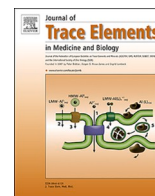




Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Delayed increase of plasma selenoproteins and absence of side effect induced by infusion of pharmacological dose of sodium selenite in septic shock: Secondary analysis of a multicenter, randomized controlled trial[☆]

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ARTICLE INFO

Keywords:

Sepsis
Sodium Selenite
Selenoprotein P
Glutathione peroxidase
Organ failure

ABSTRACT

Background: In sepsis, neutrophil respiratory bursts participate in endothelium damage, the first step to multiple organ failure. In plasma two antioxidant selenoenzymes, which protect the endothelium, decrease: selenoprotein-P, and to a lesser extent glutathione peroxidase (GPX3). Sodium selenite (Na_2SeO_3) is a Se donor, but also an oxidant chemotherapy drug depending on its concentration. In a previous published study, Na_2SeO_3 continuous infusion in septic shock patients at a pharmacological dose of 4 mg¹ Se/day on day-1, followed by a high nutritional dose of 1 mg Se/day during 9 days, showed no beneficial effect on weaning of catecholamine nor on survival. In this ancillary study, we report clinical and biological effects of such continuous infusion of Na_2SeO_3 . **Methods:** This was a multicenter, placebo-controlled, double-blind study on 60 patients. Na_2SeO_3 or placebo in continuous infusion as described above. Evolution with time of plasma Se, selenoprotein-P, GPX3, Organ dysfunction (sequential organ failure assessment SOFA scores, including PaO₂/FiO₂, for respiratory failure, and plasma lactate) and quality of life at 6 months (by SF36 scores) were analyzed using two-way (time, treatment) non-parametric repeated-measures analysis of variance (Friedman test).

Main Results: At baseline, plasma Se was about a quarter of reference values. From baseline to day-4 plasma Se, selenoprotein-P and GPX3 significantly increased by 3.9, 2.7 and 1.8 respectively in the Na_2SeO_3 group as compared with placebo and remained elevated by 2.3, 2.7 and 2.1 at day-14 respectively ($p < 0.001$). Na_2SeO_3 did not affect global and organ by organ SOFA Scores and plasma lactate concentration at day-1 and later up to day-14. The evolution of PaO₂/FiO₂ until day-14 was similar in the two groups. Quality of life in the surviving patients at 6 months was similar between the two groups.

Abbreviations: Sodium selenite, NaSe; Selenoprotein P, SELENOP, SelP; Glutathione peroxidase 3, GPX3; Organ Failure, OF.

[☆] Name of the registry: Centre Hospitalier de Meaux. Trial registration: NCT00207844. URL of the trial registry record: www.ch-meaux.fr

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¹ The administrated dose was 4 mg Se/day the first day and 1 mg Se/day the days after.

<https://doi.org/10.1016/j.jtemb.2022.127031>

Received 30 January 2022; Received in revised form 14 June 2022; Accepted 24 June 2022

Available online 27 June 2022

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Conclusion: Continuous infusion of Na_2SeO_3 at 4 mg Se at day-1 seems to have neither beneficial nor toxic effect at day-1 or later and induces a late increase of selenoprotein-P at day-4. Preclinical studies are required to confirm the use of Na_2SeO_3 as a cytotoxic drug against neutrophils and protection of the endothelium by selenoprotein-P.

1. Introduction

Septic shock is a major public health issue characterized by multiple organ failure (MOF), high mortality rate in intensive care [1–3], and frequent reduced long-term quality of life [2,4]. It is the first cause of mortality in hospitals, and one of the main causes of death worldwide, even outside of a pandemic, as with COVID 19, with which severe forms correspond to a sepsis state [5–8]. It remains to date without specific treatment, at the exception of low dose steroids [9,10]. At the early phase of sepsis, endothelial dysfunction is a major event [2,11,12]. This dysfunction is notably related to the neutrophil respiratory bursts [12–16]. The selenium (Se) is required for the vital antioxidant selenoenzymes under the form of selenocystein aminoacid [17,18]. With about 0.3 mg Se, plasma Se represents only 2 % of body Se content, which is 10–15 mg Se in a Se-repleted person [19,20]. The antioxidant selenoprotein-P accounts for about 60 % of plasma Se, and glutathione peroxidase (GPX3) for about 30 %. The rest of the plasma Se is incorporated into proteins under the non-biologically active form selenomethionine [19, 21–24]. The plasma selenoproteins are a critical point in sepsis between activated leukocytes, including neutrophils and endothelium [19,23]. Like many small selenocompounds, sodium selenite (Na_2SeO_3) - despite being a good Se provider at low concentration - is oxidant and cytotoxic above a micromolar threshold [25]. Na_2SeO_3 has been studied for a long time as a chemotherapeutic agent as the more the cells are activated the lower the cytotoxicity threshold [26–30].

At the acute phase of sepsis, there is an early and major decrease in plasma Se and selenoprotein-P concentrations in mammal models and in patients [24, 31–36]. This has been the basis the hypotheses of: (i) a selenium deficiency at the acute phase of sepsis and (ii) a need to increase selenium supply in sepsis patients. However such hypotheses were not based on preclinical data [35–40]. On the contrary several elements or experiments are against such hypotheses: (i) there is no increased losses of selenium atom during sepsis [41]; (ii) there is an increased protein catabolism at the acute phase of sepsis that should release selenium incorporated into selenomethionine and selenocystein [36]; and (iii) recent experimental data have confirmed a major multi-step down-regulation of selenoprotein-P synthesis and hepatic release at the acute phase of sepsis [36]. This is likely to be the major factor for the decrease of selenoprotein-P plasma concentration together with an increased binding to endothelium at the acute phase of sepsis [36]. In our previous SÉRÉNITÉ study the first day Na_2SeO_3 infusion, corresponding to 4 mg Se, was performed with the goal of reaching the cytotoxicity threshold for hyperactivated neutrophils [41], contrary to most sepsis studies performed on the hypothesis of a Se deficiency, requiring Se supplementation for selenoenzyme synthesis [37–40]. Our Na_2SeO_3 first day dose was based on preclinical data, reporting this dose as the maximum safe single oral dose for cancer research [42] and administrated by continuous infusion in the absence of preclinical studies allowing a bolus injection. Performed in patients with a 70 % expected mortality rate at 6 months, our study should be considered as a fast-track study similar to drug studies performed in advanced-stage cancer patients with unmet medical needs. This first day infusion was followed by 1 mg Se/day as Na_2SeO_3 during 9 days. We observed no effect on the weaning of catecholamine and on survival [43]. Some authors suggested that this first day dose toxicity might have overwhelmed the beneficial effect of Se supplementation by increasing the incidence of MOF and especially respiratory failure due to Na_2SeO_3 toxicity [44,45].

To further investigate the clinical and biological effects of this

Na_2SeO_3 infusion on oxidation and plasma selenoproteins, we performed a secondary analysis of the SÉRÉNITÉ study. Our aim was to assess if this Na_2SeO_3 infusion had an impact on plasma selenoproteins, and especially on selenoprotein-P, and if it had any clinical side effect during the early 24 h of pharmacological continuous dose infusion [43].

2. Methods

The SÉRÉNITÉ trial (high doses of selenium, as sodium selenite, in septic shock, NCT00207844) was a multicenter, placebo-controlled, randomized, double-blind study performed in 60 patients with documented septic shock. Main inclusion criteria were: severe community or nosocomial infection, need for mechanical ventilation, septic shock with at least one hour of 0.2 $\mu\text{g}/\text{kg}/\text{min}$ norepinephrine after fluid loading of at least 1.000 ml, Simplified Acute Physiologic Score II (SAPS II score) of 25 or more and written informed consent. Patients with limitation of care, previous circulatory failure, or with urinary infection or peritonitis related to dialysis or trauma, and pregnant women were excluded. Modalities of selenite infusion as well as main efficacy and safety endpoints have been described elsewhere [43].

The sample size was based on the assumption of 60 % of patients would be free of catecholamine at day 10 (end of the study treatment) the sample size would have allowed the detection of an absolute increase of 25 % of this percentage of patients with a type I error of 5 % and a power of 80 %. Analyses were performed according to the intention to treat principle. Time to vasopressor therapy withdrawal was analyzed using cumulative event curves constructed by the Kaplan–Meier method and the effect of treatment using the log-rank test and similarly for mortality. Otherwise, comparisons between groups were performed using the Student t test or Wilcoxon rank sum test as appropriate for continuous variables, and using the chi-square test, the Fisher exact test or the Cochran–Mantel–Haenzel test as appropriate for categorical variables. All reported p are two sided and $p < 0.05$ was considered significant [43].

Patients were randomly assigned in a 1:1 manner to receive either sodium selenite or matching placebo for 10 days. Treatments (Laboratoires Aguettant, Lyon, France) were conditioned in ampoules containing 1 mg selenium as sodium selenite diluted in 48 ml saline and were administered intravenously by continuous infusion (2 ml/hour) at the following

doses, expressed in selenium content: 4000 μg on the first day and 1000 $\mu\text{g}/\text{day}$ on the nine following days. Randomization was stratified on each centre by blocks of four. All patients, medical and nursing staff, and pharmacists remained blinded throughout the study period [43].

2.1. Organ dysfunctions

Organ dysfunctions were assessed through the computation of global and of specific organ Sequential Organ Failure Assessment (SOFA) scores [46,47]. Lactate concentrations were also measured. Quality of life at 6 months was evaluated using the French Short Form (36) Health Survey (SF-36) [48].

2.2. Supportive care

Cardio-vascular: cardiac pressures and indexes (measured using a Swan-Ganz catheter), fluid loading requirements and occurrence of arrhythmias were recorded.

Pulmonary: partial pressure arterial oxygen and fraction of inspired

oxygen ratio (PaO₂/FiO₂), partial pressure of carbon dioxide (PCO₂), level of positive end-expiratory pressure (PEEP), use of nitric oxide (NO) or of prone position, and duration of mechanical ventilation (MV) were recorded.

Renal: number of patients requiring renal replacement therapy (RRT), duration of RRT and number of RRT free days were recorded.

2.3. Nosocomial infections

The occurrence of the following nosocomial infections were recorded: nosocomial pneumonia during mechanical ventilation by using broncho-alveolar lavage and protected specimen brush [49,50], post-operative wound infection [51], catheter infection using Brun-Buisson technique [52], and bacteremia using blood cultures. The number of infections per patient was also recorded.

2.4. Plasma Se and plasma selenoproteins

Plasma Se concentrations were determined using graphite furnace atomic absorption spectrometry (GFAAS) using Unicam 989 QZ Solaar apparatus and the values were expressed as nanogram per milliliter [53]. The method was validated using SeronormTM lyophilized human reference serum samples (Nycomed Pharma AS, Oslo, Norway, batch No. 605113) and through participation in an inter-laboratory quality assurance program. The mean serum Se level of reference was 78.0 µg/L while that obtained in our laboratory was 77.4 ± 5.0 µg/L.

Plasma selenoprotein-P concentration was determined by sandwich Elisa procedure. Ninety six well microplates Maxisorp (Nunc) were coated overnight with anti-human selenoprotein-P monoclonal antibody (Anti-selenoprotein P 37A1; AbFRONTIER (Korea), cat. no. LF-MA0141) at room temperature, and were further incubated at 37 °C with plasma samples and with biotinylated anti-human selenoprotein-P antibody. Color reaction development was achieved with alkaline phosphatase conjugated with streptavidin and p-nitrophenylphosphate (pNPP) as the substrate. The reaction was stopped with 0.1 M Ethylenediaminetetraacetic acid (EDTA) and absorbance measured on a micro-plate reader at wave length $\lambda = 405$ nm.

Plasma samples were diluted 1:1000–1:2000 with PBS-Tween buffer before analysis. Pooled human plasma was used as standard. Selenoprotein-P concentrations were expressed as arbitrary unit/ml. Plasma selenoprotein-P concentration was calculated using exponential standard curve.

GPX3 activities were assayed by the coupled method of Paglia and Valentine with t-butyl hydroperoxide as the substrate [54]. The reaction was carried out at 25 °C in a spectrophotometer fitted with constant-temperature cell housing. The method based on a nicotinamide adenine dinucleotide phosphate (NADPH)-coupled reaction whereby oxidized glutathione produced by GPX3 and exogenous t-butyl hydroperoxide was reduced by exogenous glutathione reductase and NADPH. Enzymatic activities were expressed as units per ml of plasma. One unit of enzyme was defined as 1 µmol NADPH oxidized per minute per ml of plasma. The intra-assay coefficient of variation for both materials (six to eight analyses) was below 3 %.

2.5. Biological variables

Plasma lactate and C-reactive protein concentration, aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities were measured using standard biochemical methods.

2.5.1. Inflammation

Circulating plasma interleukin-6 (IL-6) and vascular-endothelial-growth-factor (VEGF) levels were measured by enzyme-linked immunosorbent assays (ELISA) according to manufacturer's recommendations (IL-6 and VEGF Duoset kit R&D systems, Abingdon, UK). Detection thresholds were 3 pg/ml and 5 pg/ml for IL-6 and VEGF, respectively.

Circulating plasma procalcitonin (PCT) levels were measured using time-resolved amplified cryptate-emission technology (Kryptor[®] procalcitonin; Brahms AG, Hennigsdorf, Germany) with an assay sensitivity of 0.06 ng/ml.

2.5.2. Lipid peroxidation

Lipid peroxidation was assessed according to the method proposed by Yagi [55], by adding 4.0 ml of sulfuric acid (N/12) and 500 µL of phosphotungstic acid (10 %) to 100 µL of plasma. After 5 min, the tubes were centrifuged at 1600 g for 10 min, and the supernatant discarded. The residue was suspended in sulfuric acid (2.0 ml) and phosphotungstic acid (300 µL) for 10 min. The tubes were centrifuged at 1600 g for 10 min, and the supernatant discarded again. The residue was weighed and dissolved in 4.0 ml of water and 1.0 ml of a thiobarbituric acid reagent in acetic acid (335 mg in 50.0 ml of water diluted 50:50 in acetic acid 99 %). The tubes were incubated for 1 h at 95 °C. The solutions were cooled and the product of the reaction was extracted by 5.0 ml of n-butanol. The fluorescence of the organic layer was measured at 553 nm with an excitation at 515 nm. The concentration of the total lipid peroxide (expressed in nanomoles per milligram of residue) was calculated with the slope obtained from a gradient concentration of tetramethoxypropane pure standard diluted in 4.0 ml of water and 1.0 ml of the thiobarbituric acid reagent.

2.6. Statistical analysis

The statistical analysis was performed using Statistical Analysis System (SAS) statistical software V9.3 (SAS Institute, Cary, NC). Variables are expressed as mean ± standard deviation unless otherwise noted. All available data were included in the statistical analysis. Qualitative variables were compared between the two groups using the Chi square or Fisher Exact test if necessary and quantitative variables were compared using the Student t test or Wilcoxon rank sum test if necessary. The evolution with time of quantitative variables was analyzed using two-way (time, treatment) non-parametric repeated-measures analysis of variance (Friedman test). In case of significant group effect, time-by-time comparisons were performed by use of the least-squares means procedure with a Bonferroni correction to adjust for multiple comparisons. In time by time comparisons, reported effects are calculated from median values in placebo and Na₂SeO₃ groups. For all analyses, a p value < 0.05 was considered significant. All reported p values are two-sided.

3. Results

3.1. Organ dysfunctions and supportive care

Global SOFA score (Fig. 3a) and plasma lactate concentration (Fig. 3b) significantly decreased with time (p < 0.001 and p = 0.026, respectively) but without significant difference between the two groups (p = 0.625 and p = 0.485, respectively). The evolution with time of PaO₂/FiO₂, AST and ALT until day-14 was similar in the two groups (p = 0.845, p = 0.831, and p = 0.961, respectively see electronic [Supplementary material, Fig. S2a, S2b, and S2c](#)).

There was no difference between the Na₂SeO₃ and placebo groups in terms of number of patients requiring renal replacement therapy (13/31 vs. 16/29, respectively, p = 0.545), number of days free of renal replacement therapy (37 ± 55 vs. 26 ± 49, respectively, p = 0.303), and duration of ventilation (34 ± 54 vs. 25 ± 43 days, respectively, p = 0.762).

The quality of life at 6 months was evaluated in 6/10 and 8/12 of the surviving patients in the two groups. Global, physical and behavioral SF-36 scores were similar between the two groups (p = 0.846, 0.953 and 0.905, respectively, data not shown).

3.2. Acute tolerance of Na₂SeO₃ on specific organ dysfunctions

There was no deleterious effect of Na₂SeO₃ on cardiac and respiratory functions as assessed by the SOFA cardiologic and respiratory scores especially at day-1 (see Table S1). In the same way, there was no hepatotoxicity in the Na₂SeO₃ group (as assessed by AST and ALT, see electronic [Supplementary material S2b and S2c](#)).

3.3. Nosocomial infections

The occurrence of the first nosocomial infection was delayed in the Na₂SeO₃ group as compared to the placebo group (34 ± 28 vs. 18 ± 24

days, respectively, p < 0.001). Mean time before bacteremia, first catheter and surgical wound infection was delayed in the Na₂SeO₃ treated group (p < 0.05), but not the first VAP (p = 0.27). However, the number of infections was similar in the two groups (1.8 ± 3.9 vs. 2.1 ± 3.7 infections per patient (p = 0.924)).

3.4. Plasma selenium and selenoproteins

There were significant treatment effects on plasma Se concentrations (Fig. 1a, p < 0.001), plasma selenoprotein-P concentrations (Fig. 1b, p < 0.001), and GPX3 activity (Fig. 1c, p < 0.001). Na₂SeO₃ infusion significantly increased plasma Se and selenoprotein-P concentrations,

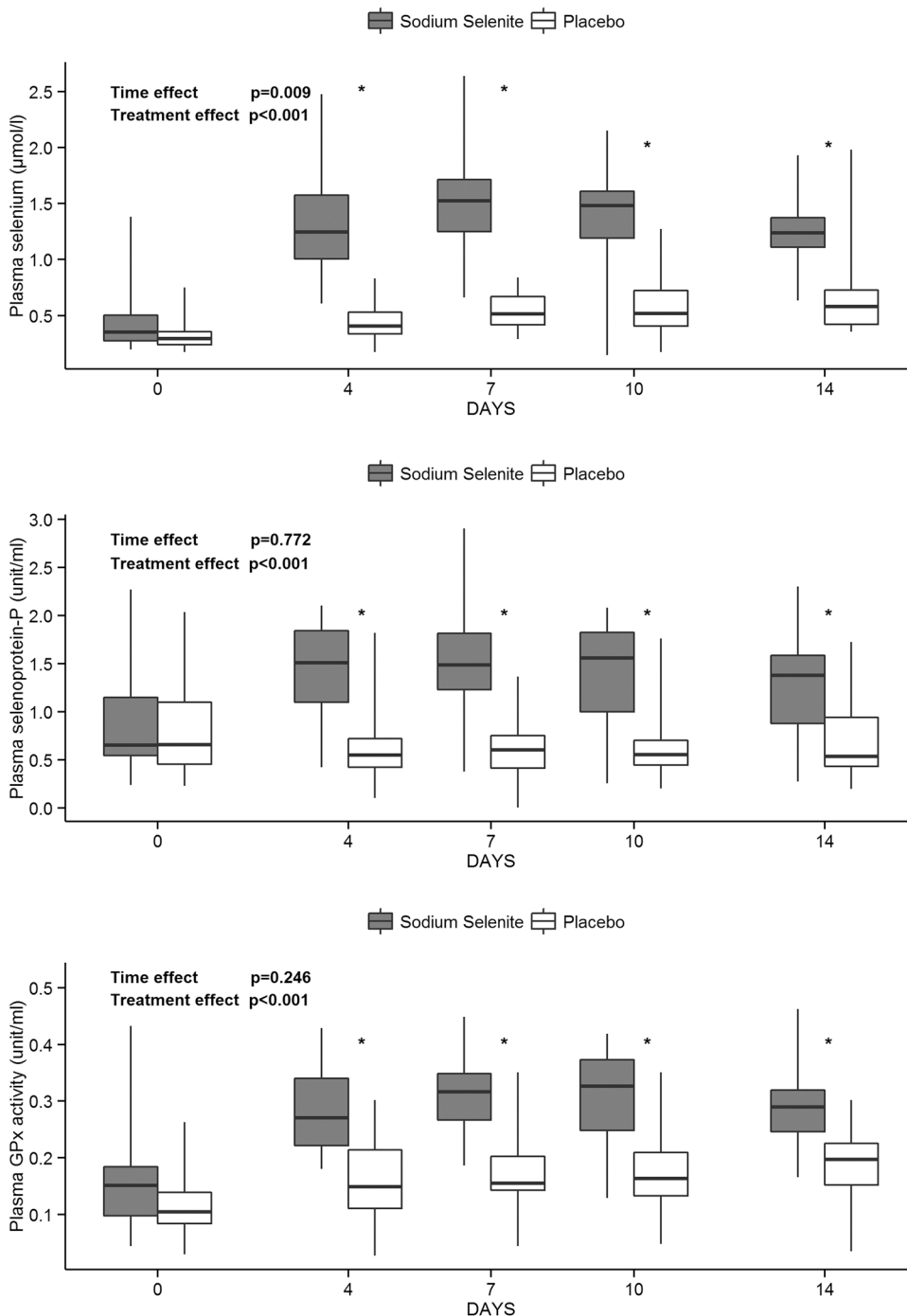


Fig. 1. Evolution of plasma selenium (1a), selenoprotein-P (1b), and Glutathione Peroxidase activity (1c). Plasma GPx: Plasma Glutathione Peroxidase (or GPX3). Data are presented as box plots. The lower and higher boundaries of the box indicate the 25th and 75th percentile. The line within the box marks the median, and error bars below and above the box respectively indicate Q1-1.5 × Interquartile and Q1 + 1.5 × Interquartile. Reference values for plasma selenium concentration in the French area are 1.00 ± 0.15 µmol/L. *, p < 0.05 vs placebo.

and GPX3 activity from day-4 to day-14 as compared to placebo ($\times 3.9$ on day-4 and $\times 2.3$ on day-14 for Se, $\times 2.7$ on day-4 and $\times 2.6$ on day-14 for selenoprotein-P, and $\times 1.8$ on day-4 and $\times 2.1$ on day-14 for GPX3 activity). Mean plasma selenium concentrations were above the reference value from day-4 to day-14 (i.e. 4 days after the end of Na_2SeO_3 administration).

3.5. Inflammation and lipid peroxidation

Plasma procalcitonin concentration significantly decreased with time ($p < 0.001$) but without significant difference between the two groups (Fig. 2a, $p = 0.869$). The evolution with time of IL6, and VEGF until day-14 was similar in the two groups ($p = 0.769$ and $p = 0.398$, respectively, see electronic Supplementary material, Fig. S1a and S1b). Similarly, there was no difference on lipid peroxidation at any time point (Fig. 2b, $p = 0.110$) Fig. 3.

4. Discussion

The additional clinical and biological data of the SÉRÉNITÉ study showed no evidence for clinical or biological effects in severe septic shock patients of continuous infusion of Na_2SeO_3 at a dose corresponding to 4 mg Se the first day followed by 1 mg/day during 9 days. There was in particular no argument in favor of pulmonary toxicity of Na_2SeO_3 as shown by the similar evolution of $\text{PaO}_2/\text{FiO}_2$ ratio, especially from day1 to day3, and duration of ventilation. SOFA score, cardiac and respiratory SOFA scores, and plasma lactate also have a similar evolution. Cardio-respiratory distress is a critical point of acute Na_2SeO_3

toxicity, which is concentration and dose dependent [30,56]. There is therefore no argument in favor of a toxic effect of continuous infusion of Na_2SeO_3 at a dose corresponding to 4 mg Se the first day.

As constantly seen in septic shock patients, we observed a profound decrease of plasma Se concentration at inclusion, which is between half to three-quarter of the reference value [12,24,32,33,36,39]. In contrast with the absence of clinical effect, we observed a marked increase of plasma Se selenoprotein-P concentrations as well as GPX3 activity with Na_2SeO_3 administration until day-4 that seemed to have no impact on plasma inflammatory markers and lipid peroxidation. This increase at day4 of plasma Se and selenoenzymes is in accordance with the reported increase in GPX3 activity at day3 after Na_2SeO_3 administration at the dose of 2 mg Se in 1-hour bolus followed by 1.5 mg Se/day continuously during 14 days [57]. No clinical effect (neither positive, nor toxic) was reported in this study, which was based on the hypothesis of Se deficiency [57]. Similarly, in the phase III study of Bloss et al., based on a similar hypothesis, a marked increase of plasma Se concentration was observed in the treated group at Day4 and after [39].

Plasma procalcitonin (PCT) concentration - a precursor hormone of calcitonin - decreases according to recovery of bacterial sepsis [58]. We recorded increased interleukin 6 (IL6) concentrations at D0, - an important marker of cytokine driven inflammation but with huge individual variations as observed in our study. Mean IL6 concentration is known to be associated with the severity and progression of sepsis, leading to COVID-19 treatment proposal [2, 59–61]. The analogous decrease of PCT and IL-6 between groups observed in our study is in accordance with an absence of effect of the selenite infusion despite septic shock being characterized by an acute oxidative stress [12],

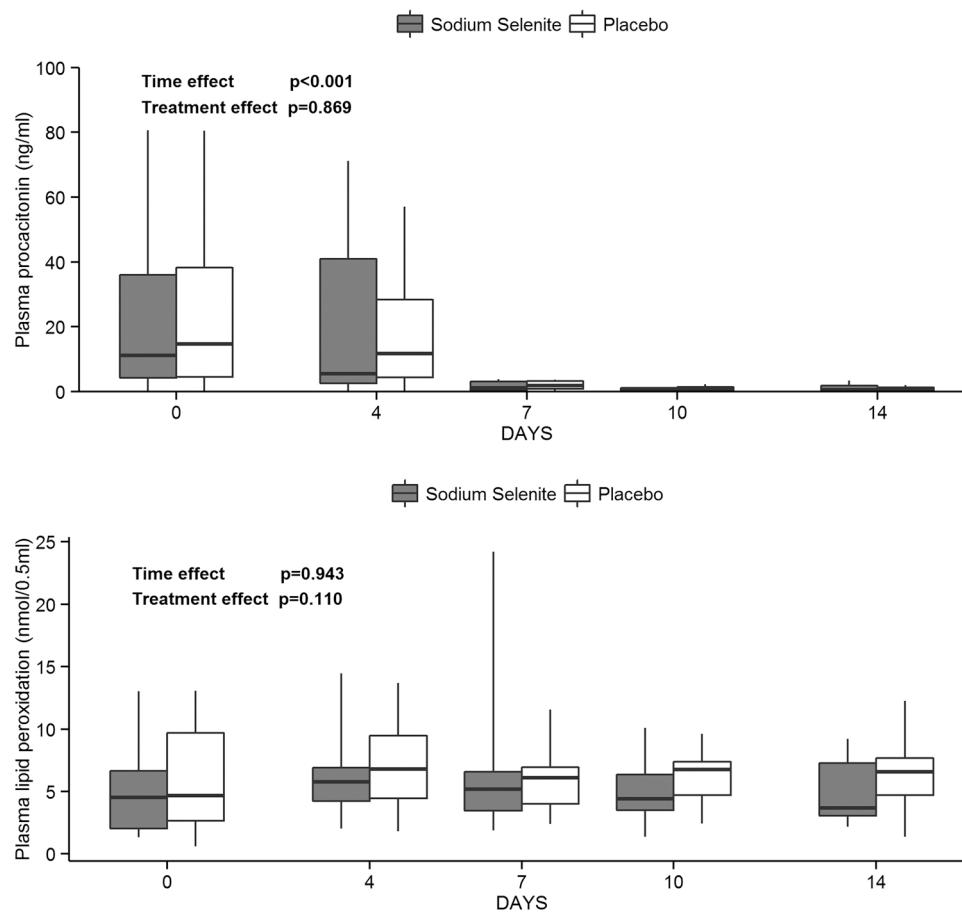


Fig. 2. Evolution of plasma procalcitonin (2a) and lipid peroxidation (2b). Data are presented as box plots. The lower and higher boundaries of the box indicate the 25th and 75th percentile. The line within the box marks the median, and error bars below and above the box respectively indicate $Q1 - 1.5 \times \text{Interquartile}$ and $Q1 + 1.5 \times \text{Interquartile}$.

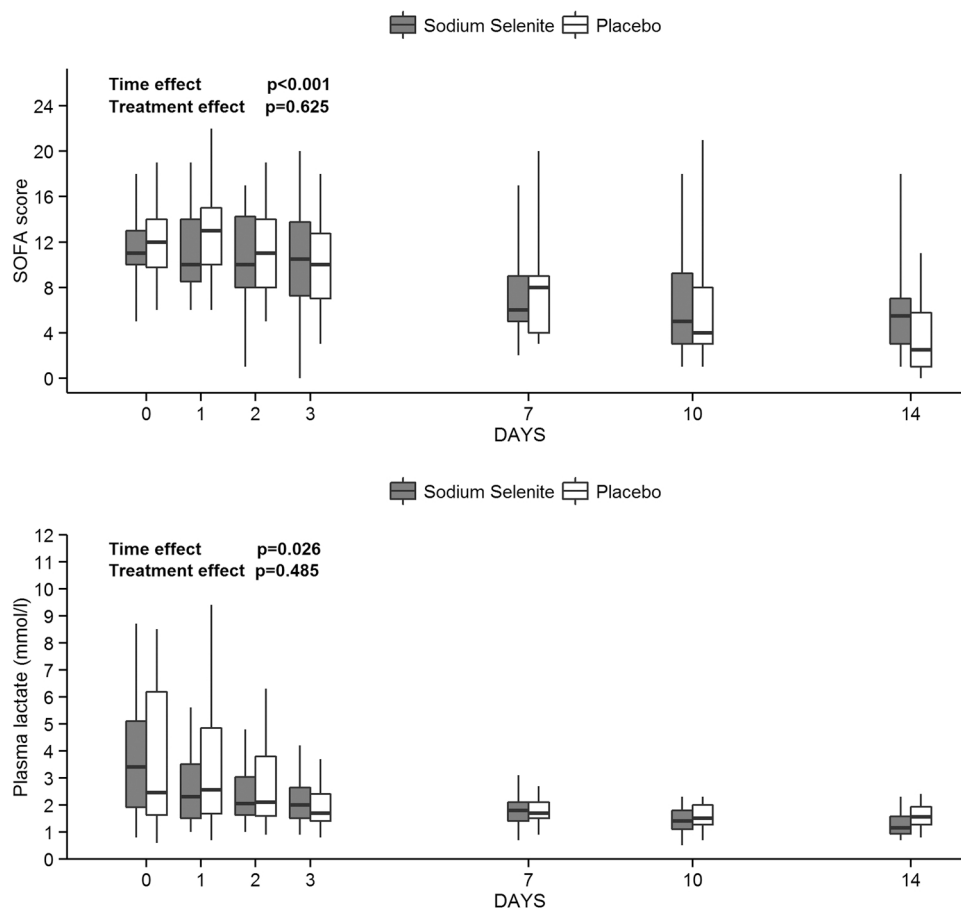


Fig. 3. Evolution of SOFA score (3a) and plasma lactate (3b). SOFA: Sequential Organ Failure Assessment. Data are presented as box plots. The lower and higher boundaries of the box indicate the 25th and 75th percentile. The line within the box marks the median, and error bars below and above the box respectively indicate $Q1-1.5 \times$ Interquartile and $Q1 + 1.5 \times$ Interquartile.

plasma lipid peroxidation has seldom been studied in sepsis patients [62–64]. An increased plasma lipid peroxidation was not observed in either group. A marked increase in thiobarbituric acid reactive substance (TBARS) has been previously described, especially measured in erythrocytes, which was not examined in our study [64]. Please, go to the line before in order to understand the impact of our continuous infusion of Na_2SeO_3 the first day in 2009 we performed a similar continuous infusion in a resuscitated peritonitis sheep model (4 $\mu\text{g}/\text{kg}\cdot\text{h}$ Se as Na_2SeO_3 ; e.g. Se 2.4 mg/24 h in these 25 kg body weight animals) with sequential measurements of plasma Se concentration [31]. This continuous infusion of Na_2SeO_3 was performed 9 h after the induction of the peritonitis by injection of autologous feces. It was performed one hour before the onset of septic shock in this resuscitated animal model. These intubated and ventilated sheep received fluid loading based on the data of arterial pressure and swan ganz catheter (including central venous pressure) with an algorithm similar to that of ICU patients [31]. At baseline, sheep have slightly lower plasma Se concentrations than humans. Plasma Se decreased rapidly after the onset of peritonitis and at H9 was at about a quarter of its baseline value [31]. Despite the Na_2SeO_3 dose above our given dose in septic shock patients, continuous infusion of Na_2SeO_3 was unable to increase the plasma Se concentration above 1 $\mu\text{mol}/\text{L}$ [31]. The obtained plasma Se concentration by continuous infusion of Na_2SeO_3 remained thus below the oxidant cytotoxic concentration threshold for Na_2SeO_3 , contrary to our expectation. The Na_2SeO_3 cytotoxicity threshold is above 1–5 $\mu\text{mol}/\text{L}$ as Se depending on cell activity. This cytotoxicity threshold is all the more lower when the cells are activated and detached [25–29]. The continuous infusion peritonitis sheep group had a similar evolution to that of the control

group in terms of microcirculatory perfusion, cardiac function, blood lactate concentration and survival time. This is in accordance with the lack of any benefits or side effect of our therapeutic intervention.

The brain seems to have a specific sensitivity to oxidative stress as shown by the susceptibility to damage of the glycocalyx of the hippocampal region in sepsis, alteration of the blood-brain barrier, and the neuronal dysfunction due to excessive microglial activation leading to long term neurological sequelae [2,65,66]. It is also one of the privileged organs for intracellular antioxidant selenoenzymes, with a specific cycle of selenoprotein-P from the astrocytes to the neurons especially in cortical, striatum as well as hippocampal regions [18,19,21,67]. Nevertheless, in our study no beneficial effect was observed in long-term brain sequelae in surviving patients. This result represents an absence of early selenoenzyme induction in brain, especially brain selenoprotein-P, during the acute phase of sepsis despite a massive Se administration. Such induction might have protected the neurons before their damage.

Decrease of plasma Se has been proposed as a protective mechanism against pathogens, as most of them require Se for growth and antioxidant defense [36, 68–70]. In the treated patient group, nosocomial infections did not increase; on the contrary, the first nosocomial infection was delayed. This could be related to a slight improvement of immunodepression following septic shock, or a cytotoxic effect of Na_2SeO_3 on pathogens.

The absence of beneficial effect of the selenite infusion on any clinical and biological parameter despite the 4 mg Se intake in the first day goes against the hypothesis of a relative Se deficiency. This hypothesis is debated at the early phase of sepsis due to the low plasma Se and selenoprotein-P concentration observed at this phase, such as observed

in our study at baseline and other studies [32,33,37]. According to such hypothesis, a large intake of Se is required at the early phase of sepsis for restoring antioxidant defense especially selenoprotein-P concentration [37,40]. As indicated in the introduction, numerous elements oppose this hypothesis [71]. One can cite: (i) the absence of extra Se losses in sepsis [32]; (ii) the increased protein catabolism at acute phase of sepsis increasing the Se availability [19, 36, 71–73]; (iii) the content of only 2 % of body Se within the plasmatic compartment [19,20,71]; (iv) the rapid decrease of plasma Se in multiple animal models after lipopolysaccharide injection [19,31,34,71], associated with a major and multi-step downregulation of hepatic selenoprotein-P synthesis and excretion [34,71,74]. In the peritonitis sheep model previously cited numerous blood sampling could be performed during the first hours after the peritonitis induction and the initiation of selenite infusion or injection [31]. In the continuous infusion peritonitis sheep group with no beneficial effect, the plasma Se concentration remained below the baseline value despite a similar Se administration of the one provided the first day in our treated group (slightly above our given dose in septic shock patients), which is against an early induction of selenoprotein-P synthesis [31]. Together with the negative phase III study of Bloos [39], there seems to be no interest in using Na₂SeO₃ in septic shock patients as the Se donor for selenoenzyme induction, including selenoprotein-P. But this did not rule out the interest of Na₂SeO₃ as a cytotoxic drug against hyperactivated leukocytes that may need rapid IV injection, nor the interest of selenoprotein-P administration. Selenoprotein-P is known to protect the endothelium against oxidative stress through peroxynitrite (ONOO⁻) detoxification [75,76]. It might also protect endothelium in sepsis (submitted data) and may thus increase the Na₂SeO₃ margin of safety. However, in order to protect the endothelium, an early administration of a recombinant selenoprotein-P seems to be required before endothelium damage occurs [77,78].

In order to achieve blood cytotoxic concentration, from 4 to 14 μM/L Se under the form of Na₂SeO₃ a bolus injection of Na₂SeO₃ at a dose corresponding to 2 mg Se was required in peritonitis sheep weighing between 23 and 28 kg. This pharmacologic administration of Na₂SeO₃ leads to transient beneficial effects from lactate to micro-, macro-circulation and survival time [31]. Such 4–14 μM/L Se concentrations are above the cytotoxic threshold of Na₂SeO₃ in in-vitro cancer studies [26–29, 31, 36]. However, its effect is limited in time (few hours) [31]. The general toxicity is a threat. The toxicity is characterized by cardio-respiratory distress symptoms similar to those of sepsis [30,56]. In addition Na₂SeO₃ cytotoxicity seems increased in sepsis as observed in a rat lipopolysaccharide (LPS) model. The minimum lethal doses were between 0.3 and 0.6 Se mg/kg as Na₂SeO₃ in LPS rats, compared to 3 mg/kg Se as Na₂SeO₃ in healthy rats [30,56]. Our present study illustrates that pharmacological use of Na₂SeO₃ cytotoxicity against hyperactivated neutrophils requires preclinical drug development to determine the adapted dose and delivery (e.g. rapid intravenous injection vs. continuous infusion) for an efficient and safe treatment.

On one hand, selenoprotein metabolisms in sepsis may be further explored by studies focusing on genomic and proteomic expression in circulating cells of broad panel proteins including selenoproteins - under selenocompound administration at nutritional or pharmacological ranges - such has been done in sepsis patients for other purpose [79,80].

Several studies in LPS mice on selenoprotein expression have already been performed in hepatic and in a lesser extent lung, kidney and spleen tissues [71,74]. But further studies might be of great interest on modification of genomic expression of selenoproteins in sepsis using knock-out models focusing on endothelium, brain -especially hypothalamus-, thyroid, renal and muscular tissues similar to studies in mice or rats investigating metabolic syndrome [81,82], thyroid disease [83, 84] or pregnancy - especially in preeclampsia [85].

On the other hand, for oxidant direct cytotoxic effects of high blood concentrations of selenite studying its effect on circulating cells, especially their binding to endothelium, might be a key point. Studies on selenoprotein-P direct protective effects on endothelium against sepsis-

induced hyper hyperoxidation might be also of great interest.

5. Conclusions

In conclusion, the absence of beneficial effects observed in the SÉRÉNITÉ study using high doses of Na₂SeO₃ in septic shock may be explained by (i) a low plasma Na₂SeO₃ concentration below the cytotoxic concentration of hyperactivated leukocytes and (ii) a delayed increase of plasma selenoprotein-P occurring after initiation of endothelium damage. Further preclinical studies need to be conducted to explore antioxidant protection of selenoprotein-P on septic endothelium, and the effects of rapid injection of Na₂SeO₃, for cytotoxic purposes on hyperactivated leukocytes. Furthermore, it might be useful to study if a combination of selenoprotein-P infusion and rapid intravenous injection of Na₂SeO₃, leads to a cytotoxic effect on leukocytes while protecting endothelium. The selenoprotein-P infusion might in addition reduce the pulmonary side effects and increase the Na₂SeO₃ margin of safety by limiting endothelium activation.

5.1. Additional files

Evolution of cardiac and respiratory SOFA scores, Table S1.

Evolution of IL6, and VEGF until day-14 in the two groups, see electronic [Supplementary material, Fig. S1a and S1b](#). Evolution of PaO₂/FiO₂, AST and ALT until day-14 in the two groups, see electronic [Supplementary material, Fig S2a, S2b, and S2c](#).

Declarations

Ethics approval and consent to participate

The study has been approved by the ethics committee (*Comité consultatif de protection des personnes dans la recherche biomédicale*, CCPPRB) of Saint Germain en Laye on the 10th of May, 2001; A statement of intention for the study of a test drug has been filed with the French Office for the safety of health products (*Agence Française de Sécurité Sanitaire des Produits de Santé* AFSSAPS), in May 2001.

The study has received the labels of the French Speaking Society of Intensive Care (*Société de Réanimation de Langue Française*, SRLF) in Nov 1999, and the French Speaking Society of Study and Research on Essential and Toxic Elements (*Société Francophone d'Etude et de Recherche sur les Eléments Toxiques et Essentiels*, SFRÉTE) in May 2001.

Consent for publication

Acceptation.

Authors' contributions

XF obtained the financing, developed the link with the administrative staff of Meaux Hospital – especially for pharmaceutical aspects – and the coordination between centers, participated in the study design and execution, in interpretation of the data, and in the writing of the manuscript. BL coordinated the monitoring of the study, performed the medical analysis of adverse events, and participated in the statistical analysis, in the interpretation of the data, and in the writing of the manuscript. BL, JG, AB, EP, PVA, and DA, participated in the execution of the study and in the interpretation of the data. EB realized the methodology of the study, coordinated the monitoring, data management, statistical analysis, interpretation of the data, and analysis of adverse events, and participated in the writing of the manuscript. All authors read and approved the final manuscript.

Author statement

I have the written consent from all authors to submit this manuscript

and all authors accept complete responsibility for the contents. This manuscript is not currently under consideration elsewhere and the reported work will not be submitted for publication elsewhere until a final decision has been made as to its acceptability. The manuscript is a truthful, original work.

Statements

The authors state that informed consent was obtained for this experimentation in septic shock patients. The privacy rights of human subjects have been always observed.

The work described has not been published previously and is not under consideration for publication elsewhere. Its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright holder.

Funding

This study has been carried out with financial support from the French Ministry of Health (PHRC 98; *Programme Hospitalier de Recherche Clinique* 98). It does not necessarily reflect its views and in no way anticipates the Ministry of Health's future policy in this area.

Competing interests

Dr Xavier Forceville created a very small start-up in 2005 (Sérénité-Forceville). In 2015, Pharm. V Cotereau volunteer manager of Sérénité-Forceville, former vice president of the French pharmacist association, filed a new patent for the treatment of sepsis entitled: "Kit for treating sepsis and/or any systemic (SIRS) or damaging cellular hyper-inflammation". Its reference numbers are: PCT number PCT/FR2016/051569, European demand 16742342.5, US demand Attorney Docket Number: 0727-1267.

I am the main shareholder of this company. One of my brothers is also a shareholder.

The other authors (BL, JG, AB, PVA, EP, DA, and EB) declare that they have no competing interests.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgements

The authors would like to thank the members of the study group: Dr Gérard Bleichner, Jean-Michel Korach, Emmanuel Cantais, Hugues Georges, Jean-Louis Soubirou, Monique Dehoux, Wojciech Wasowicz, and Alain Combes. Dr Alain Combes should be particularly thanked for his discreet, and efficient help during all the study period and Dr Dominique Vitoux for participating in the study design. We thank Mrs Stuart Birom and Jason Speck for English editing and Ms Evelaine Guilbert for designing the graphical abstract.

The authors would like to thank the members of the committee constituted by XF, who met three times for brainstorming before the planning of the study: Prof. Eric Bellissant, Prof. Jean Carlet, Dr Catherine Claise, Dr Alain Combes, Prof. Isabelle Durand-Zaleski, Dr Remy Gauzit, Prof. Jean-Claude Melchior, Dr Benoît Misset, Prof. Jean Nève, Prof. Jean-Louis Pourriat, Dr Alain Tenaillon, Mr François Thuillier, Dr Dominique Vitoux and Dr Jean-Fabien Zazzo. They would also like to thank the ICU, Biochemistry and Pharmaceutical staff of all participating hospitals and especially Françoise Perrot Pharmacist at Meaux hospital, as well as the staff of Rennes University Hospital Clinical

Research Centre, and especially the research assistant Christelle Tual. The authors are also grateful to the Ministry of Health for its grant and to the administrative staff of Meaux Hospital who completed the administrative tasks required by the study, the Laboratoires Aguettant for providing the study drug, and the SRLF and SFERETE for their kind support through Labels. Funding was received in the form of a grant from the Ministry of Health, France (PHRC 1998) to XF.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jtemb.2022.127031](https://doi.org/10.1016/j.jtemb.2022.127031).

References

- [1] K. Reinhart, M. Bauer, N.C. Riedemann, C.S. Hartog, New approaches to sepsis: molecular diagnostics and biomarkers, *Clin. Microbiol. Rev.* 25 (4) (2012) 609–634.
- [2] R.S. Hotchkiss, L.L. Moldawer, S.M. Opal, K. Reinhart, I.R. Turnbull, J.L. Vincent, Sepsis and septic shock, *Nat. Rev. Dis. Prim.* 2 (2016) 16045.
- [3] M. Singer, C.S. Deutschman, C.W. Seymour, M. Shankar-Hari, D. Annane, M. Bauer, R. Bellomo, G.R. Bernard, J.D. Chiche, C.M. Cooper-Smith, R.S. Hotchkiss, M. Levy, J.C. Marshall, G.S. Martin, S.M. Opal, G.D. Rubenfeld, T. van der Poll, J. L. Vincent, D.C. Angus, The third international consensus definitions for sepsis and septic shock (Sepsis-3), *Jama* 315 (8) (2016) 801–810.
- [4] H.C. Prescott, D.C. Angus, Enhancing recovery from sepsis: a review, *Jama* 319 (1) (2018) 62–75.
- [5] V. Liu, G.J. Escobar, J.D. Greene, J. Soule, A. Whippy, D.C. Angus, T.J. Iwashyna, Hospital deaths in patients with sepsis from 2 independent cohorts, *Jama* 312 (1) (2014) 90–92.
- [6] J.L. Vincent, J.C. Marshall, S.A. Namendys-Silva, B. Francois, I. Martin-Loeches, J. Lipman, K. Reinhart, M. Antonelli, P. Pickkers, H. Njimi, E. Jimenez, Y. Sakr, Assessment of the worldwide burden of critical illness: the intensive care over nations (ICON) audit, *Lancet Respir. Med.* 2 (5) (2014) 380–386.
- [7] M. Cevik, C.G.G. Bamford, A. Ho, COVID-19 pandemic—a focused review for clinicians, *Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis.* 26 (7) (2020) 842–847.
- [8] J.L. Vincent, COVID-19: it's all about sepsis, *Future Microbiol.* 16 (2021) 131–133.
- [9] S.M. Opal, R.P. Dellinger, J.L. Vincent, H. Masur, D.C. Angus, The next generation of sepsis clinical trial designs: what is next after the demise of recombinant human activated protein C?*, *Crit. Care Med.* 42 (7) (2014) 1714–1721.
- [10] D. Annane, A. Renault, C. Brun-Buisson, B. Megarbane, J.-P. Quenot, S. Siami, A. Cariou, X. Forceville, C. Schwebel, C. Martin, J.-F. Timsit, B. Misset, M. Ali Benali, G. Colin, B. Souweine, K. Asehnoune, E. Mercier, L. Chimot, C. Charpentier, B. François, T. Boulain, F. Petitpas, J.-M. Constantin, G. Dhonneur, F. Baudin, A. Combes, J. Bohé, J.-F. Loriferne, R. Amathieu, F. Cook, M. Slama, O. Leroy, G. Capellier, A. Dargent, T. Hissem, V. Maxime, E. Bellissant, Hydrocortisone plus fludrocortisone for adults with septic shock, *N. Engl. J. Med.* 378 (9) (2018) 809–818.
- [11] T. Merz, N. Denoix, M. Huber-Lang, M. Singer, P. Radermacher, O. McCook, Microcirculation vs. Mitochondria—what to target? *Front. Med.* 7 (416) (2020).
- [12] X. Forceville, P. Van Antwerpen, J.C. Preiser, Selenocompounds and sepsis: redox bypass hypothesis for early diagnosis and treatment: part a-early acute phase of sepsis: an extraordinary redox situation (leukocyte/endothelium interaction leading to endothelial damage), *Antioxid. Redox Signal* 35 (2) (2021) 113–138.
- [13] O. Huet, L. Dupic, A. Harrois, J. Duranteau, Oxidative stress and endothelial dysfunction during sepsis, *Front. Biosci. Landmark Ed.* 16 (2011) 1986–1995.
- [14] E. Lorne, J.W. Zmijewski, X. Zhao, G. Liu, Y. Tsuruta, Y.J. Park, H. Dupont, E. Abraham, Role of extracellular superoxide in neutrophil activation: interactions between xanthine oxidase and TLR4 induce proinflammatory cytokine production, *Am. J. Physiol. Cell Physiol.* 294 (4) (2008) C985–C993.
- [15] B. Halliwell, Phagocyte-derived reactive species: salvation or suicide? *Trends Biochem. Sci.* 31 (9) (2006) 509–515.
- [16] G.P. Novelli, Role of free radicals in septic shock, *J. Physiol. Pharmacol. Off. J. Pol. Physiol. Soc.* 48 (4) (1997) 517–527.
- [17] V.N. Gladyshev, E.S. Arnér, M.J. Berry, R. Brigelius-Flohé, E.A. Bruford, R.F. Burk, B.A. Carlson, S. Castellano, L. Chavatte, M. Conrad, P.R. Copeland, A.M. Diamond, D.M. Driscoll, A. Ferreira, L. Flohé, F.R. Green, R. Guigó, D.E. Handy, D.L. Hatfield, J. Heskest, P.R. Hoffmann, A. Holmgren, R.J. Hondal, M.T. Howard, K. Huang, H. Y. Kim, I.Y. Kim, J. Köhrle, A. Krol, G.V. Kryukov, B.J. Lee, B.C. Lee, X.G. Lei, Q. Liu, A. Lescure, A.V. Lobanov, J. Loscalzo, M. Maiorino, M. Mariotti, K. Sandeep Prabhu, M.P. Rayman, S. Rozovsky, G. Salinas, E.E. Schmidt, L. Schomburg, U. Schweizer, M. Simonović, R.A. Sunde, P.A. Tsuji, S. Tweedie, F. Ursini, P. D. Whanger, Y. Zhang, Selenoprotein gene nomenclature, *J. Biol. Chem.* 291 (46) (2016) 24036–24040.
- [18] R. Brigelius-Flohe, L. Flohe, Selenium and redox signaling, *Arch. Biochem Biophys.* 617 (2017) 48–59.
- [19] R.F. Burk, K.E. Hill, Regulation of selenium metabolism and transport, *Annu Rev. Nutr.* 35 (2015) 109–134.
- [20] M.P. Rayman, The importance of selenium to human health, *Lancet* 356 (9225) (2000) 233–241.

- [21] U. Schweizer, Selenoproteins in Nervous System: Development, Function and Degeneration, in: D.L. Hatfield, U. Schweizer, P.A. Tsuji, V.N. Gladyshev (Eds.), *Selenium: Its Molecular Biology and Role in Human Health*, Springer International Publishing, Cham, 2016, pp. 427–433.
- [22] R.F. Burk, K.E. Hill, Selenoprotein P-expression, functions, and roles in mammals, in: *Biochim Biophys Acta*, 1790, 2009, pp. 1441–1447.
- [23] R. Brigelius-Flohe, A. Banning, K. Schnurr, Selenium-dependent enzymes in endothelial cell function, *Antioxid. Redox Signal* 5 (2) (2003) 205–215.
- [24] X. Forceville, V. Mostert, A. Pierantoni, D. Vitoux, P. Le Toumelin, E. Plouvier, M. Dehoux, F. Thuillier, A. Combes, P. Selenoprotein, Rather than glutathione peroxidase, as a potential marker of septic shock and related syndromes, *Eur. Surg. Res.* 43 (4) (2009) 338–347.
- [25] C.S. Hoefig, K. Renko, J. Kohrle, M. Birringer, L. Schomburg, Comparison of different selenocompounds with respect to nutritional value vs. toxicity using liver cells in culture, *J. Nutr. Biochem* 22 (10) (2011) 945–955.
- [26] S. Berthier, L. Larrouquère, P. Champelovier, E. Col, C. Lefebvre, C. Cottet-Rousselle, J. Arnaud, C. Garrel, F. Laporte, J. Boutonnat, P. Faure, F. Hazane-Puch, A. New, Patient-derived metastatic glioblastoma cell line: characterisation and response to sodium selenite anticancer agent, *Cancers* 11 (1) (2018).
- [27] A.P. Fernandes, V. Gandin, Selenium compounds as therapeutic agents in cancer, *Biochim Biophys. Acta* 1850 (8) (2015) 1642–1660.
- [28] K. Rezacova, K. Canova, A. Bezrouk, E. Rudolf, Selenium induces DNA damage and specific mitochondrial degeneration in human bladder cancer cells, *Toxicol. Vitr. Int. J. Publ. Assoc. BIBRA* 32 (2016) 105–114.
- [29] M.S. Stewart, J.E. Spallholz, K.H. Neldner, B.C. Pence, Selenium compounds have disparate abilities to impose oxidative stress and induce apoptosis, *Free Radic. Biol. Med.* 26 (1–2) (1999) 42–48.
- [30] O. Brodin, S. Eksborg, M. Wallenberg, C. Asker-Hagelberg, E.H. Larsen, D. Mohlkert, C. Lenneby-Helleday, H. Jacobsson, S. Linder, S. Misra, M. Bjornstedt, Pharmacokinetics and toxicity of sodium selenite in the treatment of patients with Carcinoma in a phase I clinical trial: the SECAR study, *Nutrients* 7 (6) (2015) 4978–4994.
- [31] Z. Wang, X. Forceville, P. Van Antwerpen, M. Piagnerelli, D. Ahishakiye, P. Macours, D. De Backer, J. Neve, J.L. Vincent, A large-bolus injection, but not continuous infusion of sodium selenite improves outcome in peritonitis, *Shock* 32 (2) (2009) 140–146.
- [32] X. Forceville, D. Vitoux, R. Gauzit, A. Combes, P. Lahilaire, P. Chappuis, Selenium, systemic immune response syndrome, sepsis, and outcome in critically ill patients, *Crit. Care Med.* 26 (9) (1998) 1536–1544.
- [33] Y. Sakr, K. Reinhart, F. Bloos, G. Marx, S. Russwurm, M. Bauer, F. Brunkhorst, Time course and relationship between plasma selenium concentrations, systemic inflammatory response, sepsis, and multiorgan failure, *Br. J. Anaesth.* 98 (6) (2007) 775–784.
- [34] K. Renko, P.J. Hofmann, M. Stoedter, B. Hollenbach, T. Behrends, J. Kohrle, U. Schweizer, L. Schomburg, Down-regulation of the hepatic selenoprotein biosynthesis machinery impairs selenium metabolism during the acute phase response in mice, *Faseb J.* 23 (6) (2009) 1758–1765.
- [35] W. Alhazzani, J. Jacobi, A. Sindi, C. Hartog, K. Reinhart, S. Kokkoris, H. Gerlach, P. Andrews, T. Drabek, W. Manzanares, D.J. Cook, R.Z. Jaeschke, The effect of selenium therapy on mortality in patients with sepsis syndrome: a systematic review and meta-analysis of randomized controlled trials, *Crit. Care Med* 41 (6) (2013) 1555–1564.
- [36] X. Forceville P. Van Antwerpen D. Annane J.L. Vincent Selenocompounds and Sepsis: redox bypass hypothesis: Part B- selenocompounds in the management of early Sepsis Antioxid. Redox Signal, ahead of print.
- [37] M.M. Berger, W. Manzanares, Micronutrients early in critical illness, selective or generous, enteral or intravenous? *Curr. Opin. Clin. Nutr. Metab. Care* 24 (2) (2021) 165–175.
- [38] W. Manzanares, M. Lemieux, G. Elke, P.L. Langlois, F. Bloos, D.K. Heyland, High-dose intravenous selenium does not improve clinical outcomes in the critically ill: a systematic review and meta-analysis, *Crit. Care* 20 (1) (2016) 356.
- [39] F. Bloos, E. Trips, A. Nierhaus, J. Briegel, D.K. Heyland, U. Jaschinski, O. Moerer, A. Weyland, G. Marx, M. Grundling, S. Kluge, I. Kaufmann, K. Ott, M. Quintel, F. Jelschen, P. Meybohm, S. Rademacher, A. Meier-Hellmann, S. Uetzolino, U. X. Kaisers, C. Putensen, G. Elke, M. Ragaller, H. Gerlach, K. Ludewig, M. Kiehnopf, H. Bogatsch, C. Engel, F.M. Brunkhorst, M. Loeffler, K. Reinhart, Effect of sodium selenite administration and procalcitonin-guided therapy on mortality in patients with severe sepsis or septic shock: a randomized clinical trial, *JAMA Intern. Med.* 176 (9) (2016) 1266–1276.
- [40] M.W. Angstwurm, L. Engelmann, T. Zimmermann, C. Lehmann, C.H. Spes, P. Abel, R. Strauss, A. Meier-Hellmann, R. Insel, J. Radke, J. Schuttler, R. Gartner, Selenium in Intensive Care (SIC): results of a prospective randomized, placebo-controlled, multiple-center study in patients with severe systemic inflammatory response syndrome, sepsis, and septic shock, *Crit. Care Med.* 35 (1) (2007) 118–126.
- [41] X. Forceville, The effect of selenium therapy on mortality in patients with sepsis syndrome: simple selenium supplementation or real (5 H₂O).Na₂SeO₃ pharmacological effect? *Crit. Care Med.* 41 (6) (2013) 1591–1592.
- [42] O.E. Olson, Selenium toxicity in animals with emphasis on man, *Int. J. Toxicol.* 5 (1) (1986) 45–70.
- [43] X. Forceville, B. Laviolle, D. Annane, D. Vitoux, G. Bleichner, J.M. Korach, E. Cantais, H. Georges, J.L. Soubirou, A. Combes, E. Bellissant, Effects of high doses of selenium, as sodium selenite, in septic shock: a placebo-controlled, randomized, double-blind, phase II study, *Crit. Care* 11 (4) (2007) R73.
- [44] D.K. Heyland, Selenium supplementation in critically ill patients: can too much of a good thing be a bad thing? *Crit. Care* 11 (4) (2007) 153.
- [45] P. Singer, M.M. Berger, G. Van den Berghe, G. Biolo, P. Calder, A. Forbes, R. Griffiths, G. Kreyman, X. Lerverve, C. Pichard, Espen, ESPEN Guidelines on Parenteral Nutrition: intensive care, *Clin. Nutr.* 28 (4) (2009) 387–400.
- [46] J.L. Vincent, R. Moreno, J. Takala, S. Willatts, A. De Mendonca, H. Bruining, C. K. Reinhart, P.M. Suter, L.G. Thijs, The SOFA (Sepsis-related organ failure assessment) score to describe organ dysfunction/failure. On behalf of the working group on sepsis-related problems of the European Society of intensive care medicine, *Intensive Care Med.* 22 (7) (1996) 707–710.
- [47] R. Moreno, J.L. Vincent, R. Matos, A. Mendonça, F. Cantraine, L. Thijs, J. Takala, C. Sprung, M. Antonelli, H. Bruining, S. Willatts, The use of maximum SOFA score to quantify organ dysfunction/failure in intensive care. Results of a prospective, multicentre study. Working Group on Sepsis related Problems of the ESICM, *Intensive Care Med.* 25 (7) (1999) 686–696.
- [48] A. Leplege, E. Ecosse, A. Verdier, T.V. Perneger, The French SF-36 Health Survey: translation, cultural adaptation and preliminary psychometric evaluation, *J. Clin. Epidemiol.* 51 (11) (1998) 1013–1023.
- [49] J. Chastre, J.Y. Fagon, M. Bornet-Lecso, S. Calvat, M.C. Dombret, R. Al Khani, F. Basset, C. Gibert, Evaluation of bronchoscopic techniques for the diagnosis of nosocomial pneumonia, *Am. J. Respir. Crit. Care Med.* 152 (1) (1995) 231–240.
- [50] J.J. Griffin, G.U. Meduri, New approaches in the diagnosis of nosocomial pneumonia, *Med. Clin. North Am.* 78 (5) (1994) 1091–1122.
- [51] R.A. Garibaldi, D. Cushing, T. Lerer, Risk factors for postoperative infection, *The American journal of medicine* 91(3B) 158S–163S 1991.
- [52] C. Brun-Buisson, New technologies and infection control practices to prevent intravascular catheter-related infections, *Am. J. Respir. Crit. Care Med* 164 (9) (2001) 1557–1558.
- [53] J. Neve, S. Chamart, L. Molle, Optimization of a direct procedure for the determination of selenium in plasma and erythrocytes using Zeeman effect atomic absorption spectroscopy, in: P. Bratter, P. Schramel (Eds.), *Trace elements analytical chemistry in medicine and biology*, Walter de Gruyter, Berlin, 1987, pp. 349–358.
- [54] D.E. Paglia, W.N. Valentine, Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase, *J. Lab. Clin. Med* 70 (1) (1967) 158–169.
- [55] K. Yagi, A simple fluorometric assay for lipoperoxide in blood plasma, *Biochem Med* 15 (2) (1976) 212–216.
- [56] X. Forceville, S. Touati, P. Le Toumelin, V. Ducros, F. Laporte, Y. Chancerelle, D. Agay, Elements of margin of safety, toxicity and action of sodium selenite in a lipopolysaccharide rat model, *J. Trace Elem. Med Biol.* 28 (3) (2014) 303–310.
- [57] L. Chelkeba, A. Ahmadi, M. Abdollahi, A. Najafi, M.H. Ghadimi, R. Mosaed, M. Mojtahedzadeh, The effect of parenteral selenium on outcomes of mechanically ventilated patients following sepsis: a prospective randomized clinical trial, *Ann. Intensive Care* 5 (1) (2015) 29.
- [58] B. Hamade, D.T. Huang, Procalcitonin: where are we now? *Crit. Care Clin.* 36 (1) (2020) 23–40.
- [59] L. Ghosn, A. Chaimani, T. Evrenoglou, M. Davidson, C. Graña, C. Schmucker, C. Bollig, N. Henschke, Y. Sguassero, C.H. Nejtgaard, S. Menon, T.V. Nguyen, G. Ferrand, P. Kapp, C. Riveros, C. Ávila, D. Devane, J.J. Meerpohl, G. Rada, A. Hróbjartsson, G. Grasselli, D. Tovey, P. Ravaud, I. Boutron, Interleukin-6 blocking agents for treating COVID-19: a living systematic review, *Cochrane Database Syst. Rev.* 3 (3) (2021) Cd013881.
- [60] S. Kang, T. Kishimoto, Interplay between interleukin-6 signaling and the vascular endothelium in cytokine storms, *Exp. Mol. Med.* 53 (7) (2021) 1116–1123.
- [61] M. Shankar-Hari, C.L. Vale, P.J. Godolphin, D. Fisher, J.P.T. Higgins, F. Spiga, J. Savovic, J. Tierney, G. Baron, J.S. Benbenishty, L.R. Berry, N. Broman, A. B. Cavalcanti, R. Colman, S.L. De Buysier, L.P.G. Derde, P. Domingo, S.F. Omar, A. Fernandez-Cruz, T. Feuth, F. Garcia, R. Garcia-Vicuna, I. Gonzalez-Alvaro, A. C. Gordon, R. Haynes, O. Hermine, P.W. Horby, N.K. Horick, K. Kumar, B. N. Lambrecht, M.J. Landray, L. Leal, D.J. Lederer, E. Lorenzi, X. Mariette, N. Merchante, N.A. Misnan, S.V. Mohan, M.C. Nivens, J. Oksi, J.A. Perez-Molina, R. Pizov, R. Porcher, S. Postma, R. Rajasuriar, A.V. Ramanan, P. Ravaud, P.D. Reid, A. Rutgers, A. Sancho-Lopez, T.B. Seto, S. Sivapalasingam, A.S. Soin, N. Staplin, J. H. Stone, G.W. Strohbehn, J. Sundén-Cullberg, J. Torre-Cisneros, L.W. Tsai, H. van Hoogstraten, T. van Meerten, V.C. Veiga, P.E. Westerweel, S. Murthy, J.V. Diaz, J. C. Marshall, J.A.C. Sterne, Association between administration of IL-6 antagonists and mortality among patients hospitalized for COVID-19: a meta-analysis, *Jama* 326 (6) (2021) 499–518.
- [62] I. Schimke, N. Richter, H. Wauer, U. Rohr, A.S. Petersson, A. Wennmalm, H. Kuppe, W.J. Kox, High and low response in relation to nitric oxide formation but not to lipid peroxidation in patients with sepsis, *Crit. Care Med.* 31 (1) (2003) 65–72.
- [63] L.B. Ware, J.P. Fessel, A.K. May, L.J. Roberts 2nd, Plasma biomarkers of oxidant stress and development of organ failure in severe sepsis, *Shock* 36 (1) (2011) 12–17.
- [64] O. Huet, R. Obata, C. Aubron, A. Spraul-Davit, J. Charpentier, C. Laplace, T. Nguyen-Khoa, M. Conti, E. Vicaut, J.P. Mira, J. Durantéau, Plasma-induced endothelial oxidative stress is related to the severity of septic shock, *Crit. Care Med.* 35 (3) (2007) 821–826.
- [65] M.J. Butler, C.J. Down, R.R. Foster, S.C. Satchell, The pathological relevance of increased endothelial glycocalyx permeability, *Am. J. Pathol.* 190 (4) (2020) 742–751.
- [66] R. Sonnevile, F. Verdonk, C. Rauturier, I.F. Klein, M. Wolff, D. Annane, F. Chretien, T. Sharshar, Understanding brain dysfunction in sepsis, *Ann. Intensive Care* 3 (1) (2013) 15.
- [67] S. Seeher, B.A. Carlson, A.C. Miniard, E.K. Wirth, Y. Mahdi, D.L. Hatfield, D. M. Driscoll, U. Schweizer, Impaired selenoprotein expression in brain triggers

- striatal neuronal loss leading to coordination defects in mice, *Biochem J.* 462 (1) (2014) 67–75.
- [68] Z. Li, J. Tan, L. Shao, X. Dong, R.D. Ye, D. Chen, Selenium-mediated protection in reversing the sensitivity of bacterium to the bactericidal antibiotics, *J. Trace Elem. Med. Biol.* 41 (2017) 23–31.
- [69] V.N. Gladyshev, G.V. Kryukov, Evolution of selenocysteine-containing proteins: significance of identification and functional characterization of selenoproteins, *Biofactors* 14 (1–4) (2001) 87–92.
- [70] T.C. Stadtman, New biologic functions–selenium-dependent nucleic acids and proteins, *Fundam. Appl. Toxicol. Off. J. Soc. Toxicol.* 3 (5) (1983) 420–423.
- [71] X. Forceville, P. Van Antwerpen, D. Annane, J.-L. Vincent, Selenocompounds and Sepsis: Redox Bypass Hypothesis: Part B- Selenocompounds in the Management of Early Sepsis, *Antioxid Redox Signal* (2022) ahead of print.
- [72] K. Bierla, M. Dernovics, V. Vacchina, J. Szpunar, G. Bertin, R. Lobinski, Determination of selenocysteine and selenomethionine in edible animal tissues by 2D size-exclusion reversed-phase HPLC-ICP MS following carbamidomethylation and proteolytic extraction, *Anal. Bioanal. Chem.* 390 (7) (2008) 1789–1798.
- [73] R.R. Wolfe, Herman Award Lecture, 1996: relation of metabolic studies to clinical nutrition—the example of burn injury, *Am. J. Clin. Nutr.* 64 (5) (1996) 800–808.
- [74] L.G. Sherlock, K. Sjostrom, L. Sian, C. Delaney, T.E. Tipple, N.F. Krebs, E. Nozik-Grayck, C.J. Wright, Hepatic-specific decrease in the expression of selenoenzymes and factors essential for selenium processing after endotoxemia, *Front. Immunol.* 11 (2020) 2901.
- [75] J.B. Atkinson, K.E. Hill, R.F. Burk, Centrilobular endothelial cell injury by diquat in the selenium-deficient rat liver, Laboratory investigation; a journal of technical methods and pathology 81, 3, 2001, pp. 193–200.
- [76] R.F. Burk, K.E. Hill, Selenoprotein P: an extracellular protein with unique physical characteristics and a role in selenium homeostasis, *Annu. Rev. Nutr.* 25 (2005) 215–235.
- [77] R.F. Burk, K.E. Hill, J.A. Awad, J.D. Morrow, T. Kato, K.A. Cockell, P.R. Lyons, Pathogenesis of diquat-induced liver necrosis in selenium-deficient rats: assessment of the roles of lipid peroxidation and selenoprotein P, *Hepatology* 21 (2) (1995) 561–569.
- [78] R.F. Burk, K.E. Hill, M.E. Boeglin, F.F. Ebner, H.S. Chittum, Selenoprotein P associates with endothelial cells in rat tissues, *Histochem. Cell Biol.* 108 (1) (1997) 11–15.
- [79] J. Hawiger, Heartfelt sepsis: microvascular injury due to genomic storm, *Kardiol. Pol.* 76 (8) (2018) 1203–1216.
- [80] B.P. Scicluna, L.A. van Vught, A.H. Zwinderman, M.A. Wiewel, E.E. Davenport, K. L. Burnham, P. Nürnberg, M.J. Schultz, J. Horn, O.L. Cremer, M.J. Bonten, C. J. Hinds, H.R. Wong, J.C. Knight, T. van der Poll, Classification of patients with sepsis according to blood genomic endotype: a prospective cohort study, *Lancet Respir. Med.* 5 (10) (2017) 816–826.
- [81] D.J. Torres, M.W. Pitts, L.A. Seale, A.C. Hashimoto, K.J. An, A.N. Hanato, K.W. Hui, S.M.A. Remigio, B.A. Carlson, D.L. Hatfield, M.J. Berry, Female mice with selenocysteine tRNA deletion in AgRP neurons maintain leptin sensitivity and resist weight gain while on a high-fat diet, *Int. J. Mol. Sci.* 22 (20) (2021).
- [82] K. Day, L.A. Seale, R.M. Graham, B.R. Cardoso, Selenotranscriptome network in non-alcoholic fatty liver disease, *Front. Nutr.* 8 (2021), 744825.
- [83] J. Chiu-Ugalde, E.K. Wirth, M.O. Klein, R. Sapin, N. Fradejas-Villar, K. Renko, L. Schomburg, J. Kohrle, U. Schweizer, Thyroid function is maintained despite increased oxidative stress in mice lacking selenoprotein biosynthesis in thyroid epithelial cells, *Antioxid. Redox Signal* 17 (6) (2012) 902–913.
- [84] J. Kohrle, Selenium and the thyroid, current opinion in endocrinology, Diabetes, *Obes.* 22 (5) (2015) 392–401.
- [85] C. Hogan, A.V. Perkins, Selenoproteins in the human placenta: how essential is selenium to a healthy start to life? *Nutrients* 14 (3) (2022).