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RESEARCH ARTICLE

Effects of High-Dose Selenium on Mortality of Sepsis and Septic Shock Patients with Severe Selenium Deficiency in Taiwan

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ABSTRACT

The relationship between serum selenium levels and mortality was investigated in septic patients with severe selenium deficiency (baseline selenium ≤ 80 ng/mL). Eligible patients of sepsis or septic shock were randomized to receive Placebo or High-Dose Selenium (1,000 μg/day) via intravenous injection. Safety, serum selenium, mortality, SOFA, and Glasgow Coma Scale (GCS) scores were monitored. Among all 330 subjects, 27.9% subjects (n=92) had severe selenium deficiency (mean serum selenium = 66.5 ng/mL). Mortality of severe selenium deficiency patients was 27.2%, significantly higher than 17.9% of all subjects. In severe selenium deficiency Placebo group (n=45), 62% subjects showed gradual increase of selenium levels to ~110 ng/mL (mortality ~21.4%), while 38% subjects remained at low selenium ≤ 110 ng/mL throughout study (mortality ~41.2%). Mortality for Placebo subjects with normal baseline selenium ≥ 110 ng/mL was 13.6%. With High-Dose Selenium treatment, 91% of severe selenium deficiency subjects showed quick selenium increase to ~110 ng/mL (mortality 25.5%). Mortality was reduced to 8.6% for High-Dose Selenium subjects with baseline selenium ≥ 110 ng/mL. The odds ratio showed significantly greater survival of High-Dose Selenium subjects with baseline selenium ≥ 110 ng/mL (91.4%) than severe selenium deficiency Placebo subjects (74.1%). Mean baseline SOFA scores for severe selenium deficiency patients were 9.1–9.4, decrease of SOFA scores in High-Dose Selenium subjects was significantly greater than Placebo subjects, along with significant improvement of GCS scores. Repeated infusion of High-Dose Selenium in severe selenium deficiency patients for 14 days was safe and well-tolerated. Mortality for patients with sepsis was clearly affected by serum selenium concentrations. High mortality (41–50%) was observed in the sepsis patients constantly with low selenium ≤ 80 ng/mL; mortality was reduced to 21–23% if their serum selenium could be increased to ≥ 110 ng/mL. High-Dose Selenium resulted in rapid restoration of serum selenium and improved the survival of severe selenium deficiency septic patients. Low mortality (9–14%) was observed in the sepsis patients starting with baseline selenium ≥ 110 ng/mL. Overall this study demonstrates the significant impact of insufficient selenium levels on the mortality of septic patients. Treatment with high-dose selenium reduced the mortality of severe selenium deficiency septic subjects.

Keywords: Selenium deficiency, sepsis and septic shock, mortality, SOFA score, Asian.

Introduction:

Sepsis is a life-threatening disease characterized by a dysregulated host response to infection and results in organ dysfunction represented by the sequential organ failure assessment (SOFA) score of two points or more[1]. It is a prominent cause of overall mortality in the critically ill and remains a leading cause of death in most of countries[2,3], accounting for approximately 20% of all deaths worldwide[4].

No specific anti-sepsis treatments exist due to the complexity of the cause, pathophysiology, and immunological mechanisms of sepsis; disease management thus relies on early diagnosis and appropriate interventions to control source infection, fluid resuscitation, and/or other therapeutic measures[5].

Several previous clinical studies showed that selenium (Se) as a potent antioxidant may play an important role in reducing the mortality of the patients suffering from sepsis or septic shock[6,7]. Se is known as an essential micronutrient involved in antioxidant defense, regulation of thyroid and insulin metabolism, and immunomodulation[8]. Substantially decreased bodily Se stores have been observed in patients with sepsis, trauma, or critical illness and are inversely correlated with disease severity and outcomes[9]. In this study, severe selenium deficiency (SSD) was defined as having Se levels less than or equal to 80 ng/mL in serum, where the same deficiency level of serum Se is defined by the German government[10] stating that the patients with Se level below 80 ng/mL would be required to administer with intravenous injection of selenite products.

Early studies reported that the mean value of serum Se was determined to be approximately 110 ng/mL, where the low and high ends of normal range of Se in serum/plasma have been generally reported to be 60–107.5 ng/mL and 108–150.5 ng/mL, respectively. Median values of the normal range are therefore determined to be approximately 84–130 ng/mL, regardless of the difference in ethnicity. The reference values of Se levels in humans are summarized in Table 1:

Table 1: Reported Normal Range of Selenium (Se) Levels in Humans

	Study Title	Normal Range of Se (ng/mL)	Ethnicity	References
1	Influence of Selenium supplements on the post-traumatic alterations of the thyroid axis: a Placebo-controlled trial [11]	63 – 126 ng/mL (0.8 – 1.6 µmol/L) in serum	Switzerland	Intensive Care Med (2001) 27:91-100
2	Selenium blood concentrations in patients undergoing elective cardiac surgery and receiving perioperative sodium selenite [12]	100–140 µg/L in blood 80–120 µg/L (ng/mL) in serum	Germany	Nutrition 29 (2013) 158–165
3	Serum selenium concentration is associated with metabolic factors in the elderly: a cross-sectional study[13]	72 – 108 ng/mL 1.14 ± 0.23 µmol/L (µM)	Taiwan	Yang *et al. Nutrition & Metabolism* 2010, **7**:38
4	Sodium selenite and cancer related lymphedema: Biological and pharmacological effects. [10]	selenium deficit: < 100 µg/L selenium in whole blood (~ 80 ng/mL in serum)	Germany	Pfister: Journal of Trace Elements in Medicine and Biology Vol 37, Sep. 2016, P. 112
5	Selenium, Iron, Copper, and Zinc Levels and Copper-to-Zinc Ratios in Serum of Patients at Different Stages of Viral Hepatic Diseases [14]	107.5 – 150.5 ng/mL 129.0 ± 21.5 µg/L	Taiwan	Lin: Biological Trace Element Research, Vol. 109, 2006, 15-23
6	Selenium Depletion in Patients on Home Parenteral Nutrition [15]	60 – 115 ng/mL (0.75 – 1.46 µmol/L) in plasma	Denmark	Rannem: Biological Trace Element Research, Vol. 39, 1993, 81-89
7	A short-term intervention trial with selenate, selenium-enriched yeast and selenium-enriched milk: effects on oxidative defense regulation [16]	98 – 124 ng/mL 110.7 ± 12.9 µg/L	Denmark	Ravn-Haren: British Journal of Nutrition (2008), 99, 883–892

Note: 1.0 µM = 79 ng/mL Selenium (or 100 ug/L = 1.266 µM Se)

Pharmaconutrition using high-dose Se reconstitution in the septic patients has been previously investigated in several clinical trials; however, the overall outcomes were

inconclusive, mainly due to diverse subject selection criteria and differences in dosing regimen. Although clinical benefits have been reported on various indices of mortality, inflammation, organ failure, and consumption of hospital resources with selenium supplementation[17–18], no large-scale selenium clinical trials have been conducted in Asia. High-Dose Selenium, containing sodium selenite pentahydrate, was therefore investigated in a clinical trial in Taiwan as an add-on therapy for patients with sepsis and septic shock. In this subgroup analysis, we reported the 28-day all-cause mortality of the septic patients with severe selenium deficiency (baseline selenium level ≤ 80 ng/mL when entering this clinical study), with or without treatment with injection of High-Dose Selenium.

Materials and Methods

STUDY DESIGN

The main trial was registered (Study ID: MOFI-001, registration number: 1046085311) in Taiwan Clinical Trials and performed in accordance with the principles of the Declaration of Helsinki, institutional guidelines, and national regulations. Informed consent was obtained from all participants. The clinical study was designed as a randomized, double-blind, placebo-controlled, multicenter trial in seven medical centers in Taiwan to assess the therapeutic efficacy and safety of High-Dose Selenium in intensive care unit (ICU) patients with sepsis or septic shock. The total sample size was 330, randomized 1:1 into High-Dose Selenium or Placebo treatment and stratified by disease severity within each clinical site.

The selection criteria are briefly described below. Inclusion criteria were patients of age ≥ 20 years admitted to the hospital who met the definition of sepsis or septic shock according to Sepsis-3[1], had at least one organ system SOFA score ≥ 2 and life expectancy ≥ 72 hours. Exclusion criteria included patients with known allergy to selenium; sepsis due to surgical complications or malignant neoplasms; had severe head trauma or history of heart failure or third-degree burns covering > 20% of body surface area; on extracorporeal membrane oxygenation treatment, regular hemodialysis or peritoneal dialysis treatment, continuous immunosuppressant agent therapy, or anti-cancer medication; pregnancy/lactation.

STUDY INTERVENTIONS

High-Dose Selenium is an injectable solution containing sodium selenite pentahydrate (equivalent to 50 μg Se/mL), and the first dose of High-Dose Selenium or Placebo was administered intravenously within two hours after enrollment. The patients in High-Dose Selenium group received an intravenous loading dose of 1,000 μg Se within 30 min, followed by a slow infusion of 1,000 μg Se (mixed with 100 mL normal saline [0.9% NaCl] prior to use) to 24 h on day 1 and the same 1,000 μg slow infusion on days 2–14 or until discharge from ICU. The patients randomized to the Placebo group received only injectable normal saline solution by the same dosing regimen. The medical conditions of individual subjects were monitored up to a maximum of 28 days.

Patients were treated with the standard of care for sepsis and septic shock according to the guidelines recommended by the Surviving Sepsis Campaign.[20] Single interruption of study drug administration > 2 hours or accumulative interruption of study drug

administration > 6 hours in one day was not allowed, except for subjects requiring acute renal replacement therapy or due to emergency according to investigator's judgement.

Pharmacokinetic serum samples were collected from all patients at various timepoints and analyzed by a validated method using ICP-MS instrument (Agilent 7800) for the serum concentrations of total Se. The lower limit of quantification for serum selenium level was 5 ng/mL.

STUDY SUBJECTS

Subjects who met the inclusion criteria at initial assessment were screened for eligibility and enrolled within 48 hours of diagnosis of sepsis or septic shock by the principal investigator.

Upon confirmation of meeting all eligibility criteria, subjects were randomized into High-Dose Selenium or Placebo treatment at a 1:1 ratio stratified by disease severity within each clinical site. Subjects received a random code that dictated the assigned treatment at each site by their incoming order. Both the investigator and the patient remained blinded of the treatment assignment throughout the study.

STATISTICAL ANALYSIS

The descriptive statistics was applied to compare the 28-day call-cause mortality in the septic patients with or without treatment of high-dose selenium. Particularly the mortality was evaluated in those patients with SSD in both Placebo and High-Dose Selenium groups. Other therapeutic effectiveness included (1) mean change from baseline in total SOFA scores on days 1, 7, 14, 21, and/or 28 and (2) Glasgow Coma Scale (GCS) scores. Student's t-test and chi-square test were used to assess statistical difference between the two treatment groups for those factors of interest. Statistical analyses were performed by Microsoft Excel (Microsoft 365 version 2206).

All efficacy analysis was carried out on the modified intention-to-treat (mITT) population. Safety of the study intervention was evaluated based on the records of adverse events, vital signs, and electrocardiography and laboratory assessments throughout the 28-day study period or at hospital discharge. Selected pharmacokinetics results of serum Se and selenite concentrations were calculated and reported.

Kaplan-Meier method and Z-test (Greenwood method) were used for the estimation of 7-day, 14-day, and 21-day all-cause mortality incorporating the censored information and treatment comparison, respectively. Chi-square test was performed to compare the mortality in ICU and that of SSD subjects between treatment groups. In addition, Cox regression model was used to estimate the adjusted hazard ratio (HR) with 95% confidence interval of the treatment groups (High-Dose Selenium/Placebo).

Results

SUBJECT BASELINE CHARACTERISTICS

A total of 330 patients completed the study without violating the eligibility criteria (mITT population). More male subjects than female subjects (ratio ~2:1) participated in this study, but the proportion in each treatment group was consistent. The mean age of all patients was 72.0 ± 14.9 years. Among them, 92 patients (45 patients in Placebo group and 47 patients in High-Dose Selenium group) started

with baseline serum selenium levels ≤ 80 ng/mL, ~27.9% of the overall 330 subjects. Overall, the baseline characteristics of the two study groups were well balanced (Table 2).

Table 2: Baseline Characteristics of the Subjects with Se Level ≤ 80 ng/mL

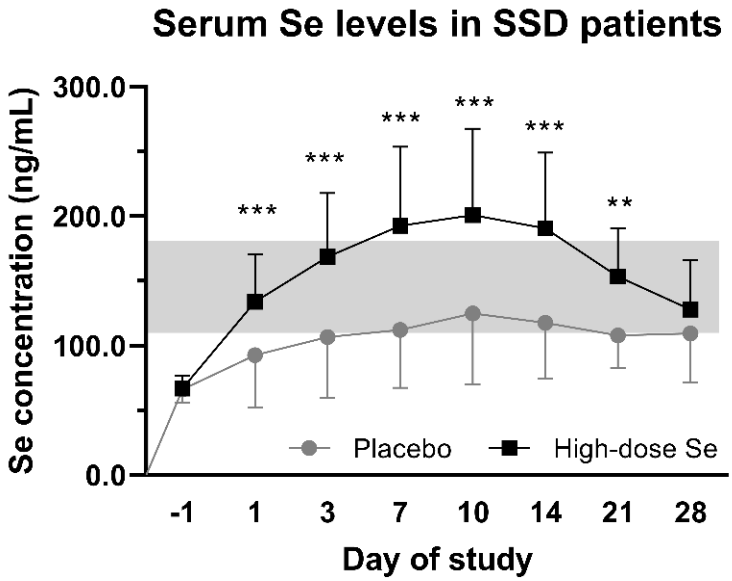
Parameter	Placebo N=45	High-Dose Selenium N=47	All N=92
Demographics			
Gender, n (%)			
Male	34 (75.6)	31 (66.0)	65 (70.7)
Female	11 (24.4)	16 (34.0)	27 (29.3)
Age (y), mean (SD)	71.4 (17.4)	72.5 (12.3)	72.0 (14.9)
Body mass index (kg/cm^2), mean (SD)	21.9 (4.3)	22.5 (5.1)	22.2 (4.7)
Severity of illness			
Septic shock, n (%)	17 (37.8)	18 (38.3)	35 (38.0)
Sepsis, n (%)	28 (62.2)	29 (61.7)	57 (62.0)
APACHE II score, mean (SD)	25.6 (7.9)	25.4 (8.4)	25.5 (8.1)
SOFA score, mean (SD)	9.1 (3.5)	9.4 (3.8)	9.2 (3.6)
CGS score, mean (SD)	9.1 (3.4)	9.2 (3.2)	9.2 (3.3)
No. of organ failure, n (%)			
1–3	33 (73.3)	33 (70.2)	66 (71.7)
4–6	12 (26.7)	14 (29.8)	26 (28.3)
Pneumonia, n (%)			
Yes	31 (68.9)	29 (61.7)	60 (65.2)
No	14 (31.1)	18 (38.3)	32 (34.8)
Source of infection, n (%)			
Culture negative (source unknown)	20 (44.4)	14 (29.8)	34 (37.0)
Culture positive	25 (55.6)	33 (70.2)	58 (63.0)
Drug-resistant pathogen positive [a]	1 (4.0)	4 (12.1)	5 (8.6)
Drug-resistant pathogen negative	24 (96.0)	29 (87.9)	53 (91.4)
Laboratory values, mean (SD)			
WBC (10^3/μL)	16.0 (7.6) [b]	16.2 (9.7)	16.1 (8.7) [b]
PCT (ng/mL)	21.8 (35.6)	41.7 (55.2)	32.0 (47.4)
L-lactate (mEq/L)	18.3 (16.7)	21.9 (26.2)	20.1 (22.0)
CRP (mg/L)	144.1 (96.8)	165.2 (112.3)	154.9 (105.0)
Selenium level (ng/mL)	66.0 (10.3)	66.9 (9.6)	66.5 (9.9)

[a] Subjects whose source of infection was on the priority list of drug-resistant pathogens defined by World Health Organization; SD: standard deviation; APACHE II: Acute Physiology and Chronic Health Evaluation II; SOFA: Sequential Organ Failure Assessment; CGS: Glasgow Coma Scale; WBC: white blood cell count; PCT: procalcitonin; CRP: C-reactive protein.
[b] The WBC values of these two subjects (S202: 10,700 10^3/μL, S205: 8,800 10^3/μL) in the Placebo group were extremely high (outliers) and not included in the mean calculation.

SERUM SELENIUM LEVELS IN SEPTIC PATIENTS

Among Placebo group (total of 166 patients), there were 44 subjects (~26.5%) with Se baseline ≥ 110 ng/mL, and 45 patients (~27.1%) started with Se baseline ≤ 80 ng/mL. Similarly, in High-Dose Selenium group (total of 164 patients), 35 subjects (~21.3%) had Se baseline ≥ 110 ng/mL and 47 patients (~28.7%) had Se baseline ≤ 80 ng/mL. The average serum concentration of Se in all 92 patients with severe selenium deficiency (SSD, Se baseline ≤ 80 ng/mL) was 66.5 ± 9.9 ng/mL (Mean ± SD). The serum concentration-time profiles of Se are presented in Figure 1.

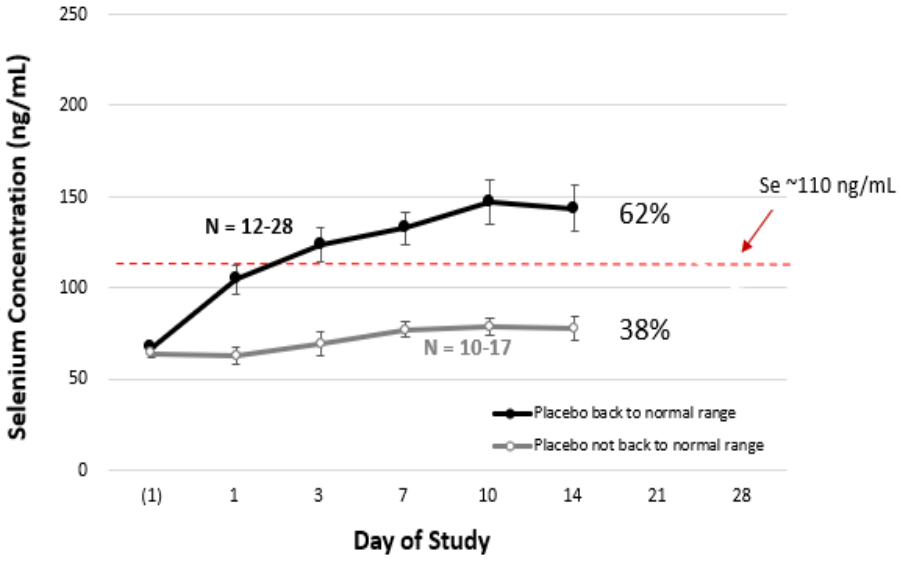
Figure 1: Serum Concentrations of Se in SSD Septic Patients with Baseline Se ≤ 80 ng/mL. Data are presented as Mean ± SD. The gray rectangle overlay in figure indicates the normal range (110–180 ng/mL) of Se levels.



** $p < 0.01$ and *** $p < 0.001$, statistically different between groups. SSD: baseline Se level ≤ 80 ng/mL.

With High-Dose Selenium treatment in SSD septic patients, almost all of the subjects (> 91%) showed quick increase of their Se levels to ~110 ng/mL within 2.0 days of therapy in average. The Se levels in High-Dose Selenium treatment group were significantly higher than Placebo throughout the end of the study period. The study results showed that the serum Se levels in 62% (n = 28) of these 45 Placebo control SSD patients gradually increased to the range of ~110 ng/mL (after ~2 days in ICU), whereas 38% (n = 17) of all 45 SSD patients would remain at the Se levels lower than 110 ng/mL throughout the study period (Figure 2).

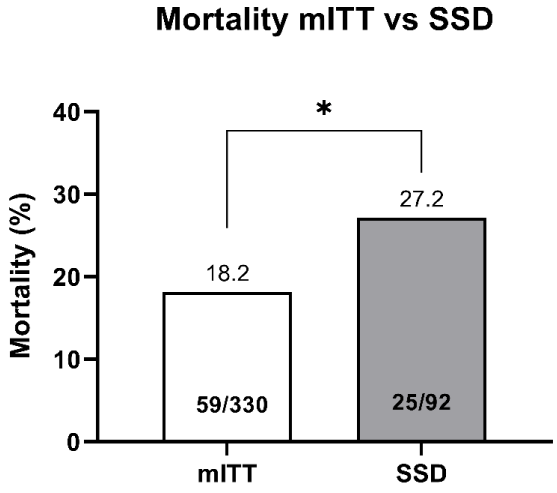
Figure 2: Sepsis Patients with Severe Selenium Deficiency (Se ≤ 80 ng/mL) in Placebo Group (45/166, ~27.1%). Data are presented as Mean ± SEM.



MORTALITY IN PATIENTS WITH SEVERE SELENIUM DISEASES

The study results showed that the overall 28-day all-cause mortality of all 330 septic patients in either Placebo or High-Dose Selenium group was less than 20% and the difference between groups was not statistically different. However, patients with septic shock had a significantly lower serum Se levels and higher mortality rate than the patients with sepsis (84.3 ng/mL vs 98.2 ng/mL, $p < 0.001$ for baseline Se levels and 32.5% vs 13.4%, $p < 0.001$ for mortality). The results also revealed that the mortality rate was significantly higher in the SSD population than that in the mITT population consisting of all subjects (27.2 vs 17.9%, $p = 0.048$) (Figure 3).

Figure 3: Comparison of Mortality of Septic Patients with Se Level ≤ 80 ng/mL vs. All Patients in the Study.



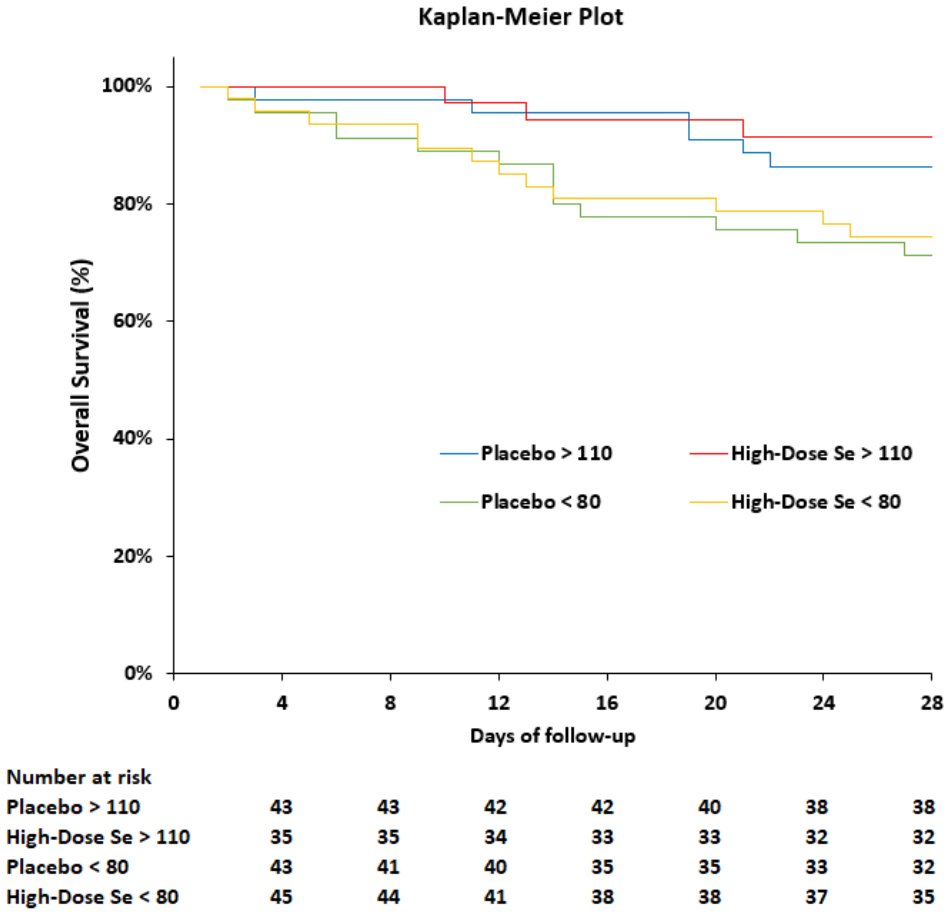
SSD: Serum Se level ≤ 80 ng/mL

Further analysis for the SSD patients with baseline Se levels ≤ 80 ng/mL, the patients in Placebo group without treatment with High-Dose Selenium resulted in the mortality of 28.9% (n = 45), more than 2-fold higher than those patients (n = 44, mortality ~13.6%) with the normal baseline Se levels (≥ 110 ng/mL) on day 0. These results suggest the baseline Se levels of patients suffering from sepsis could have impact on their recovery; where the patients started with low baseline Se levels resulted in greater mortality under current standard ICU treatment.

Kaplan-Meier analysis was conducted to compare the survivals between Placebo and High-Dose Selenium groups for the subjects with baseline Se level ≥110 ng/mL versus ≤ 80 ng/mL. Overall, the mortality rate was significantly higher in the SSD population, as compared to those subjects with baseline Se level ≥ 110 ng/mL (Figure 4). High-Dose Selenium treatment resulted in higher survival than Placebo in the SSD subjects (74.5% vs. 71.1%), however the difference was not statistically significant.

Interestingly, the survival rate of Placebo subjects with baseline Se ≥ 110 ng/mL was greater than that of the High-Dose Selenium treated subjects with baseline Se level ≤ 80 ng/mL (86.4% vs. 74.5%, odds ratio = 0.46, p ~ 0.08), although not statistically significant. High-Dose Selenium treated subjects with baseline Se level ≥ 110 ng/mL resulted in the highest survival rate (91.4%).

Figure 4: Kaplan-Meier Plots of Survival Rate in Septic Patients with Se Level ≤ 80 ng/mL and ≥ 110 ng/mL in Placebo and High-Dose Selenium Groups



Summary odds ratio and hazard ratio:

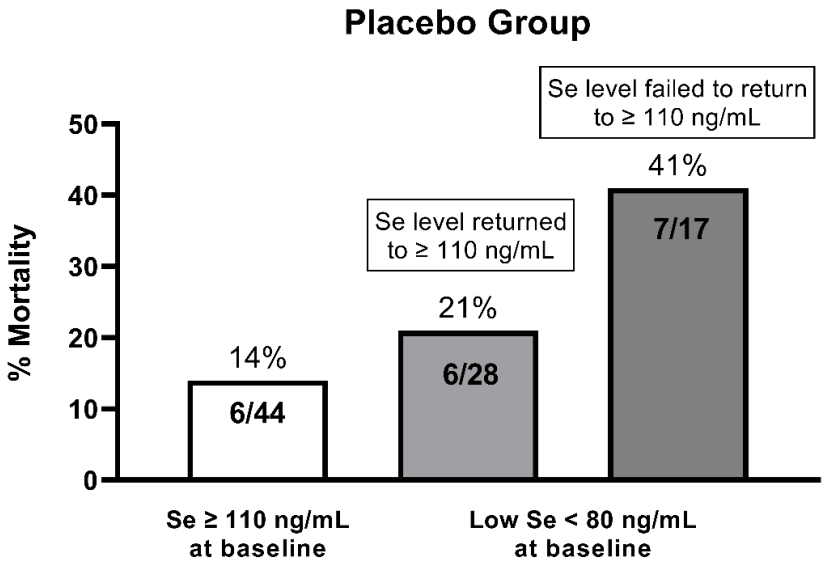
	Odds Ratio[1]		Hazard ratio[2]	
	Estimate	*p*-value	Estimate	*p*-value
High-Dose Se >110 vs. High-Dose Se <80	0.27	0.03	0.30	0.0496
High-Dose Se >110 vs. Placebo <80	0.23	0.02	0.27	0.026
Placebo >110 vs. Placebo <80	0.39	0.04	0.42	0.073
Placebo >110 vs. High-Dose Se <80	0.46	0.08	0.49	0.14

[1] Odd's Ratio: Calculated only from the 28-day all-cause mortality.

[2] Hazard Ratio: The relative ratio considering the time factor.

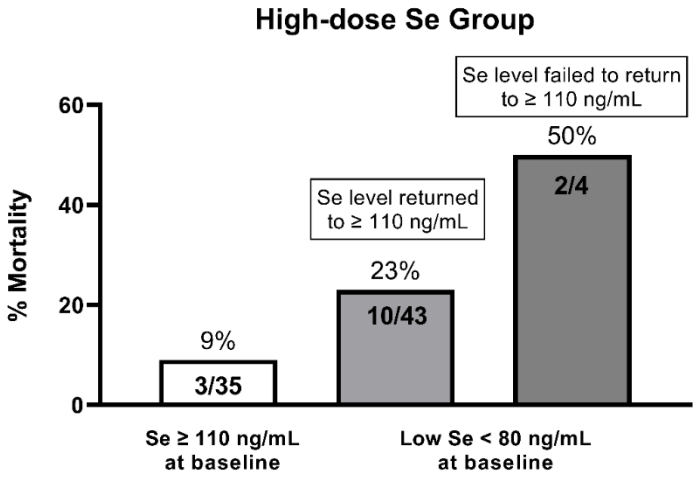
Figure 5 shows that the mortality of those SSD patients with gradual elevation of their Se levels to the normal range of ~110 ng/mL was 21.4% (n = 6 of 28), whereas high mortality of 41.2% was observed for the remaining 17 patients whose Se levels stayed low till the end of study. It was noted that the mortality rate of those patients in Placebo group with baseline Se ≥ 110 ng/mL was 14%. These results suggest that the septic patients would have better recovery (lower mortality from 41% to 21%) by ~2-fold when their serum Se levels increased to the range ≥ 110 ng/mL during the standard ICU treatment.

Figure 5: Mortality of Placebo Group Patients with Baseline Se Level < 80 ng/mL, Compared with Those Patients with Baseline Se Level ≥ 110 ng/mL.



It is also noted that, with treatment of High-Dose Seleniumto the SSD septic patients, the Se levels in almost all patients increased back to the normal range of 110 ng/mL, where the 28-day all-cause mortality was 23.3%. The mortality rate of the patients with Se baseline ≥ 110 ng/mL in High-Dose Seleniumgroup was 8.6% (3 deaths, out of 35 subjects) (Figure 6).

Figure 6: Mortality of High-Dose Selenium Group Patients with Baseline Se Level < 80 ng/mL, Compared with Those Patients with Baseline Se Level ≥ 110 ng/mL.



ORGAN FAILURE—CHANGES OF SOFA SCORES

The changes of SOFA scores were evaluated for the septic patients with Se level < 80 ng/mL. The baseline SOFA scores were 9.1 and 9.4 for Placebo control and High-Dose Selenium groups, respectively, where a very wide range of SOFA scores in these patients was observed, ranging from 2 to 18. Medium SOFA score was 9 for both groups. The SOFA scores from these patients were continuously monitored for 28 days. The results are summarized in Table 3.

Table 3: Summary of SOFA Scores in SSD Septic Patients after Treatment of High-Dose Selenium

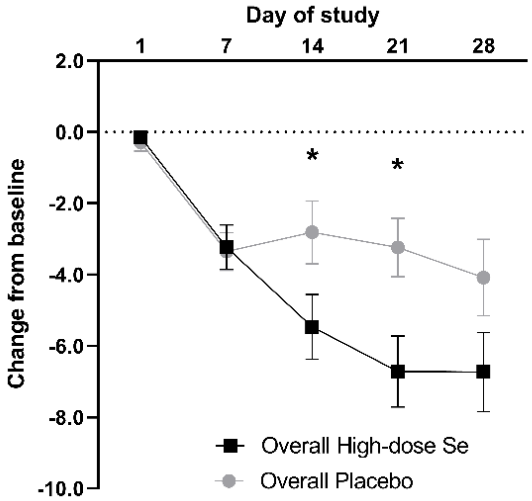
Days of Treatment	0	1	7	14	21	28
	Placebo Group					
N	45	45	40	27	17	12
Mean	9.11	8.82	5.30	5.89	4.82	3.92
SD	3.46	3.34	3.26	3.67	2.46	2.31
Range	2-17	2-17	0-16	1-17	1-10	0-9
Median	9	9	5	5	4	3.5
	High-Dose Selenium Group					
N	47	47	43	32	18	15
Mean	9.36	9.21	5.91	4.25	4.00	3.53
SD	3.77	3.77	3.77	3.99	3.03	2.47
Range	3-18	3-18	0-16	0-18	0-11	0-10
Median	9	8	5	3	3	3

The SOFA scores appeared to remain relatively unchanged for both groups after day 7. Although the trend of overall SOFA scores started to decrease after day 14, there were no statistical differences between Placebo and High-Dose Selenium groups through to the end of treatment on Day 28. However, the decreases of SOFA scores from baseline were more significant in the High-Dose Selenium treatment groups, as compared with Placebo group (Table 4). The differences in decreases of SOFA scores were statistically significant on Days 14 and 21 ($p < 0.05$).

Table 4: Summary of the Changes of SOFA Scores in SSD Septic Patients after Treatment of High-Dose Selenium

Days of Treatment		1	7	14	21	28
	Placebo Group					
Mean		-0.29	-3.35	-2.81	-3.24	-4.08
SD		1.60	3.40	4.59	3.38	3.73
	Overall High-Dose Selenium Group					
Mean		-0.15	-3.23	-5.47	-6.72	-6.73
SD		1.02	4.08	5.14	4.23	4.32
p-value		0.62	0.89	0.04	0.01	0.11

Figure 7: Changes of SOFA Scores in SSD patients with Se Level ≤ 80 ng/mL in Placebo and High-Dose Selenium Treatment Groups. Data are presented as Mean ± SEM.

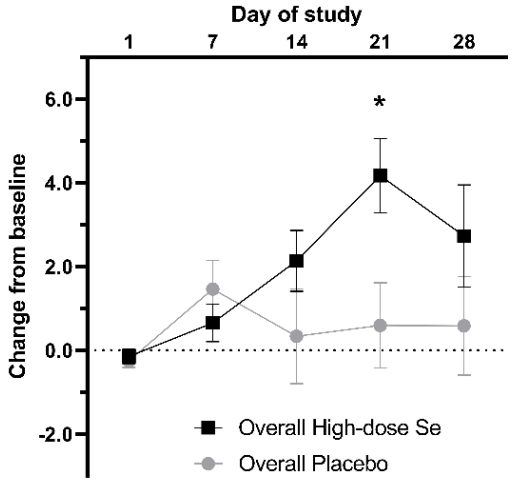


CHANGES OF GLASGOW COMA SCALE (GCS) SCORES

Along with the decrease of SOFA scores by the therapy with or without High-Dose Selenium, GCS scores increased over the duration of study in both groups. The mean baseline GCS scores were 9.1 (± 3.4) and 9.2 (± 3.2) for the septic patients in Placebo and High-Dose Selenium groups, respectively. However, for SSD septic patients, High-Dose

Selenium appeared to result in greater decrease in SOFA scores and increase of GCS scores, as compared to Placebo (Figure 8). The difference on Day 21 was statistically significant ($p < 0.05$) (Figure 8). Overall, the study results indicated that treatment with High-Dose Selenium for SSD septic patients significantly improved the recovery from coma.

Figure 8. Changes of GCS Scores in SSD Septic Patients with Se Level ≤ 80 ng/mL in Placebo and High-Dose Selenium Treatment Groups. Data are presented as Mean ± SEM.



*$p < 0.05$ by Student's t-test on each pair of sampling day.

SAFETY OF SEVERE SELENIUM DEFICIENCY PATIENTS RECEIVING HIGH-DOSE SELENIUM TREATMENT

Overall, the safety profiles of the severe selenium deficiency patients were comparable between High-Dose Selenium and Placebo groups, suggesting High-Dose Selenium was well-tolerated. A total of 2 subjects (4.3%) experienced High-Dose Selenium-related adverse events (AEs, such as phlebitis, rash) and no subject discontinued to receive High-Dose Selenium administration due to High-Dose Selenium-related AEs. No treatment-related serious SAEs occurred in High-Dose Selenium group.

There was only 1 injection site reaction reported in Placebo group and none in High-Dose Selenium group. No pyrexia was observed in either Placebo or High-Dose Selenium group.

PHARMACOKINETICS OF SELENIUM IN SEPTIC PATIENTS

The pharmacokinetics of Se was evaluated in septic patients following intravenous injection of high-dose selenium. The study results showed that very low serum concentrations of selenite were detected in all patients after receiving intravenous administration of high-dose selenium, indicating a very rapid conversion of selenite to selenium in human subjects.

The study results showed that the baseline serum Se levels in patients with sepsis or septic shock was low, 95.3 ± 30.0 ng/mL (mean ± SD, n = 167) in Placebo group and 94.0 ± 30.0 ng/mL in High-Dose Selenium group (n = 165). On day 1, all patients in High-

Dose Selenium group received a 30-min rapid intravenous infusion of 1,000 μg/day, followed by a slow infusion of 1,000 μg/day to 24 hr, and slow 24 h-infusion of 1,000 μg/d on subsequent days of treatment. The estimated steady state serum concentrations (Css) of Se in High-Dose Selenium-treated patients were in the range of ~211 ng/mL (~32% CV). The calculated systemic clearance (CL) was ~91.2 mL/day/kg, and the volume of distribution of Se at steady state (Vdss) was 590 mL/kg. The time of slow 24-h infusion required to reach steady state was 1.9 days, where the terminal half-life was ~4.5 days.

Discussion

In several early clinical investigations, the uses of selenite from low to high doses of selenium for treating sepsis and septic shock have disparate conclusions[21-27], likely due to the multiple major factors associated with the invasive surgery and/or pathological nature of the diseases[28].

In a previous study, a selenium formulation containing sodium selenite was investigated in a placebo-controlled randomized clinical trial in Germany to determine whether high-dose sodium selenite treatment, and procalcitonin-guided anti-infectious therapy in patients with severe sepsis affect mortality. The dose of 1,000 μg selenium daily was administered intravenously until discharge from the intensive care unit, but not longer than 21 days[27]. The study showed that neither high-dose intravenous administration of sodium selenite nor anti-infectious therapy guided by a procalcitonin algorithm effectively improved the therapeutic outcomes in patients with severe sepsis. These findings did not seem to support the high-dose sodium selenite in these patients, and the application of a procalcitonin-guided algorithm is treating sepsis was unclear.

A systemic review and meta-analysis study conducted by Manzanares on 21 randomized clinical trials revealed that the high dose selenium administered intravenously as monotherapy failed to improve the clinical outcomes such as mortality in the patients suffering from clinical illness[29].

However, our early study reported that the decrease in GPx-3 bioactivity observed in the septic patients was resulted from the significant sepsis-related decline of GPx-3 protein concentrations[30]. Serum glutathione peroxidase-3 (GPx-3) is known as a key selenoprotein with antioxidant properties. GPx-3 deficiency, along with low level of serum selenium, has been associated with sepsis. The mean GPx-3 bioactivity was 78.13U/l for patients with sepsis, significantly lower than normal subjects with 108.21U/l (p <0.0001). Similarly, the GPx-3 protein concentration was significantly lower in patients with sepsis than in normal subjects, with the mean GPx-3 value of 0.78 vs 3.10 μg/ml, respectively (p <0.0001).

Xia investigated the effectiveness of repeated administration of selenium as selenite via oral route in selenium-deficient Chinese subjects[31]. The results showed that the plasma selenium levels rose in direct proportion to the amount selenium administered as sodium selenite, also resulted in the increased in GPx activity in direct proportion to the amount of selenium until it reached optimal level. The study concluded that sodium selenite normalized the plasma selenium levels in selenium-deficient Chinese subjects and

consequently contributed to the increase of GPx activity levels.

Similar clinical findings were reported by Manzanares that high-dose of selenite increased the selenium concentration to physiologic and safe range in patients with systemic inflammatory response syndrome (SIRS) but were independent of dose[32]. The pharmacodynamic profiles showed that the maximum GPx-3 activity was reached after 3 – 7 days. No adverse events attributable to high-dose selenite were observed.

From above clinical results with the uses of high-dose selenium in critically ill patients such as sepsis, we therefore designed this study by recruiting a more homogenous patient population, while excluding the patients admitted for trauma or surgery and patients of malignant diseases. In addition, patients were administered with an initial bolus loading dose of selenium and a longer treatment duration.

Subjects with low Se levels due to the increased utilization, redistribution, and effluent losses from fluid resuscitation are common in the critically ill and are associated with increased oxidative stress and inflammation in septic patients[33,34]. In this study, the serum Se concentrations (94.9 ± 28.9 ng/mL) in all septic patients were confirmed to be lower than the normal range (e.g., 110–180 ng/mL). The patients with septic shock even had significantly lower baseline serum Se levels than the patients with sepsis (84.3 vs 98.2 ng/mL), with corresponding higher mortality rate of septic shock (32.5 vs 13.4%). All of the study results and clinical evidence suggest the importance of maintaining baseline Se levels at ≥ 110 ng/mL.

It was interested to observe that ~ 62% of patients in Placebo group had gradual increase of their serum Se concentrations up to 110 ng/mL level, without treatment of High-Dose Selenium or other Se product. These results indicate the slow redistribution or rebalance of Se from tissues/organs into systemic circulation in septic patients. With treatment of High-Dose Selenium, ~91% of patients with severe selenium deficiency were found to rapidly recover to the normal range of 110 ng/mL; however, surprisingly ~9% of patients remained at the low Se level, even receiving the repeated high doses of Se injections. The underlying causes of Se deficiency in sepsis patients remain to be further investigated.

The SOFA score evaluates the degree of organ dysfunction or failure, a notable feature of sepsis resulting from insufficient tissue perfusion and oxygen delivery[35]. Compared to Placebo whose SOFA scores remained relatively unchanged after day 7, High-Dose Selenium treatment resulted in a significant decrease from the baseline SOFA scores on days 14 and 21, particularly in the patients with SSD. The decrease of SOFA scores indicates the amelioration of organ dysfunction and thus suggests High-Dose Selenium improved disease recovery in these patients.

With regards to aggravation of renal impairment which is considered a concern in previous studies of high-dose Se supplementation[36,37], no negative effects on renal function were reported in septic patients after treatment with high dose of High-Dose Selenium. This study demonstrated the injection of high-dose selenium to be safe and well-tolerated by the patients with sepsis/septic shock, similar safety profile

reported by Manzanares[32], and only a few adverse events were observed throughout the study. Some patients had their serum Se level above 500 ng/mL, but no high-dose selenium-related adverse effects were reported. The patients with low Se levels appeared to be most benefited from the Se supplementation therapy.

In the future studies, the other biomarkers such as procalcitonin (PCT), C-reactive protein, and inflammatory cytokines like tumor necrosis factor-α (TNFα) may be applied to assess the recovery of patients with sepsis/septic shock after the treatment of High-Dose Selenium[38]. PCT is a more sensitive and specific biomarker for the diagnosis and monitoring of antimicrobial treatment efficacy for sepsis.

Conclusion

Mortality for patients with sepsis was apparently affected by the serum Se concentrations, particularly for the severe selenium deficiency (SSD) septic patients with baseline Se levels ≤ 80 ng/mL. High mortality rate 41–50% was observed in the sepsis patients constantly with low Se level ≤ 80 ng/mL, however the mortality was reduced to 21–23% when their serum Se levels were increased to ≥ 110 ng/mL. High-Dose Selenium treatment resulted in rapid restoration of Se levels and thus improved the survival of SSD septic patients. Relatively low mortality rate of 9–14% was observed in the sepsis patients starting with sufficient serum Se ≥ 110 ng/mL. Overall, the study results demonstrate the impact of insufficient selenium (e.g., ≤ 80 ng/mL) on the mortality of patients suffering from sepsis or septic shock. Treatment with high dose of selenium reduced the mortality of the septic subjects with baseline Se level ≤ 80 ng/mL.

Conflict of Interest:

Chin Ming Chen received financial support from TaiRx, Inc. for an investigator-initiated trial. The authors Ting-Yu Chao, Chun-Man Chen, and Yen-Ling Chen are employees of TaiRx, Inc., and Yi-Wen Chu and Du-Shieng Chien have direct ownership of TaiRx, Inc., which holds responsibility and the right for the pharmaceutical development of injection product of the high-dose selenium.

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References:

1. Singer M, Deutschman CS, Seymour CW, et al. The third international consensus definitions for sepsis and septic shock (sepsis-3). *JAMA*. 2016;315(8):801-810.

2. Cohen J, Vincent JL, Adhikari NKJ, et al. Sepsis: a roadmap for future research. *Lancet Infect Dis*. 2015;15(5):581-614.

3. Schlapbach LJ, Kissoon N, Alhawsawi A, et al. World Sepsis Day: a global agenda to target a leading cause of morbidity and mortality. *Am J Physiol Lung Cell and Mol Physiol*. 2020;319(3):L518-L522.

4. Rudd KE, Johnson SC, Agesa KM, et al. Global, regional, and national sepsis incidence and mortality, 1990–2017: analysis for the Global Burden of Disease Study. *The Lancet*. 2020;395(10219):200-211.

5. Jarczak D, Kluge S, Nierhaus A. Sepsis—Pathophysiology and therapeutic concepts. *Front Med (Lausanne)*. 2021;8:609.

6. Mahmoodpoor A, Faramarzi E, Reyhanifard A, et al. The effects of selenium supplementation on inflammatory markers in critically ill patients. *SN Appl Sci*. 2022;4 (12):326.

7. Berger MM, Chioléro RL. Antioxidant supplementation in sepsis and systemic inflammatory response syndrome. *Crit Care Med*. 2007;35(9).

8. Roman M, Jitaru P, Barbante C. Selenium biochemistry and its role for human health. *Metallomics*. 2013;6(1):25-54.

9. Manzanares W, Langlois PL, Heyland DK. Pharmaconutrition with selenium in critically ill patients. *Nutrition in Clinical Practice*. 2015;30(1):34-43.

10. Pfister C, Dawzcynski H, Schingale FJ. Sodium selenite and cancer related lymphedema: Biological and pharmacological effects. *J of Trace Elem in Med and Biol*. 2016;37:111-116.

11. Berger MM, Reymond MJ, Shenkin A, et al. Influence of slenium supplements on the post-traumatic alterations of the thyroid axis: a placebo-controlled trial. *Intensive Care Med*. 2001;27:91-100.

12. Stoppe C, Spillner J, Rossaint R, et al. Selenium blood concentrations in patients undergoing elective cardiac surgery and receiving perioperative sodium selenite. *Nutrients*. 2013;29(1):158-165.

13. Yang KH, Lee LT, Lee YS, et al. Serum selenium concentration is associated with metabolic factors in the elderly: a cross-sectional study. *Nutrition & Metabolism*. 2010;7:38.

14. Lin CC, Huang JF, Tsai LY, Huang YL. Selenium, Iron, Copper, and Zinc Levels and Copper-to-Zinc Ratios in Serum of Patients at Different Stages of Viral Hepatic Diseases. *Biological Trace Element Research*. 2006;109: 15-23.

15. Rannem T, Ladefoge K, Hylander E, et al. Selenium Depletion in Patients on Home Parenteral Nutrition. *Biological Trace Element Research*. 1993;39:81-89.

16. Ravn-Haren G, Bügel S, Krath BN, et al. A short-term intervention trial with selenate, selenium-enriched yeast and selenium-enriched milk: effects on oxidative defense regulation. *British Journal of Nutrition*. 2008;99(1):883-892.

17. Huang JF, Hsu CP, Ouyang CH, et al. The impact of selenium supplementation on

trauma patients—systematic review and meta-analysis. *Nutrients*. 2022;14(2).

18. Kong L, Wu Q, Liu B. The impact of selenium administration on severe sepsis or septic shock: a meta-analysis of randomized controlled trials. *Afr Health Sci*. 2021;21(1): 277-285.

19. Strachan S, Wyncoll D. Why might selenium supplementation be beneficial in sepsis? *J Intensive Care Soc*. 2009;10(1):38-43.

20. Dellinger RP, Levy M, Rhodes A, et al. Surviving sepsis campaign: International guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med*. 2013;39(2).

21. Zimmermann T, Albrecht S, Kühne H, et. al., Selensubstitution bei Sepsispadenten. *Medizinisehe Klinik*. 1997;92(Suppl. III):3-4.

22. Angstwurm MW, Engelmann L, Zimmermann T, et al. 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*. 2007;35:118-126.

23. Manzanares W, Biestro A, Torre M, et al. High-dose selenium reduces ventilator-associated pneumonia and illness severity in critically ill patients with systemic inflammation. *Intensive Care Med*. 2011;37: 1120-1127.

24. Andrews PJ, Avenell A, Noble DW, et al. Randomised trial of glutamine, selenium, or both, to supplement parenteral nutrition for critically ill patients. *BMJ*. 2011;342:d1542.

25. González CM, Luna AH, Villalobos JA, et al. Efecto antiinflamatorio del selenio en pacientes sépticos. *Rev Asoc Mex Med Crit y Ter Int*. 2009;23(4):199-205.

26. Forceville X, Laviolle B, Annane D, et al. Effects of high doses of selenium, as sodium selenite, in septic shock: a placebo-controlled, randomized, double-blind, phase II study. *Critical Care* 2007;11(4):R73.

27. Bloos F, Trips E, Nierhaus A, et al. 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*. 2016;176(9):1266-76.

28. Huang TS, Shyu YC, Chen HY, et al. Effect of Parenteral Selenium Supplementation in Critically Ill Patients: A Systematic Review and Meta-Analysis. *PLoS One*. 2013;8(1):e54431.

29. Manzanares W, Lemieux M, Elke G, et al. High-dose intravenous selenium does not improve clinical outcomes in the critically ill: a systematic review and meta-analysis. *Critical Care* 2016;20:356.

30. Lee WJ, Chen YL, Chu YW, Chien DS. Comparison of glutathione peroxidase-3 protein expression and enzyme bioactivity in normal subjects and patients with sepsis. *Clin Chim Acta* 2019;489:177-182.

31. Xia Y, Hill KE, Byrne DW, et al. Effectiveness of selenium supplements in a low-selenium area of China. *Am J Clin Nutr*. 2005;81(4):829-34.

32. Manzanares W, Biestro A, Galusso F, et al. High-dose selenium for critically ill patients with systemic inflammation: Pharmacokinetics and pharmacodynamics of selenious acid: A pilot study. *Nutrition*. 2010;26:634-640.

33. Mertens K, Lowes DA, Webster NR, et al. Low zinc and selenium concentrations in

sepsis are associated with oxidative damage and inflammation. *Br J Anaesth*. 2015;114(6):990-999.

34. Fiaccadori E, Sabatino A, Barazzoni R, et al. ESPEN guideline on clinical nutrition in hospitalized patients with acute or chronic kidney disease. *Clin Nutr*. 2021;40(4):1644-1668.

35. Gyawali B, Ramakrishna K, Dhamoon AS. Sepsis: The evolution in definition, pathophysiology, and management. *SAGE Open Med*. 2019;7:205031211983504.

36. Bloos F, Trips E, Nierhaus A, et al. 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*. 2016;176(9):1266-1276.

37. Chelkeba L, Ahmadi A, Abdollahi M, et al. The effect of high-dose parenteral sodium selenite in critically ill patients following sepsis: a clinical and mechanistic study. *Indian J Crit Care Med*. 2017;21:287-293.

38. Vijayan AL, Ravindran S, Saikant R, Lakshmi S, Kartik R, Manoj G. Procalcitonin: a promising diagnostic marker for sepsis and antibiotic therapy. *J Intensive Care*. 2017;5(1).