, an iron scavenger, or allopurinol, an xanthine inhibitor). We will focus on compounds that are both endogenous and exogenous.

Selenium. Selenium's AOX activity is as a constituent of the different GSHPx selenoenzymes and a series of other selenoproteins (46). It has repeatedly been shown that plasma selenium is depressed in SIRS and in sepsis (7, 47). Plasma GSHPx activity declines in parallel with plasma selenium, while selenium supplementation restores the activity of the enzyme (7) and is associated with improved clinical outcome. In a controlled randomized trial with 42 patients receiving moderate selenium supplementation (maximum, 535 μg/day) for 9 days, a reduction of acute renal failure and the need for renal replacement therapy was observed (7). The same German group recently completed a multicenter prospective randomized selenium supplementation study in patients with severe sepsis (8). The intervention consisted of an intravenous supplement of 1000 μg selenium vs. placebo daily for 2 wks after a loading dose. While there was no significant difference in intention to treat mortality, the authors found a significant reduction of 28-day mortality in the subgroup of patients with the highest quartile Acute Physiology and Chronic Health Evaluation III scores from 81.5% to 55.6%, and in patients in septic shock with disseminated intravascular coagulation from 66.7% to 40.5%. By contrast with the first study (7), there was no reduction in the need for renal replacement therapy, nor in organ dysfunction, vasopressor therapy, or nosocomial pneumonia. There are shortcomings to this study, and particularly patient enrollment was difficult and prolonged, with large between-center variations, suggesting selection bias (48). In addition, there seems to be a contradiction between the lack of effect on organ failure and the effect on mortality of a subgroup of patients. Further investigation is required to address these important questions.

Zinc. This trace element long has been known to be involved in immunity, but single trials are scarce. A randomized trial conducted in Bangladesh including 270 young children showed that zinc supplementation (20 mg/day) reduced the duration of pneumonia and the length of hospital stay (12). The authors concluded that the improved immune defense associated with zinc therapy might stimulate host antimicrobial resistance by decreasing the exposure to multiple antibiotics.

Trace Element Combinations. A recent meta-analysis investigated whether supplementing critically ill patients with AOX micronutrients might positively influence survival (41). Eleven trials met the inclusion criteria, including 886 ICU patients. Results suggest that overall AOX was associated with a significant reduction in mortality (risk ratio [RR], 0.65; $p = .03$). Only the studies using parenteral AOXs were associated with a significant reduction in mortality (RR, 0.56; $p = .02$), whereas those using enteral AOX were not. Selenium-containing supplements were those associated with a reduction in mortality. However, most of the investigations were small single-center studies. The above multicenter German selenium study (8), therefore, strengthens the conclusion of this meta-analysis.

Our group recently has confirmed that restoring a near normal status of copper, selenium, and zinc using intravenous supplements combining the three elements achieved a significant reduction of infectious complications in severely burned patients. The study consisted of an aggregation of two randomized placebo-controlled trials including 41 patients with major burns. Septic morbidity was reduced; particularly, we found a 65% reduction in nosocomial pneumonia (9). Wound healing also was improved, and ICU stay was shortened (10).

Vitamin E. Positive animal trials on vitamin E are numerous, while human

ICU data remain rare. In septic rats, short-term, high-dose enteral supplementation of vitamin E modulates the monocyte and macrophage response to endotoxin (14). In septic rats, supplementation ameliorated the altered expression of vasodilators (6). Most human supplementation trials address elderly patients, or viral pathologies such as human immunodeficiency virus and hepatitis, with some biological clinical benefits.

In a prospective randomized study including 35 patients with major burns, vitamin E plasma levels decreased most significantly from days 6–8 postburn in all burned patients, while the concomitant serum lipid peroxides increased significantly. In the vitamin E–treated patients, the serum levels increased and lipid peroxides decreased to the levels of healthy controls, but no clinical end points were reported (49). One trial investigating vitamin E pharmacokinetics in patients with acute respiratory distress syndrome receiving enteral supplements of 3 g per day for 10 days showed a doubling of the plasma concentrations, but again, no clinical end point was mentioned (50).

Vitamin C. Work in septic rats suggests that high-dose ascorbic acid supplementation may protect cells from free radical injury and improve survival. A pharmacokinetic study in 14 critically ill patients confirmed extremely low plasma levels of ascorbic acid following trauma and infection (51). The plasma levels were unresponsive to 2 days on 300 mg per day supplementation, and approached low normal plasma levels following 2 days on 1 g per day, while a significant increase was noted following 2 days on 3 g per day. The authors conclude that early repletion of vitamin C requires rapid pool-filling early in the postinjury period using supraphysiologic doses for 3 or more days. A very interesting, although nonrandomized, trial in major burn patients showed that megadoses of ascorbic acid provided during the first 24 hrs after injury were able to reduce the capillary leak and the volume of fluid required for hemodynamic stabilization (15). The AOX mechanism of this intervention has been confirmed in burned sheep (52). The potential risk of acute renal failure with high dose vitamin C should be underlined. The risk is due to oxalate deposition in the interstitium and renal tubules. Vitamin C should therefore not be viewed as a benign, water-soluble substance, but rather as a potentially toxic drug for both normal and diseased kidneys (53).

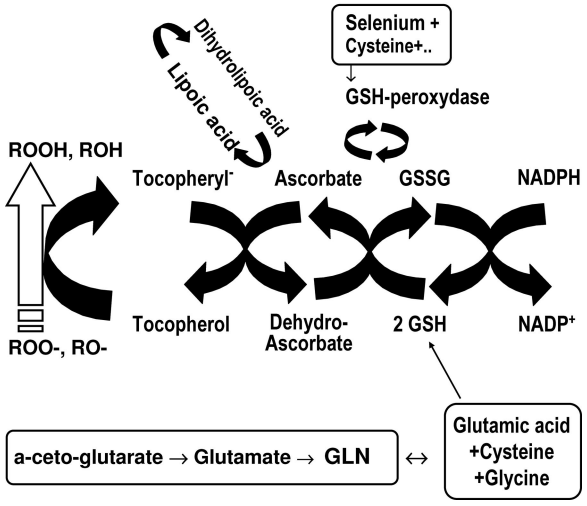

Figure 1. Conceptual description of interactions between selected antioxidants, showing the interactions in the antioxidant spiral of the various endogenous antioxidants. *GSH*, glutathione; *ROOH*, hydroperoxides; *ROH*, hydroxy derivatives of hydroperoxides; *ROO*, cumene peroxyl; *RO*, alkoxy radical; *GSSG*, oxidized glutathione; *NADPH*, nicotinamide adenine dinucleotide phosphate in reduced state; *NADP*, nicotinamide adenine dinucleotide phosphate in oxidized state.

Vitamin Combinations. In 595 critically ill patients of which 91% were trauma cases (13), the early administration of AOX supplementation using α-tocopherol (3 g) and ascorbic acid (3 g) daily for >14 days reduced the incidence of organ failure (RR, 0.81) and shortened ICU length of stay, but the results remained only strong trends, due to insufficient power. Another European trial testing enteral feeds enriched with a combination of vitamins (A, C, and E) showed enhanced resistance to oxidative stress in *ex vivo* tests, but no clinical impact (54).

Trace Element Plus Vitamin Combinations. Trauma patients are exposed to negative micronutrient balances during the first days after injury (55) and are, therefore, candidates for multimodal substitution. Eighteen critically ill trauma patients were randomized to a control group or an AOX group treated with N-acetylcysteine, selenium, and vitamins C and E for 7 days (11). Fewer infectious complications (8 vs. 18) and organ failures (0 vs. 9) were observed in the AOX group. A randomized trial recently completed in our center including 70 trauma patients on AOX mixture (selenium, zinc, and vitamins B1 and C) failed to show any significant reduction in infectious complications, but was associated with a significantly shorter hospital stay in patients without brain injury (−11 days; $p = .017$) (56).

Glutamine. The most abundant amino acid in the body, glutamine is considered conditionally essential in critical illness. Because glutamine is a precursor for GSH (Fig. 1), its supplementation in the enteral and parenteral nutrition solutions can be used to maintain high levels of GSH and prevent oxidative stress damage (45). A series of supplementation trials carried out in ICU patients showed significant infection prevention (18–20). The latest was a multicenter trial including 114 critically ill patients, which showed that alanine- and glutamine-supplemented parenteral nutrition was associated with a lower incidence of complicated outcome ($p < .05$), which was mainly the result of a reduced infection rate per patient (21). Glutamine has multiple additional metabolic effects beyond the scope of this review (21).

Glutathione. In addition to its AOX scavenger activities, GSH has a number of important functions in amino acid transport across membranes, in protein synthesis and degradation, in gene regulation, and in cellular redox regulation (45). GSH turnover has been shown to be increased in septic animals, with an increased utilization (57). Therefore, the possibility of manipulating its availability has become attractive, but we are still lacking trials with clinically relevant end points.

Eicosapentaenoic and Docosahexaenoic Acids. In an experimental model of sepsis using endotoxin injection in healthy volunteers, our group recently

showed that oral fish oil supplementation was able to blunt several aspects of the response to endotoxin in a very reproducible manner (43). The same blunting of fever, tumor necrosis factor-α, and the endocrine (corticotropin) and sympathetic (epinephrine) responses was observed using two doses of intravenous fish oil (0.5 g/kg) (58). In critically ill patients suffering either sepsis or acute respiratory distress syndrome, two trials have tested enteral feeding solutions enriched with eicosapentaenoic acid, γ-linolenic acid, and antioxidant micronutrients. They found significant clinical benefits in terms of oxygenation, ventilator-free days, and mortality rate (absolute mortality reduction = 19.4%) compared with patients fed with the control diet (16, 17). A recent large nonrandomized study showed that the outcome benefits appear to be diagnosis and dose dependent, optimal effects appearing with doses of 0.15–0.2 g per kg daily (59).

R-α-lipoic Acid. The natural scavenger R-α-lipoic acid decreases lipid peroxidation. In endotoxemic mice, the combination of R-α-lipoic acid and glutamine supplementation is effective in increasing systemic and intestinal B lymphocytes (23). In addition, R-α-lipoic acid augmented the intracellular GSH in the small intestine. Such promising preliminary experimental data require further animal and human studies.

Melatonin. This tryptophan derivative melatonin produced by the pineal gland exhibits several important physiologic functions (24). Several properties make it unique: it is both lipophilic and hydrophilic, passing all biobarriers; it is widely distributed in all compartments; it is endogenously produced, although this declines with age; and it is provided by food. It is able to scavenge a variety of free radicals, including nitric oxide metabolism (60). It differs from other electron donors by being a terminally suicidal AOX; once loaded with free radicals, it undergoes metabolism to end products that are excreted in urine. It, thereby, preserves the other AOXs such as GSH and the vitamins C and E, and even increases GSHPx activity.

N-Acetylcysteine. Based on a convincing rationale although it is not an endogenous AOX, N-acetylcysteine has been included in multiple studies in experimental sepsis models and in human trials since the 1990s (61). Despite many promising preliminary data with significant positive impact on biological end points, larger clinical trials in sepsis and septic shock have been disappointing (22).

Dose-Response Curves of Micronutrients

More is not always better. Trace elements and vitamins have dose response curves, with toxicity at high levels of intake (4, 62). As previously mentioned, the capacity to scavenge free radicals is associated with the transformation of the scavenger into a free radical itself. The potential harm of large doses is particularly releveant for selenium and vitamin E when delivered for prolonged periods.

- Large-dose selenium supplementation deserves a word of caution. Chronic intakes above 450 μg per day are associated with depressed activity of the type I iodothyronine 5' deiodinase (5'DI), another important selenoenzyme that is considered a better indicator of safe selenium intakes than GSHPx. It catalyses the production of triiodothyronine (63). Without reaching acute toxicity, the 1000-μg doses used by Dr. Angstwurm and colleagues (8) may nonetheless have been excessive. In burn trials, the beneficial clinical effects were reached with lower doses (300–550 μg/day) (64). The optimal acute selenium dose may range somewhere between 500 and 750 μg/day, while ideal duration of supplementation is between 1 and 3 wks, depending on severity of disease (10).
- Vitamin E supplementation has long been believed to be safe. The cytotoxic activity of the vitamin E analogues was inversely proportional to their AOX activity, and the pro-oxidant activity may cause hepatic toxicity (65). AOX intervention is most likely to benefit patients with prior or acute depletion. The enteral route appears less efficient than the intravenous route. Acute short-term interventions (2–3 wks) appear free from deleterious effects, while long-term doses >150 mg per day should be avoided.

These limitations are probably valid for all of the endogenous AOXs. Therefore, before considering any supplementation trial, dose-finding studies should be conducted (34).

CONCLUSIONS

Plasma AOX micronutrient concentrations are depressed during critical illness and especially during sepsis, as the result of losses, low intakes, dilution by resuscitation fluids, and the SIRS-mediated redistribution to tissues, creating a circulating AOX defense deficit. The evidence is increasing that AOX supplementation contributes in limiting tissue and organ damage caused by sepsis mediators. Indeed, the best approach to sepsis appears to be support to prevent failure (1).

Interestingly, among the candidates, glutamine, GSH, and selenium are linked with the most potent antioxidant enzyme family—GSHPx—at some stage of their synthesis and metabolism, while lipoic acid might be a generic regenerator. This link probably should be considered important indirect evidence in favor of multimodal AOX support.

Three AOX nutrients already have undergone successful clinical testing reaching a top level of evidence: a) selenium improves clinical outcome (infections, organ failure); b) glutamine reduces infectious complication in large trials; and c) the association of eicosapentaenoic acid and micronutrients blunted endotoxin response. Other AOXs remain at the clinical benchmark level, awaiting well-designed clinical trials. Although studies have shown clinical benefits of early AOX micronutrient supplementation in critically ill patients with different conditions during the last decade, controversies remain regarding the indications for this therapy, the combination of antioxidants, the doses, and the timing of supplementation.

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