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Oxidative stress as a novel target in pediatric sepsis management

Bettina von Dessauer MD[a], Jazmina Bongain MD[a], Víctor Molina MD[b], Julio Quilodrán MD[a], Rodrigo Castillo MD[b], Ramón Rodrigo Msc[b,*]

[a]*Pediatric Intensive Care Unit, Doctor Roberto del Río Children's Hospital, Santiago, Chile*
[b]*Laboratory of Renal Pathophysiology, Molecular and Clinical Pharmacology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, 70058, Santiago 7, C.P. 8380453, Chile*

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Abstract Sepsis with secondary multisystem organ dysfunction syndrome is the leading cause of death in the pediatric intensive care unit. Increased reactive oxygen species may influence circulating and endothelial cells, contributing to inflammatory tissue injury and explaining the tissue hypoxia paradigm based on microvascular dysfunction. An impaired mitochondrial cellular oxygen utilization, rather than inadequate oxygen delivery, was claimed to play a more important role in the development of multisystem organ dysfunction syndrome. Anyway, it seems plausible that reactive oxygen species can mediate the pathophysiologic processes occurring in sepsis. However, the consensus guidelines for the management of patients with these conditions do not include the enhancement of antioxidant potential. Therefore, further investigation is needed to support interventions aimed to attenuate the severity of the systemic compromise by abrogating the mechanism of oxidative damage. Antioxidant supplementation currently in use lacks a mechanistic support. Specific pharmacologic targets, such as mitochondria or Nicotinamide Adenine Dinucleotide Phospate-Oxidase (NADPH) oxidase system, need to be explored. Furthermore, the early recognition of oxidative damage in these seriously ill patients and the usefulness of oxidative stress biomarkers to define a cut point for more successful therapeutic antioxidant interventions to be instituted would offer a new strategy to improve the outcome of critically ill children.

1. Introduction

Severe sepsis with secondary multisystem organ dysfunction syndrome (MODS) is the leading cause of death in all intensive care units (ICUs). Sepsis in adults cause more than 200 000 deaths per year in the United States, thus equaling the number of patients dying from myocardial infarction. Mortality rates in septic patients have been demonstrated to range as high as 40% to 60%. In children, sepsis and septic shock are also major causes of morbidity and mortality, with an estimated 42 000 cases per year in the United States and associated mortality rate of 10% [1]. In the United Kingdom, infection accounts for more than 10% of deaths in children younger than 4 years. Approximately 1000 children with severe sepsis are admitted to pediatric intensive care units (PICUs) annually and up to 20% of those having severe sepsis die [2]. In addition, each year, an unknown number of children die from septic shock in emergency departments before reaching a PICU.

* Corresponding author. Tel.: +56 2 9786126; fax: +56 2 9786126.
E-mail address: rrodrigo@med.uchile.cl (R. Rodrigo).

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During recent years, several new treatment strategies have improved sepsis survival; but the related mortality remains high in this group. Compliance with sepsis evidence-based guidelines has led to a significant reduction of sepsis-related mortality in adults and children [3]. Pediatric septic shock has an adverse impact on survival in a time-dependent manner; and the early recognition of this condition, followed by immediate aggressive therapy, has been identified as one of the most important therapeutic measures to reduce its related complications and mortality [4]. Oxidative stress has been implicated in the pathogenesis of multiple highly prevalent diseases. Improvement in the understanding of sepsis and septic shock pathophysiology has contributed to establish a relationship between these settings and the occurrence of oxidative stress. However, the consensus guidelines for the management of patients with these conditions do not include the enhancement of antioxidant potential. Oxidative injury from pathologic conditions such as sepsis may have more serious consequences in pediatric patients than in older people because of the lower functional reserve and because of the need for subsequent tissue growth to match somatic and normal development [5]. In this article, the evidence pointing to a role of oxidative stress in pediatric sepsis is reviewed; and the potential therapeutic implication of this association is discussed.

1.1. Reactive oxygen species and oxidative stress

Reactive oxygen species (ROS) include superoxide anion ($O2\cdot^-$), hydroxyl radical, hydrogen peroxide (H_2O_2), and oxygen singlet. Reactive nitrogen species (RNS) include nitric oxide (·NO), nitrogen dioxide radicals, and peroxynitrite anion. Whereas ·NO has important vasorelaxing and antiproliferative properties, peroxynitrite anion, resulting from the reaction between ·NO and $O2\cdot^-$, is highly toxic. Nitrogen dioxide can undergo diffusion-controlled radical-radical termination reactions with biomolecules, resulting in nitrated compounds such as those containing nitrotyrosine residues. There are several sources of ROS in mammalian cells, including Fenton reaction, Haber-Weiss reaction, and enzymatic activity of xanthine oxidase, NADPH oxidase (Nox), nitric oxide synthases, myeloperoxidase, and cytochrome P450. Some of these sources can be induced in certain pathophysiologic conditions [6]. Reactive oxygen species can mediate cell damage through several ways. Lipid peroxidation is a known ROS-mediated cellular damage phenomenon. This injury mechanism includes the oxidation of polyunsaturated fatty acid residues of phospholipids present in cell membrane and further damage through the generation of aldehydes that can diffuse and attack targets intracellularly or extracellularly. Some damage mechanisms include mitochondrial membrane damage and decrease of NO bioavailability. Antioxidant molecules work as scavengers, this way reducing ROS bioavailability. They can be produced endogenously or provided exogenously and include vitamin C, vitamin E, selenium, carotenoids, polyphenols, uric acid, and bilirubin, among others. The antioxidant defense system includes the endogenous antioxidant enzymes superoxide dismutase, catalase, and glutathione peroxidase (GSH-Px). Superoxide dismutase converts $O2\cdot^-$ to H_2O_2, which is a substrate for catalase and GSH-Px. Catalase metabolizes H_2O_2 to water and oxygen, and GSH-Px reduces both H_2O_2 and organic hydroperoxides when reacting with glutathione (GSH).

Under normal conditions, a balance is established between ROS production and antioxidant defenses. This balance can be disturbed through variable extent of increased ROS production and/or impaired antioxidant defense mechanisms. The prooxidant-antioxidant imbalance, in favor of the former, is known as *oxidative stress*.

2. Contribution of ROS/RNS to the pathophysiology of sepsis and septic shock

Sepsis is a complex inflammatory syndrome with a wide spectrum of severity. It is defined as a systemic inflammatory response syndrome (SIRS) in the presence of infection [7]. Severe sepsis occurs when organ dysfunction is associated. Multiple organ dysfunction syndrome or MODS is defined as the simultaneous impairment of at least 2 organ systems. *Septic shock* is defined in adults as persisting hypotension despite adequate volume resuscitation. In children, hypotension is a late feature of the syndrome; and septic shock is characterized by tachycardia with signs of decreased perfusion, including decreased peripheral pulses, impaired alertness, flash or slow capillary refill, mottled or cool extremities, and decreased urine output [3]. Although these features have been included in this definition, they occur sequentially along with disease progression. In children, septic shock onset includes tachycardia and mild signs of hypoperfusion, such as impaired alertness. Once compensatory mechanisms become overwhelmed, clear signs of hypoperfusion appear, including cold extremities, slow capillary refill, a worsening impairment of consciousness, and decreased urine output with final eventual hypotension.

There is a prevailing theory that sepsis represents an uncontrolled inflammatory response with loss of the normal homeostatic balance between systemic inflammation and a counterbalancing anti-inflammatory response. A balance between the intensity of tissue inflammation and the anti-inflammatory response in blood enables the body to concentrate activated neutrophils and other effectors in the area of infection, preventing at the same time the damage in distant organs. The inflammatory response to infection is initiated with cell activation by the binding of microbial products such as the bacterial cell wall components lipopolysaccharide (LPS) and peptidoglycans. Indeed, LPS behaves as an inflammatory mediator in sepsis, together with tumor necrosis factor–α and interleukins (ILs), mainly

IL-1β and IL-6. In concert with the activation of complement pathways, the cytokine stimulation of circulating and resident immune cells and endothelial cells results in increased production of ROS and RNS, such as $O2^{\cdot -}$ and ·NO. Superoxide is a weak ROS but can activate nuclear factor (NF)–κB translocation, induce endothelial apoptosis, suppress endothelial nitric oxide synthase, and potentiate the inflammatory process. The ROS increment occurs early in the development of sepsis because LPS induces respiratory burst and macrophages use ROS to kill pathogens. Therefore, this step may be decisive in the outcome of sepsis and consists of the regulation of key cytokines and chemokines, which further modulate the inflammatory response, during which ROS and RNS induce phagocytosis, gene expression, and apoptosis. Under prooxidant imbalance, such as sepsis and acute lung injury, ROS levels may influence circulating and endothelial cells, contributing to inflammatory tissue injury. It should be noted that oxidative stress onset implies changes in immune functions, such as decreased chemotaxis and increased phagocytosis [8,9].

The prevalent hypothesis is that these pathologic conditions are caused by an excessive defensive and inflammatory response characterized by massive production of ROS, RNS, and inflammatory cytokines [10]. As a consequence, a systemic damage of vascular endothelium occurs. Because the vascular endothelium plays a central role in the control of microvascular flow, it has been proposed that widespread vascular endothelial activation, dysfunction, and eventually injury occur in septic shock, ultimately resulting in MODS [11]. It is of interest to remark that despite fluid resuscitation and both adequate arterial blood oxygenation and cardiac output, this impairment is accompanied by decreased density of perfused capillaries and increased proportion of non-perfused capillaries [12]. Therefore, it can lead to tissue hypoxia and may explain why microvascular dysfunction is a strong predictor of death and why one third of severe sepsis adult patients die of MODS, even when shock is prevented [13]. Although the mechanism accounting for these pathophysiologic events remains to be elucidated, a major role of ROS has been suggested from clinical and experimental evidence. Recently, this tissue hypoxia paradigm has been challenged. It has been proposed that impaired cellular oxygen utilization, termed *cytopathic hypoxia*, rather than inadequate oxygen delivery, may play a more important role in the development of MODS [14]. The key issue here is that, although oxygen therapy may deliver adequate oxygenation of hemoglobin and tissue tensions as measured by oximetry standards, the oxygen may still not be able to be used at the mitochondrial level. As mitochondria are the primary consumers of cellular oxygen, increasing attention has now started to be paid to the role of mitochondrial function and dysfunction in the establishment of sepsis-related MODS. Understanding the precise effect of sepsis on mitochondrial function and the involvement of mitochondria in the development of multiple organ failure is fundamental. More human studies are thus necessary to evaluate mitochondrial dysfunction in early and late phases of sepsis development before testing therapeutic strategies targeting this organelle [15]. Although the contribution of either oxygen delivery or its mitochondrial metabolism remains to be debated, it seems plausible that ROS can

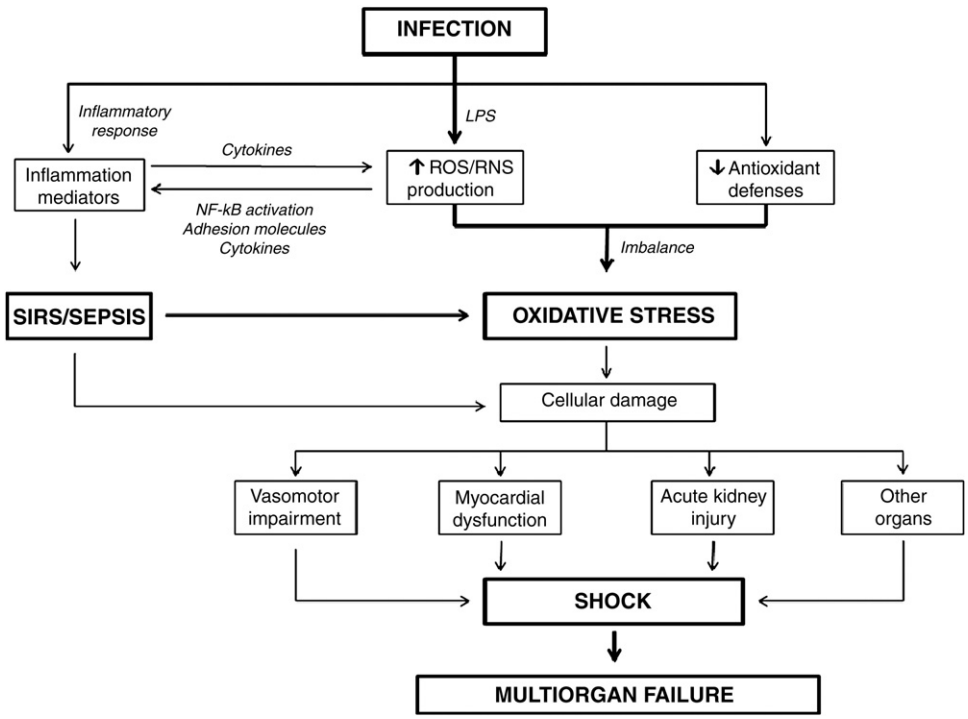

Fig. 1 Scheme illustrating the hypothesis of the involvement of ROS and RNS in sepsis pathogenesis.

mediate the pathophysiologic processes occurring in sepsis, as depicted in Fig. 1.

3. Role of oxidative stress-related biomarkers

Several studies in adults confirm the occurrence of severe oxidative stress in patients with SIRS as demonstrated by reduced values of plasma total radical-trapping antioxidant parameter and its components (uric acid, protein SH groups, unconjugated bilirubin, vitamin C, vitamin E, and plasma unidentified antioxidants) [16]. Furthermore, elevated markers of lipid and protein oxidative damage have been observed in septic patients. In addition, it has been reported that there is an increased plasma concentration of nitrite plus nitrate, NO reaction products, in patients with septic shock. Patients with sepsis present reduced levels of GSH, α-tocopherol, selenium, vitamin A, β-carotene, lycopene, and ascorbic acid [17].

Plasma antioxidant potential in septic patients has been shown to be lower than that in healthy subjects at the beginning of the disease, later increasing to normal or even supranormal levels in septic patients who survived, but not in patients who died [18]. Lipid peroxidation products levels are higher, as shown by increased levels of thiobarbituric acid-reactive substances, in septic patients who develop MODS. In addition, increased levels of malondialdehyde, another marker of lipid peroxidation, have been reported in survivors in relation to nonsurvivors [19]. Xanthine oxidase is an enzymatic source of ROS that has been shown to be activated in sepsis, pointing to a failure of microvasculature control that leads to underperfusion and ischemia. In children, a potential association between markers of oxidative modifications and intensive care disease severity scores, such as PIM 2 (Paediatric Index of Mortality 2), PELOD (Paediatric Logistic Organ Dysfunction) and PELODd (Paediatric Logistic Organ Dysfunction daily) [20], has not been established.

4. Role of antioxidants in sepsis management

Interventions with antioxidants could have an impact in improving the prognosis and survival of these patients, depending on the moment at which the support is instituted in the course of the disease.

4.1. Vitamins

Vitamin A is obtained from the diet either as *all-trans*-retinol, retinyl esters, or β-carotene. It has long been known to have antioxidant and immunomodulatory properties. Accordingly, deficiency of this vitamin is associated with inflammation. Vitamin A supplementation reduces the pathophysiologic effects of endotoxin in animals [21], an anti-inflammatory effect that could depend, at least in part, on NF-κB inhibition [22]. However, although vitamin A has known antioxidant properties, there is evidence pointing also to effects through binding to nuclear receptors.

Vitamin E is the collective name for molecules that exhibit the biological activity of α-tocopherol. Both naturally occurring and synthetic forms of vitamin E are present. Naturally occurring forms of vitamin E include 4 tocopherols and 4 tocotrienols (α, β, γ, and δ).

Vitamin C (ascorbic acid) is a 6-carbon lactone that is synthesized from glucose in the liver of most mammalian species, but not in humans. Consequently, when humans do not ingest vitamin C in their diets, a deficiency state occurs that manifests as scurvy. Vitamin C is an electron donor and therefore a reducing agent. However, vitamin C itself is oxidized in the process, generating ascorbyl radical, which has a very low reactivity.

4.1.1. Evidence supporting the role of vitamin supplementation in sepsis management

The effect of vitamin C supplementation has been studied in relation to capillary blood flow, which is impaired in severe sepsis because of compromised microvascular responsiveness. Animal and in vitro models show that vitamin C can act in vascular endothelial cells through the inhibition of Nox and inducible nitric oxide synthase, both of which are induced in sepsis [23,24]. In addition, in a study involving septic abdominal surgery patients, treatment with 3 intravenous doses of 450 mg/d of vitamin C resulted in the reduction of caspase-3 and poly(ADP-ribose) polymerase levels and the increment of Bcl-2 levels, all of which points to an antiapoptotic effect on peripheral blood neutrophils [25]. This effect could be relevant in light of evidence showing a possible role of ROS in neutrophil apoptosis and of apoptosis in MODS development. In an animal model of sepsis, supplementation with vitamin E reduced monocyte-macrophage cytokine production in response to endotoxin. Furthermore, the association of vitamin E to simvastatin has been shown to reduce $O2^{\cdot -}$ levels in septic patients through Nox inactivation [26]. Other studies in animals show a possible protective effect of vitamins C and E in sepsis-induced hepatic dysfunction, as shown by a reduction in hepatic vascular stress genes and a prevention of cytochrome P450 dysfunction following vitamin E and vitamins C to E supplementation, respectively [27]. Myocardial damage can also be reduced through antioxidant supplementation, as showed by the attenuation of sepsis-related myocardial contractile dysfunction in rats, following the administration of vitamin C, vitamin E, vitamin A, and zinc [28]. In addition, in an animal model, supplementation with vitamin C and with vitamins A to C prevented endotoxin-induced renal oxidative tissue damage [29]. A randomized, double-blind, placebo-controlled trial studied the supplementation of 500 mg/d of vitamin C and 400 IU/d

of vitamin E through enteral feeding in critically ill patients, showing a significant reduction in mortality [30]. Accordingly, in another prospective, randomized trial, supplementation with vitamin E (1 000 IU, 3 times a day per naso- or orogastric tube) and vitamin C (1000 mg, 3 times a day, intravenously) was associated to a reduction in the occurrence of pulmonary morbidity and multiple organ failure, also reducing the duration of mechanical ventilation and length of ICU stay [31].

4.2. Selenium

Selenium is an essential micronutrient of special relevance in redox homeostasis. It has antioxidant properties mainly due to the action of ROS-degrading selenoenzymes, containing selenocysteine in their catalytic center. Selenoenzymes include the GSH-Pxs and thioredoxin reductases families, capable of degrading hydroperoxides. In contrast, selenocompounds have been described to have a direct prooxidant effect that could contribute to the toxicity observed at high doses.

Increasing interest in selenium supplementation to critical patients has been supported on the basis of its antioxidant properties. In critically ill surgical patients, lower plasma selenium concentrations have been found to correlate with more tissue damage, the presence of infection, organ dysfunction/failure, and increased ICU mortality. In a prospective, randomized, placebo-controlled, multiple-center trial, Angtwurm et al [32] studied the effect of the supplementation with sodium-selenite at a dose of 1000 μg initially, followed by a continuous infusion of 1000 μg/d for 14 days. An increment of GSH-Px-3 activity and a decrease in mortality rate in supplemented patients were observed. Furthermore, they noted a direct correlation between selenium concentrations and survival rate. However, in another prospective, multicenter, placebo-controlled, randomized, double-blind study, supplementation with sodium selenite at a dose of 4000 μg initially, followed by a continuous infusion of 1000 μg/d for 9 days, showed no improvement in the outcome of septic shock patients [33]. These contradictory results could be a consequence of a different timing in the initiation of supplementation, heterogeneity of patient groups, or the result of a different initial dose, through prooxidant effects or NF-κB modulation.

4.3. Melatonin

Melatonin has also been proven to have broad antioxidant properties as a radical scavenger. In animal models of sepsis, administration of melatonin has been shown to reduce circulatory failure, lung injury, and intestinal apoptotic damage, also improving survival [34]. Melatonin supplementation reduces postoperative oxidative stress in newborns. Furthermore, Gitto et al [35] studied the effect of melatonin administration in septic newborns, showing a reduction in oxidative stress parameters and a possible improvement in survival; but these conclusions are limited by the small number of enrolled patients. Finally, experimental studies have shown that melatonin ameliorates respiratory syncytial virus-induced disease and lung inflammatory injury in mice via inhibition of oxidative stress and proinflammatory cytokine production and may constitute a novel therapeutic agent in virus-induced pulmonary infection [36].

4.4. Other antioxidant-based therapies

There are a number of other antioxidant-based therapies that have shown promising results regarding a possible beneficial effect in sepsis. Among them, glutamine supplementation improves clinical outcome in critically ill patients [37], likely including in its mechanisms the antioxidant effect conferred by being a precursor of GSH. *N*-acetylcysteine has antioxidant properties through increase of GSH reserves and direct antioxidant activity through its thiol group. Supplementation with *N*-acetylcysteine has been studied in acute respiratory distress syndrome with conflicting results. There is evidence supporting a potentially beneficial effect of early administration of *N*-acetylcysteine in sepsis-related reduction of regional blood flow. On another hand, the treatment with *N*-acetylcysteine plus deferoxamine in rats has been associated with a reduction of macrophage cytokine release and mitochondrial dysfunction, thereby improving survival [38]. Statins appear to be beneficial in sepsis through restoration of microvascular tone and maintenance of microvascular integrity, an effect mediated by the increment in NO concentrations in vascular endothelium, this way reversing endothelial dysfunction [39]. Finally, polyphenols, a group of chemicals abundant in plant-based food and beverages, can exert an antioxidant effect through the promotion of some antioxidant enzymes expression. Polyphenols are thought to be a possible therapeutic resource against sepsis because of their known protective and anti-inflammatory effects in animal models of sepsis [40].

4.5. Mitochondria as a therapeutic target

Mitochondrial dysfunction has been proposed as a key early cellular event in critical illness. Taking into account the major role of mitochondrial dysfunction in the pathophysiology of sepsis-derived MODS, attention should be paid to the antioxidant therapies targeted to mitochondria. Accordingly, it was reported that antioxidant treatment reverses mitochondrial dysfunction in a sepsis animal model [41]. Moreover, restoration of mitochondrial bioenergetics was associated with improvements in hemodynamic parameters, organ function, and overall survival [14]. Endogenous antioxidants, such as reduced glutathione, superoxide dismutase, GSH-Px, among others, may counteract the

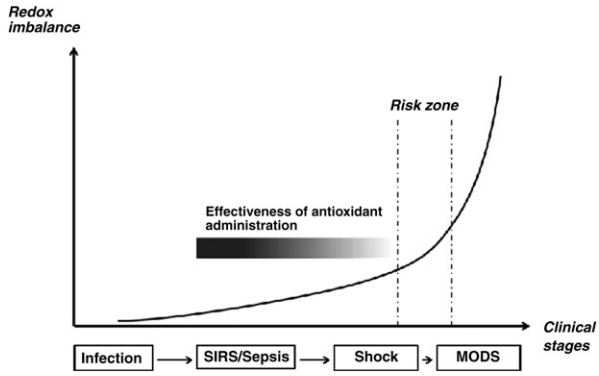

Fig. 2 Representative diagram of the hypothetical relationship between redox imbalance and clinical progression toward MODS. The vertical dotted lines represent the risk zone. Likely, within this risk zone, there is a cut point from which oxidative stress increases exponentially, giving rise to the progression surpassing the limit for reversibility. The degrading black-to-white band represents the domain at which antioxidant intervention could be useful in preventing the progression.

effects of increased ROS production, otherwise contributing to the inflammatory or apoptotic pathways activation [10]. In addition, exogenous antioxidant administration could reinforce the antioxidant defense system, thus abrogating the oxidative damage. Therefore, it should be expected that mitochondrial therapies will have an increasingly relevant role in the management of sepsis and MODS.

5. Future perspectives

There exists a large amount of evidence suggesting a role of oxidative stress in the pathophysiology of sepsis and septic shock. The oxidative modifications occurring in sepsis are probably an important promoter of sepsis progression toward shock and MODS. Likely, a cut point exists after which oxidative stress increases exponentially, in relation to the rapidly progressive and eventually irreversible clinically observed worsening of the disease. This condition offers a unique opportunity to optimize a goal-directed medical management of critically ill pediatric patients with sepsis. The measures should be aimed to reduce comorbidities, complications or secondary disease, length of the PICU stay, mortality, and overall health care costs. Assessment of oxidative stress-related biomarkers could be useful to establish prognosis and identify patients at risk for developing a more severe course. The relationship of oxidative stress biomarkers with pediatric intensive care disease severity scores needs to be established. Moreover, in the future, oxidative stress biomarkers could eventually be included in such severity scores to improve their accuracy and outcome prediction value.

Evidence of a direct association between antioxidant capacity and outcome strongly suggests the usefulness of antioxidant supplementation in critically ill patients. Previous trials evaluating the impact of antioxidant-based therapies in adults have shown conflicting, but promising, results. In our opinion, evidence regarding the appropriate timing of supplementation, as well as the useful dose of the selected agents, is still lacking. The available evidence supports the design and development of clinical trials aiming at the early administration of antioxidant-based treatment as a novel therapy for pediatric sepsis.

Taken together, these data give support to the hypothesis that the early administration of antioxidant supplementation is useful in preventing or attenuating the progression of the pathophysiologic events occurring in pediatric sepsis, in a time-dependent manner. The greater ameliorating effect should be expected to occur in the initial phases of the disease, when all the mechanisms responsible for ROS/RNS production have not yet been well established (Fig. 2). In our opinion, vitamin C, vitamin E, and selenium are between the most promising candidates for pediatric antioxidant supplementation, based on the evidence supporting their protective role in sepsis. However, the proper dosage and administration routes still need to be determined. The validation of the correlation of oxidative stress biomarkers and intensive care severity scores could warrant the usefulness of an antioxidant therapy. To our knowledge, studies of this kind have not been conducted so far.

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