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*

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Objective: **Sepsis is associated with an increase in reactive oxygen species and low endogenous antioxidative capacity. We postulated that high-dose supplementation of sodium-selenite would improve the outcome of patients with severe sepsis and septic shock.**

Design: **Prospective randomized, placebo-controlled, multiple-center trial.**

Setting: **Eleven intensive care units in Germany.**

Patients: **Patients were 249 patients with severe systemic inflammatory response syndrome, sepsis, and septic shock and an Acute Physiology and Chronic Health Evaluation (APACHE) III score >70.**

Interventions: **Patients received 1000 μg of sodium-selenite as a 30-min bolus injection, followed by 14 daily continuous infusions of 1000 μg intravenously, or placebo.**

Measurements and Main Results: **The primary end point was 28-day mortality; secondary end points were survival time and clinical course of APACHE III and logistic organ dysfunction system scores. In addition, selenium levels in serum, whole blood, and urine as well as serum gluthation-peroxidase-3 activity were measured. From 249 patients included, 11 patients had to be excluded. The intention-to-treat analysis of the remaining 238 patients revealed a mortality rate of 50.0% in the placebo group and 39.7% in the selenium-treated group ($p = .109$; odds ratio, 0.66; confidence interval, 0.39–1.1). A further 49 patients had to be excluded before the final analysis because of severe violations of the study protocol. In the remaining 92 patients of the study group, the 28-day mortality rate was significantly reduced to 42.4% compared with 56.7% in 97 patients of the placebo group ($p = .049$, odds ratio, 0.56; confidence interval, 0.32–1.00). In predefined subgroup analyses, the mortality rate was significantly reduced in patients with septic shock with disseminated intravascular coagulation (n = 82, $p = .018$) as well as in the most critically ill patients with an APACHE III score ≥102 (>75% quartile, n = 54, $p = .040$) or in patients with more than three organ dysfunctions (n = 83, $p = .039$). Whole blood selenium concentrations and glutathione peroxidase-3 activity were within the upper normal range during selenium treatment, whereas they remained significantly low in the placebo group. There were no side effects observed due to high-dose sodium-selenite treatment.**

Conclusions: **The adjuvant treatment of patients with high-dose sodium-selenite reduces mortality rate in patients with severe sepsis or septic shock. (Crit Care Med 2007; 35:118–126)**

KEY WORDS: selenium; antioxidants; systemic inflammatory response syndrome; sepsis; septic shock; organ failure

The mortality rate in patients with sepsis and septic shock is still between 28% and 50% (1), and efforts to reduce mortality are a great challenge in intensive care medicine (2, 3). Although intensive insulin treatment (4), substitution of activated protein C (5), and supplementation of hydrocortisone in patients with reduced adrenal reserve in septic shock (6, 7) have been shown to reduce the mortality rate in severe sepsis and septic shock, it is still unsatisfying.

Besides cytokine activation, oxidative stress and free oxygen species might con-

*See also p. 306.

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tribute to the development of multiple organ failure in septic shock (8). Reactive oxygen species and reactive nitrogen species have been shown to modulate cell signaling, proliferation, apoptosis, and cell protection (9, 10). The selenium-dependent glutathione-peroxidases (GPx) as well as thioredoxin reductases are important compounds responsible for the maintenance of the redox system in all cells including the immune-competent cells. According to present knowledge, the activity of these enzymes is mainly regulated by the availability of selenium (11–14). During severe oxidative stress like sepsis or septic shock, the requirement of selenium might be increased, as patients with systemic inflammatory activity (SIRS), sepsis, and septic shock exhibit low selenium and GPx activities. The GPx-3 activity, which is the main GPx activity in serum, is negatively correlated with the severity of the diseases (15, 16). In preterm infants, a selenium supplementation decreased morbidity (17, 18).

A recently published meta-analysis of all available small intervention studies with selenium in critically ill patients revealed a tendency toward mortality reduction ($Z = 1.70$; $p = .09$), with the best results obtained with high doses of sodium-selenite (19).

We present the results of a first multiple-center, prospective, double-blind, placebo-controlled study, the Selenium in Intensive Care (SIC) study, where the efficacy and safety of a high-dose selenium supplementation in patients with severe SIRS, sepsis, and septic shock are shown.

METHODS

Study Design

The study was designed as a phase III, multiple-center, double-blind, randomized placebo-controlled trial. All patients fulfilling the inclusion criteria were enrolled in the study. Eleven independent German intensive care units (medical, surgical, and anesthetic) participated in the trial. The study design was approved by the local ethic committee of each single center and conformed with ethical guidelines (Declaration of Helsinki) and the International Conference on Harmonization (ICH), and all patient files were monitored by an external institute (GKM, Gesellschaft für Therapieforschung mbH Munich, Germany). Data were collected by independent data managers and compared with the case report form.

Patients were randomly assigned to treatment (Se1) or placebo (Se0). The study group (Se1) received 1000 μg of sodium-selenite within 30 mins intravenously followed by 1000 μg of sodium-selenite during 24 hrs continuously for 14 days; thus, the total amount of selenium was 15 mg within 14 days. This dosage was chosen on the basis of efficacy in previous pilot studies (20, 21) and later was shown to be effective in a meta-analysis (17). The placebo group (Se0) received sodium chloride 0.9% in the same regimen. Additional selenium supplementation up to 100 μg of selenium per day, together with other trace elements during parenteral nutrition, was allowed in all patients.

The inclusion criteria were as follows:

Males and females ≥18 yrs with an Acute Physiology and Chronic Health Evaluation (APACHE) III score (22) ≥70 and at least two of the following criteria (23):

Rectal body temperature >38°C or hypothermia <36°C

Heart rate >90 beats/min

Respiratory frequency >20' and $Paco_2$ <32 mm Hg (<4.3 kPa)

Leukocytes >12,000/μL or <4 000/μL or >10% immature leukocytes

Decrease of platelet count >50% within the first 24 hrs or platelets <150,000/μL at admission

Admission into the study after diagnosis within 24 hrs

Beginning of treatment within 1 hr after inclusion

Informed consent either from the patient or the relative/close friend

The exclusion criteria were as follows:

Pregnancy

Missing informed consent of the patient or the relative/intimate friend of the patient

Withdrawal of informed consent by patient or next of kin after inclusion into the study

Participation in any other clinical trial currently or within the last 30 days

Prior participation in this clinical trial

Cerebral injury due to hypoxia after cardiopulmonary resuscitation

Primary concomitant disease with an expected high mortality within 2 months

Do-not-resuscitate code

Malignant primary disease as the cause of SIRS or sepsis, for example, agranulocytosis as a result of chemotherapy or idiopathic bone marrow aplasia

Hemorrhagic-necrotizing pancreatitis without infectious complications

Treatment Assignments

Patients were randomly assigned in a one-to-one ratio to receive vials containing 48 mL of study medication intravenously. The study medication had to be started within 1 hr after inclusion, with a bolus injection of one vial during 30 mins, followed by a continuous infusion (2 mL/hr) for 14 days. Patients otherwise were treated according to the best practice, including parenteral or enteral nutrition together with vitamins and trace elements as necessary. No further specific directives for medical treatment, mechanical ventilation, or dialysis procedures were provided to the study centers.

Predefined severe protocol violations were as follows: study drug administration delay of >6 hrs after inclusion, interruption of study drug administration for >6 hrs, missing bolus administration, number of vial administrations lower than defined, or administration of selenite containing solutions >100 μg/day.

End Points and Safety Criteria

The primary end point was 28-day mortality. Secondary end points were as follows:

1. Time of survival after enrollment
2. Variable part of the APACHE III score (22), percentage of change between day 1 and last visit
3. Logistic organ dysfunction system score (24) at all visits or last available visit
4. Incidence of renal failure within the 28-day survey
5. Days of dialysis or chronic veno-venous hemofiltration dialysis
6. Incidence of cardiovascular failure defined as the demand for vasoactive drugs despite volume substitution
7. Number of days with vasopressor therapy to maintain adequate tissue perfusion
8. Days of mechanical ventilation
9. Incidence of nosocomial pneumonia
10. Incidence of acute respiratory distress syndrome
11. Incidence of reinfection
12. Duration of stay (days) in the intensive care unit for all patients
13. Analyses of subgroups (age, gender, severity of illness, number of organ failure, intensive insulin treatment, source of infection, surgical vs. internal) (Table 1)

The tertiary end points were the determination of selenium levels in whole blood, serum, and 24-hr urine excretion and GPx-3 activity on days 1, 3, 7, 14, 21, and 28.

The safety criteria included all adverse events like changes in vital parameters, acid-base balance, liver and kidney function tests, and hematologic variables, especially changes in whole blood and serum selenium concentrations on days 1, 3, 7, 14, 21, and 28 as well

Table 1. Mortality rate of per-protocol population

	Se1 (n = 92)		Se0 (n = 97)		Comparison of Treatment Groups				
Visit, Days	No.	%	No.	%	χ^2 Test	*p* Value	Odds Ratio	Lower CI	Upper CI
7	20	21.7	28	28.9	1.27	.261	0.68	0.35	1.33
14	28	30.4	42	43.3	3.35	.067	0.57	0.31	1.04
21	35	38.0	51	52.6	4.02	.045	0.55	0.31	0.99
28	39	42.4	55	56.7	3.87	.049	0.56	0.32	1.00

Se1, treatment group; Se2, placebo group; CI, confidence interval.

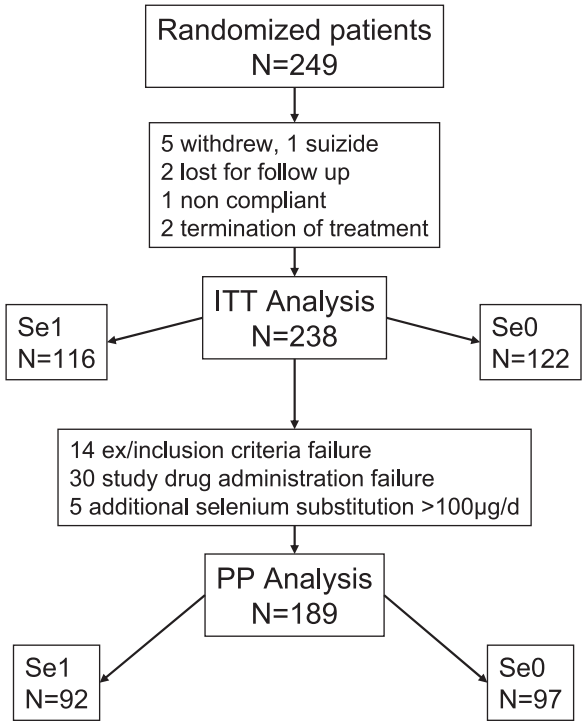

Figure 1. Trial profile. *Se1*, treatment group; *Se0*, placebo group; *ITT*, intention to treat; *PP*, per protocol.

as selenium excretion in the urine until day 21. The safety collective included all randomized patients (n = 246).

Evaluation of Patients and Laboratory Tests

Patients were followed for 28 days after inclusion. Baseline characteristics including demographic information, preexisting health conditions, organ function, markers of disease severity, infection, and laboratory tests were assessed within 24 hrs before study drug administration and on days 3, 7, 14, 21, and 28.

Probes of EDTA blood, serum, and samples of a 24-hr urinary collection were obtained at baseline and on days 3, 7, 14, 21, and 28 for the blinded determination of sodium-selenite (25) as well as GPx-3 activities (26) in a central independent reference laboratory (Institute of Clinical Chemistry, Friedrich-Schiller University Jena, Germany). The reference values for selenium for the normal population are serum selenium 0.72–1.33 μmol/L, in whole blood 0.96–1.78 μmol/L, and in 24-hr urine samples 0.02–0.79 μmol/L. The normal reference GPx-3 activity is 96–150 units/L.

Microbial samples were analyzed in the local institutes of bacteriology at the day of inclusion and throughout day 28, if new infections were suspected. All other routine laboratory tests also were determined in local laboratories.

Sepsis was defined according to the established sepsis criteria (23). Septic shock was defined as hypotension, not sufficiently responding to volume replacement, requirement for vasopressors, and decrease in platelet counts >50% within the first 24 hrs.

Statistical Analysis

Randomization was done using the program BiAS for Windows (version 7.0) and the SAS procedure PROC PLAN. Before we broke the code, the monitoring of all files and case report forms, data management, and the complete statistical analysis were done by an independent external institute (GKM, Gesellschaft für Therapieforschung mbH Munich, Germany). The data management was performed using ACCESS 2000 and the statistical analysis was performed using SAS (version 9.1).

The null hypothesis (H0) and alternative hypothesis (H1) are as follows: H0, $p1 \geq p0$; and H1, $p1 < p0$, where p1 stands for the 28-day mortality rate under sodium-selenite and p0 for the 28-day mortality rate under placebo. The chi-square test was used, which is equivalent to the two-tailed Z-test (normal approximation). According to the one-tailed testing situation, a chi-square test at a significance level of $\alpha = .05$ was used. This is equivalent to the one-tailed Z-test with $\alpha = .025$.

The sample size was calculated at the base of 80% power to detect a 20% reduction in 28-day mortality rate in the study population according to the results of the pilot studies (20, 21). For the statistical analysis of the primary efficacy criterion, a one-tailed significance test at a significance level of $\alpha = .025$ was performed. The trial was designed to enroll 196 eligible patients.

A planned interim analysis was carried out after 120 patients had been enrolled according to the method of O'Brian-Fleming with a significance level of $\alpha 1 = .0027$; the final statistical analysis was conducted with $\alpha 2 = .0246$. Thus, a global significance level of $\alpha = .025$ was guaranteed. The one-tailed significance level $\alpha 2 = .0246$ corresponds to a significance level of $\alpha 2 = .0492$ for the chi-square test. The test statistical analysis of all secondary efficacy criteria and the tertiary efficacy criterion was conducted using two-tailed significance tests with $\alpha = .05$.

For efficacy criteria, the length of stay in the intensive care unit as well as the incidence rate and number of days of acute renal failure, acute circulatory failure, development of pneumonia, and development of acute respiratory distress syndrome was assessed.

Comparisons of treatment groups with regard to incidence rates were done via statistical testing and by calculating the odds ratio (OR).

The statistical analysis of safety and tolerability criteria was performed descriptively.

RESULTS

Study Population

Between December 1999 and October 2004, 249 patients were randomized and enrolled in the study (Fig. 1). Before final analysis, three patients and the relatives of two patients withdrew consent after

inclusion (2.0%), one patient committed suicide, two patients were lost for follow-up, in one patient treatment was terminated by the physicians because of a do-not-resuscitation decision, one patient was identified to suffer from acute leukemia, and one patient was incompliant. Thus, 11 patients were excluded, leaving 238 patients randomized to selenium (n = 116) or placebo (n = 122) for the intention-to-treat analysis (Table 2).

From these, 49 patients had to be excluded before breaking the code because of not fulfilling the inclusion criteria (n = 14) or severe violation of the study protocol (n = 35): study medication delayed or interrupted for >6 hrs (n = 13), no bolus administration (n = 6), number of vial administration lower than defined (n = 11), and administration of additional sodium-selenite >100 μg/day (n = 5). Therefore, the final per-protocol population consisted of 189 patients, 92 in the study group (Se1) and 97 in the placebo group (Se0).

Characterization of Patients

The characterization of patients is shown in Table 2 for all randomized patients and in Table 3 for the patients treated per protocol. Age distribution, body mass index, and severity of illness assessed by APACHE III score or organ dysfunction defined by logistic organ dysfunction system score were comparable between the groups. The SIRS criteria were fulfilled in 97.9% of patients treated per protocol.

Due to the lower number, women were not equally distributed between Se1 (18 of 92; 19.6%) and Se0 (33 of 97; 34%; $p = .025$, chi-square-test). In addition, the mean age of women (69 ± 14.2 yrs) was higher than in men (62 ± 13.3 yrs): in Se1 33.3% and in Se0 21.2% of women were older than 80 yrs, compared with 5.4% and 9.4% of men.

The mean body mass index (BMI) was similar in both groups, but in the Se1 group 10.3% of patients had a low (<20 kg/m^2) BMI and 7.5% a high BMI (>40 kg/m^2) compared with Se0 (5.1% low BMI, 1.5% high BMI).

The mean whole blood selenium concentrations (0.74 ± 0.22 μmol/L in Se1, 0.74 ± 0.16 μmol/L in Se0) as well as serum selenium concentrations (0.48 ± 0.23 μmol/L in Se1, 0.46 ± 0.16 μmol/L in Se0) were similarly low in both groups at admission. Also, the mean C-reactive protein and procalcitonin levels were similar in both groups.

Intensive insulin treatment was received by 54 patients (25 of Se1 and 29 of Se0), documented by blood glucose levels <120 mg/dL in more than ten determinations per day. Hydrocortisone (200 mg/day) was substituted in 56 Se1 and 67 Se0 patients. No patient was treated with activated protein C.

Table 2. Characterization of randomized patients

Variable	Se1	Se0	Total
Demographics			
Age, yrs, n (mean ± SD)	116 (63.9 ± 13.8)	122 (65.3 ± 14.1)	238 (64.6 ± 14.0)
Male gender, n (%)	86 (74.1)	76 (62.3)	162 (68.1)
Body mass index, kg/m^2, n (mean ± SD)	107 (27.1 ± 6.8)	117 (26.7 ± 5.0)	224 (26.8 ± 5.9)
Severity of illness			
APACHE III score, total, n (mean ± SD)	116 (92.2 ± 19.2)	122 (91.2 ± 20.5)	238 (91.7 ± 19.8)
<80, n (%)	32 (27.6)	36 (29.5)	68 (28.6)
80–90, n (%)	25 (21.6)	32 (26.2)	57 (23.9)
90–100, n (%)	24 (20.7)	21 (17.2)	45 (18.9)
100–110, n (%)	16 (13.8)	14 (11.5)	30 (12.6)
≥110, n (%)	19 (16.4)	19 (15.6)	38 (16.0)
LOD score, total, n (mean ± SD)	116 (7.7 ± 3.1)	122 (7.7 ± 3.0)	238 (7.7 ± 3.1)
<5, n (%)	13 (11.2)	19 (15.6)	32 (13.4)
5–10, n (%)	69 (59.5)	74 (60.7)	143 (60.1)
≥10, n (%)	34 (29.3)	29 (23.8)	63 (26.5)
Number of failing organs, n (%)			
1	2 (1.7)	4 (3.3)	6 (2.5)
2	14 (12.1)	22 (18.0)	36 (15.1)
3	54 (46.6)	47 (38.5)	101 (42.4)
4	30 (25.9)	36 (29.5)	66 (27.7)
5	14 (12.1)	10 (8.2)	24 (10.1)
6	2 (1.7)	3 (2.5)	5 (2.1)
Comorbidities, n (%)[a]	116	122	238
Cardiovascular disease	85 (73.3)	96 (78.7)	181 (76.1)
COPD	36 (31.0)	39 (32.0)	75 (31.5)
Chronic renal disease	58 (50.0)	55 (45.0)	113 (47.5)
Diabetes mellitus	33 (28.4)	43 (35.2)	76 (62.2)
Liver disease	28 (24.1)	25 (20.4)	53 (22.3)
Malignant disease	23 (19.8)	27 (22.1)	50 (21.0)
Site of infection, n (%)	90	109	199
Pneumonia	59 (65.6)	67 (61.5)	127 (63.8)
Peritonitis	20 (22.2)	28 (25.7)	48 (24.1)
Pyelonephritis	1 (1.1)	1 (0.9)	2 (1.0)
Skin and soft tissue infection	9 (10.0)	11 (10.1)	20 (10.0)
Endocarditis	1 (1.1)	2 (1.8)	3 (1.5)
Pathogen, n (%)[a]			
Gram positive	42 (36.2)	31 (25.4)	73 (30.7)
Gram negative	27 (23.3)	29 (23.8)	56 (23.5)
Fungi	8 (6.9)	3 (2.5)	11 (4.6)
Not classifiable	0 (0.0)	1 (0.8)	1 (0.4)
Viruses	1 (0.4)	0 (0.0)	1 (0.2)
Parasite	1 (0.9)	0 (0.0)	1 (0.4)

Se1, treatment group; Se0, placebo group; APACHE, Acute Physiology and Chronic Health Evaluation; LOD, logistic organ dysfunction system; COPD, chronic obstructive pulmonary disorder.
[a]Patients may have more than one comorbidity or pathogen.

Efficacy

28-Day Mortality. The interim analysis after inclusion of 120 patients revealed a reduction in mortality rate from 48.4% in the placebo group (n = 62) to 37.9% in the treatment group (n = 58; $p = .25$; OR, 0.65, 95% confidence interval [CI], 0.31–1.35).

In the intention-to-treat analysis (n = 238), 46 of 116 patients in the Se1 group and 61 of 122 Se0 patients died ($p = .109$; OR, 0.66; 95% CI, 0.39–1.10). The estimated mean survival time was 20.3 days in group Se1 and 17.6 days in group Se0 ($p = .098$).

In the per-protocol analysis (n = 189), 39 of 92 (42.4%) patients in the Se1 group compared with 55 of 97 (56.7%) in the Se0 group died within 28 days

Table 3. Characterization of the per-protocol population at study entry

Variable	Se1	Se0	Total
Demographics			
Age, yrs, n (mean ± SD)	92 (64.1 ± 13.1)	97 (65.9 ± 14.0)	189 (65.0 ± 13.5)
<50, n (%)	16 (17.4)	12 (12.4)	28 (14.8)
50–65, n (%)	26 (28.3)	27 (27.8)	53 (28.0)
65–80, n (%)	40 (43.5)	45 (46.4)	85 (45.0)
>80, n (%)	10 (10.9)	13 (13.4)	23 (12.2)
Males, n (%)	74 (80.4)	64 (66.0)	138 (73.0)
Females, n (%)	18 (19.6)	33 (34.0)	51 (27.0)
Males >80 yrs, n (%)	4 (5.4)	6 (9.4)	10 (7.2)
Females >80 years, n (%)	6 (33.3)	7 (21.2)	13 (25.5)
Body mass index, kg/m^2, n (mean ± SD)	84 (27.5 ± 7.1)	94 (26.7 ± 5.2)	178 (27.1 ± 6.2)
Severity of illness			
SIRS criteria fullfilled, n (%)	89 (96.7)	96 (99.0)	185 (97.9)
APACHE III score, n (mean ± SD)	92 (94.5 ± 18.5)	97 (94.2 ± 20.1)	189 (94.3 ± 19.2)
Logistic organ dysfunction score, n (mean ± SD)	92 (7.9 ± 3.3)	97 (8.0 ± 3.0)	189 (7.9 ± 3.1)
Number of organs failing, n (%)			
1–2	11 (12.0)	20 (20.6)	31 (16.4)
3	41 (44.6)	34 (35.1)	75 (39.7)
4	26 (28.3)	30 (30.9)	56 (29.6)
5–6	14 (15.2)	13 (13.4)	27 (14.3)
Biochemical markers			
Procalcitonin (normal <0.5 μg/L), n (mean ± SD)	61 (35.0 ± 106.1)	67 (34.9 ± 76.5)	128
CRP (normal <5 mg/L), n (mean ± SD)	91 (200.3 ± 119.1)	97 (193.9 ± 124.9)	188
Serum selenium (0.72–1.33 μM/L), n (median)	91 (0.48)	97 (0.45)	188
Pathogen, n			
Gram positive	26	19	45
Gram negative	12	12	24

Se1, treatment group; Se2, placebo group; SIRS, systemic inflammatory response syndrome; APACHE, Acute Physiology and Chronic Health Evaluation; CRP, C-reactive protein.

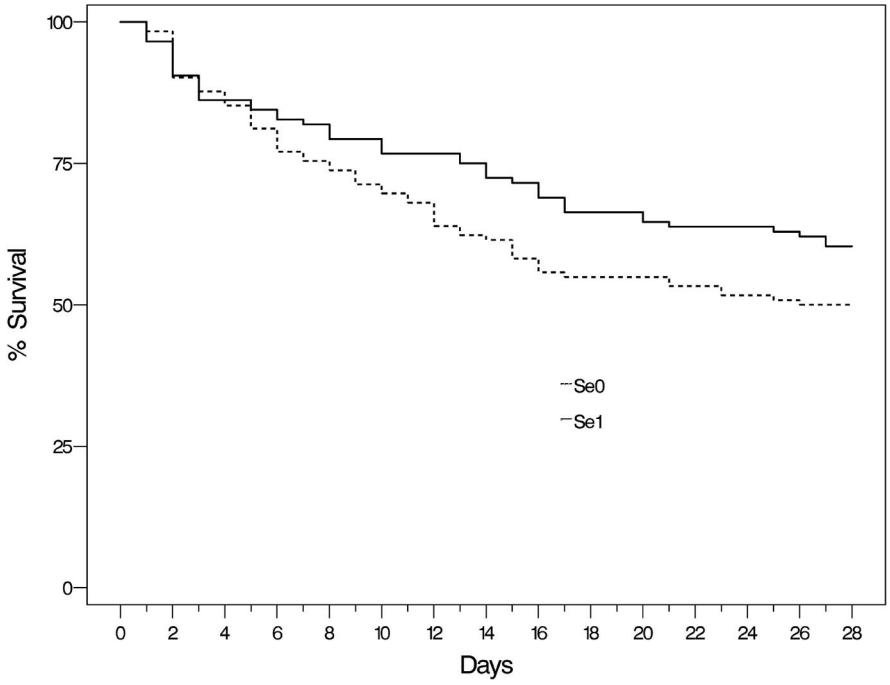

Figure 2. Survival time. Survival curves in patients of the intention-to-treat analysis were generated by the Kaplan-Meier curve. Difference between groups was calculated by the log rank test. The estimated mean survival time was 20.3 days in treated patients (*solid line*) compared with 17.6 days in the placebo group (*dotted line*) ($p = .098$). *Se1*, treatment group; *Se0*, placebo group.

($p = .049$; OR, 0.56; 95% CI, 0.32–1.00). Thus, the absolute reduction in mortality was 14.3%; the number of patients needed to treat was seven. The differences in mortality rate between both groups was already significantly lower at day 21 in the treatment group (Se1) compared with placebo ($p = .045$; OR, 0.55; 95% CI, 0.31–0.99) (Table 1). The estimated mean survival time was 19.7 days in Se1 patients compared with 16.4 days in the Se0 group ($p = .0476$) (Fig. 2). The proportion of deaths during the first 2 days after inclusion was similar in the two treatment groups. After exclusion of these deaths (16 of 92 in Se1 and 15 of 97 in Se0), the absolute mortality reduction with adjuvant selenium treatment was 17.6% ($p = .024$; OR, 0.48; 95% CI, 0.25–0.91).

Predefined Subgroup Analyses

Those patients with an APACHE III score >102 (75% quartile of all patients, n = 27 in each group) revealed a significantly lower mortality rate ($p = .040$; OR, 0.28; 95% CI, 0.08–0.97) in the Se1 group (Table 4). Those patients with more than three organ failures also had a significantly improved survival rate of 22.6% (23 of 40 in Se1 group and 15 of 43 in Se0 group; $p = .039$; OR, 0.40; 95% CI, 0.16–0.96). Patients with the sepsis criteria, a continuous decrease in platelet counts below 50,000/μL (indicating disseminated intravascular coagulation), and septic shock had a survival rate of 59.5% (22 of 37) in the Se1 group, compared with 33.3% (15 of 45) in the Se0 group ($p = .018$; OR, 0.34; 95% CI, 0.14–0.84). Patients receiving intensified insulin treatment with tight glucose control (n = 54) had a significantly lower mortality rate (−28.4%) in the Se1 group compared with placebo (−8.2%; $p = .034$; OR, 0.30; 95% CI, 0.10–0.93). There were no significant differences in the survival rate of patients identified with pneumonia or peritonitis alone, without other systemic signs of septic shock, or surgical or internal medicine patients.

Whole Blood Selenium and Mortality

Mortality rate was inversely correlated with the whole blood selenium concentrations in both groups. In Se1 patients, the mortality rate was 50.0%, if whole blood selenium was constantly within the

Table 4. 28-day mortality rate in predefined subgroups of per protocol analysis

	Se1			Se0			Absolute Difference	Significance	Odds Ratio
	No.	Deceased	%	No.	Deceased	%			
Age distribution									
<50	16	3	18.8	12	3	25.0	−6.3	0.690	0.69 (0.11–4.24)
50 to <65	26	8	30.8	27	12	44.4	−13.7	0.305	0.56 (0.18–1.71)
65 to <80	40	19	47.5	45	29	64.4	−16.9	0.116	0.50 (0.21–1.19)
>80	10	9	90.0	13	11	84.6	5.4	0.704	1.64 (0.13–21.1)
Gender									
Males	74	25	33.8	64	35	54.7	−20.9	0.014	0.42 (0.21–0.84)
Females	18	14	77.8	33	20	60.6	17.2	0.214	2.28 (0.61–8.45)
APACHE III score 75% quartile									
<102	65	24	36.9	70	33	47.1	−10.2	0.230	0.66 (0.33–1.31)
≥102	27	15	55.6	27	22	81.5	−25.9	0.040	0.28 (0.08–0.97)
No. of organs failing									
1–3	52	22	42.3	54	27	50.0	−7.7	0.427	0.73 (0.34–1.58)
4–6	40	17	42.5	43	28	65.1	−22.6	0.039	0.40 (0.16–0.96)
Type of admission									
Surgical	37	19	51.4	38	24	63.2	−11.8	0.301	0.62 (0.24–1.55)
Internal	49	19	38.8	56	30	53.6	−14.8	0.130	0.55 (0.25–1.20)
Gram staining									
Positive	26	9	34.6	19	8	42.1	−7.5	0.609	0.73 (0.22–2.46)
Negative	12	6	50.0	12	8	66.7	−16.7	0.408	0.50 (0.10–2.60)
Type of infection									
Pneumonia	38	14	36.8	44	25	56.8	−20.0	0.071	0.44 (0.18–1.08)
Peritonitis	13	5	38.5	11	6	54.5	−16.1	0.431	0.52 (0.1–2.66)
Death attributable to sepsis	87	34	39.1	91	49	53.8	−14.8	0.048	0.55 (0.30–1.00)
Septic shock with DIC[b]									
Yes	37	15	40.5	45	30	66.7	−26.1	0.018	0.34 (0.14–0.84)
No	55	24	43.6	52	25	48.1	−4.4	0.645	0.84 (0.39–1.8)
Tight blood glucose control									
Yes	25	11	44.0	29	21	72.4	−28.4	0.034	0.30 (0.10–0.93)
No	67	28	41.8	68	34	50.0	−8.2	0.339	0.72 (0.36–1.42)

Se1, treatment group; Se2, placebo group; APACHE, Acute Physiology and Chronic Health Evaluation; DIC, disseminated intravascular coagulation. [a]Mean (95% confidence interval); [b]decrease of platelet count >50% or platelet count <150,000/μL.

Table 5. Relationship between the maximal whole blood selenium concentrations after day 1 and mortality rate in selenium-treated group (Se1) and control group (Se0) except those patients who died within the first 2 days

	Se1			Se0		
		Deceased			Deceased	
Selenium Whole Blood[a] (Normal 0.96–1.78 μM/L)	No. Total	No.	%	No. Total	No.	%
<1.75	24	12	50.0			
1.75 to <2.1	25	6	24.0			
≥2.1	26	6	23.1			
			$p = .019$			
<0.88				41	27	65.8
≥0.88				37	9	24.3
						$p < .001$

[a]Maximum after day 1.

lower third of all values (<1.75 μmol/L, n = 25) but 24.0% and 23.1% when selenium was within the upper two thirds of selenium concentrations ($p = .019$). In Se0 patients, the mortality rate was 65.8% if the selenium levels were below the median of 0.88 μmol/L but 24.3% ($p < .001$) in patients with selenium levels >0.88 μmol/L (Table 5).

Secondary End Points

APACHE III Score. The variable part of the APACHE III score decreased from day 1 to day 28 in the Se1 group (−27.6%, $p = .0002$), comparable to the Se0 group (−24.1%, $p = .0002$).

Logistic Organ Dysfunction System. The resolution of organ dysfunction, calculated by changes of the logistic organ dysfunction system score during the observation time, was also similar in both groups (−2.6 ± 4.7 in Se1, −2.0 ± 4.0 in Se0).

Duration of Intensive Care Unit Stay. There was little difference between groups. The mean treatment duration was 15.1 ± 10 days in the Se1 group and 12.7 ± 9 days in the Se0 group.

Other End Points. Incidence of ventilation, hours requiring mechanical ventilation, and need for hemodialysis or vasopressor therapy were similar in both groups. The incidence of new infections was not significantly different between groups—the development of hospital-acquired pneumonia was ten (10.9%) in Se1 and ten (10.3%) in Se0 patients—and the incidence of acute respiratory distress syndrome also was not significantly different in Se1 (5.4%) and Se0 (4.1%) patients.

Tertiary End Points. C-reactive protein and procalcitonin decreased in both groups, but without a significant difference between the groups (Table 6).

The GPx-3 activity significantly increased in the Se1 group compared with

Table 6. The absolute median serum levels and their differences between days 1 and 28, or the last visit before death for glutathione peroxidase-3 (GPx-3), C-reactive protein (CRP), and procalcitonin (PCT)

	Se1		Se0	
	No.	Median	No.	Median
GPx-3 (normal 96–150 units/L)				
Day 1	90	151.5	89	155.3
Day 28/last visit	74	197.5	76	182.0
Difference	74	48.8	71	**6.0**
CRP (normal <5 mg/L)				
Day 1	91	184.0	97	176.0
Day 28/last visit	82	83.3	85	92.0
Difference	81	**−88.8**	85	**−61.2**
PCT (normal <0.5 μg/L)				
Day 1	61	9.7	67	5.7
Day 28/last visit	64	0.9	67	1.1
Difference	55	**−6.4**	57	**−3.0**

the placebo group ($p < .001$). The median change from baseline was 48.8 units/L in the Se1 group, whereas it was 6.0 units/L in Se0 patients (Table 6).

Safety

Adverse Effects. All safety criteria were analyzed including all randomized patients (n = 246) except three patients who withdrew informed consent. Without significant differences, adverse events occurred in 110 of 122 (90.2%) and 119 of 124 (96.0%) Se1 and Se0 patients, respectively. The sum of adverse events was 539 in Se1 and 591 in Se0 patients leading to an incidence of serious adverse events per patient year of 54.1 and 65.8 in Se1 and Se0, respectively. There were no specific adverse effects associated with the high-selenium supplementation.

Selenium Concentrations. Selenium levels were low at baseline (Se1, 0.48 μmol/L; Se0, 0.46 μmol/L) and increased significantly ($p \leq .001$) only in Se1 patients. In Se1 patients, the maximum serum selenium concentrations were found on day 14 with the highest value in one patient being 5.34 μmol/L; the maximum whole blood concentration was 3.57 μmol/L. The median concentrations were 2.05 μmol/L in serum and 1.83 μmol/L in whole blood. In patients with acute renal failure, selenium levels increased to a maximum median level of 1.80 μmol/L in serum and to 1.89 μmol/L in whole blood.

Urine selenium concentrations increased in Se1 patients from 0.20 to 1.90 μmol/L ($p \leq .001$), whereas in Se0 patients selenium excretion remained low (0.13 μmol/L).

Liver function assessed by the levels of albumin, liver enzymes, or global coagulation variables, as well as rates of renal or pulmonary failures, were not different between Se1 and Se0 patients and not related to high selenium levels.

DISCUSSION

The results of this randomized and placebo-controlled trial indicate that high-dose sodium-selenite supplementation is a new and important adjuvant therapeutic approach to improve outcome in sepsis and septic shock: The intention-to-treat analyses of all patients confirm the data of our previous pilot studies (20, 21) but the similar odds ratios (0.66, 0.65, and 0.64, respectively) indicate an underpowered study population. In the per-protocol analysis, however, the 28-day mortality rate was with 14.3%, significantly lower, in patients receiving adjuvant selenium treatment. This corresponds to a number needed to treat of seven patients. Assuming that about 140,000 sepsis-associated deaths occur per year in Germany, around 20,000 could be prevented with this adjuvant therapy. The total additional costs per saved life would only be around 1050 Euros. In the subgroup of patients with septic shock, the mortality rate was even 26.2% lower in Se1 patients, and the number needed to treat was four.

There was a direct correlation between selenium concentrations in whole blood and survival rate. High normal selenium concentrations associated with optimal selenoenzyme function obviously are necessary for the organism to cope with the challenges of severe sepsis. As the subgroup with the highest selenium whole blood concentrations had no further reduction in mortality, it could be speculated that lower dosages of selenium might be sufficient. However, there was no harm to these patients, and no selenium-specific side effects were observed.

In previous pilot studies, similar effects of selenium supplementation were found with a reduced mortality rate in the most critically ill patients (20, 21). However, due to low quality of data and no comparable supplementation regimens, a Cochrane analysis concluded, "There is insufficient evidence to recommend supplementation of critically ill patients with selenium or ebselen" (27). The results of this larger, multiple-centre trial now confirm the efficacy of high-dose sodium-selenite supplementation in patients with severe sepsis and septic shock. In patients with severe burn trauma, an adjuvant selenium substitution reduced mainly pulmonary infections (28). This could not be confirmed in our study, as the infectious complications were similar in both groups.

The mechanisms responsible for improved survival in sepsis and septic shock by selenium supplementation are still unknown. As a typical sign of an acute phase reaction (29), selenium levels are below normal already at admission to the intensive care unit (19, 20, 30). The severity of selenium depletion is correlated with survival as already shown (15). Selenium blood levels might be an unreliable marker of intracellular selenium and selenoenzyme content. It is supposed that high blood selenium supplies the organs with sufficient selenium to synthesize selenoenzymes (31, 32). As the difference in mortality rate between both groups was similar within the first days, selenoenzymes rather than sodium-selenite *per se* are responsible for these effects (12, 33).

Septic shock is associated with multiple organ failures and disseminated intravascular coagulation. Especially in those patients, the adjuvant selenium supplementation was most effective. One hypothesis is that under selenium supplementation, selenoprotein P is rapidly generated (13), preventing endothelial cells from oxidative damage followed by a diminished activation of these cells (34). Administration of sodium-selenite decreased tumor necrosis factor-α-induced intercellular adhesion molecule and selectin expression *in vitro* (35). In animal trials, selenium supplementation reduces oxidative stress, intranuclear nuclear factor-κB translocation, and cytokine formation as well as tissue damage (36) and

normalizes all known selenoenzymes like intracellular GPx and thioredoxin reductase activities. These enzymes reduce hydrogen peroxide, lipid, and phospholipid hydroperoxides; dampen the propagation of free radicals and reactive oxygen species; reduce hydroperoxide intermediates in the cyclo-oxygenase and lipoxygenase pathways; diminish the production of inflammatory prostaglandins and leukotrienes; and modulate the respiratory burst (37).

Endogenous glutathione plays an important role in reducing vascular hyporeactivity to exogenous norepinephrine due to its deactivation by superoxide (38) and endothelial dysfunction in response to peroxynitrite and endotoxic shock. Depletion of glutathione also enhances the cytotoxic effects of hydrogen peroxide and free oxygen radicals in endothelial cells and smooth muscle cells in shock (39) and, specifically, the peroxynitrite-induced injury (40, 41). A low activity of GPx (42) in plasma, platelets, and leukocytes in different acute and chronic illnesses (11, 12, 13) might contribute to increased oxidative stress in several compartments and contribute to multiple organ failure but might be prevented by selenium supplementation. High GPx activity regenerates the oxidized glutathione. Whether additional glutathione supplementation would augment the effect of selenium supplementation alone has to be established.

CONCLUSION

This multiple-center trial shows that an adjuvant, high-dose selenium supplementation reduced the mortality rate in patients with severe sepsis and especially in septic shock. This therapy is inexpensive, the number needed to treat is less than seven, it is easy and safe to handle, and it is not associated with overt adverse side effects. A larger trial is now needed to confirm the results of this trial.

The exact mechanisms of the beneficial effects of this adjuvant selenium supplementation are not known. There is, however, strong evidence that selenium might enhance the activities of important selenoenzymes involved in the maintenance of redox-homeostasis and immune and endothelial cell function.

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