

The effect of selenium supplementation among pediatric patients with burns

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Version: February 4, 2015

I. Background and Significance

A. Historical Background

The importance of Selenium

Selenium (Se) is an essential nutrient that is abundant in the American food supply. As a component of twelve mammalian selenoproteins, selenium plays an important role in the well being of all mammals. For example, four types of glutathione peroxidase (GPx) are indispensable in the protection against oxidative injury (1). In fact, these Se-containing enzymes provide antioxidant defense in all cell and interstititum compartments. Thioredoxin reductase, another important selenoprotein, regulates cytokine expression and therefore plays a major role in immune defense. These enzymes are expressed in almost every cell type and are dependent on the availability of Se (2). Due to its incorporation into proteins, Se affects all components of the immune system, both innate and adaptive responses (3, 4) and therefore influences the ability to respond to infection (5-7). The role of Se in innate and acquired immune responses is mediated via its incorporation into glutathione peroxidase (8) and thioredoxin reductase (9). Reduced intakes of Se produce decreases in antibody titres (7), T cell proliferation (10), and natural killer cell activity (11).

Selenium Deficiency

Although rare in developed countries, Se deficiency has been affiliated with a variety of cancers, rheumatic arthritis, muscular dystrophy, cystic fibrosis, malaria, and cardiovascular diseases (12). Additionally, low plasma Se values were found to be significantly associated with increased respiratory morbidity among very low birth weight infants (13).

Selenium Toxicity

Se toxicity is rare but can occur after high intakes over long periods of time. Reports of acute selenium toxicity typically involve unintentional ingestions (14-19) and suicides (18, 20-25). Most of these cases were fatal and 3 involved children who ingested gun-bluing solution (14, 16, 18). Studies from South China, where Se in the soil is particularly high, toxicity is only associated with intakes greater than 850 ug/day (26, 27). In a recent case series published in 2012 (28), the others retrospectively examined 9 patients who presented to a medical toxicology clinic with symptoms of selenosis after consuming a nutritional supplement. Analyses revealed that the supplement contained 200 times the reported amount of selenium. This resulted in an average Se intake of 1.3 g over

10-60 days. Symptoms of selenium toxicity began within 1 week of supplement intake and included alopecia, fingernail changes, GI symptoms, and memory loss. All patients survived and none required hospitalization.

We did not find any clinical trials that reported selenium toxicity during parenteral selenium administration.

High blood levels of Se (greater than 100 ug/dL) can result in a condition called selenosis (29). Symptoms of selenosis include gastrointestinal upsets, hair loss, white blotchy nails, garlic breath odor, fatigue, irritability, and mild nerve damage (30).

The Institute of Medicine of the National Academy of Sciences (IOM) (31) has set a tolerable upper intake level (UL) for Se that ranges between 90 and 400 ug/day for healthy children 1-18 years old (Table 1). The UL is not known with a great deal of certainty and is conservatively calculated. Se UL among children is determined by extrapolating adult data.

It is important to note that the IOM (31) state in their report that the UL does not apply to patients under medical supervision and that doses of Se above the UL should not be discouraged in clinical trials. Se toxicity has not been reported in trials administering Se supplements to adults with doses up to 1000 ug per day for short periods of time (1-3 weeks).

Table 1. UL values for Se among children (31)

Age Group (years)	UL for Se (ug/day)
1-3	90
4-8	150
9-14	280
14-18	400

Selenium Compounds

Selenium is incorporated into selenoproteins. There are 4 naturally occurring oxidation states of selenium: selenide (Se^{2-}), elemental selenium (Se^0), selenite (Se^{+4}), and selenate (Se^{+6}). Selenite can associate with other elements to form selenious acid.

Dietary Se (organic) exists in two compounds, selenomethionine (from plants) and selenocysteine (from animals). Supplemental Se (inorganic) exists as selenate or selenite. A study conducted in 1995 (32) suggested that the organic forms of Se increased blood Se concentration to a greater extent than inorganic forms. However, it did not significantly improve the activity of the Se-dependent enzyme, glutathione peroxidase (32).

Selenium Supplements

Organic supplements can only be administered via the enteral route, while inorganic supplements can be given both enterally and parenterally. No data is available to compare the effect of enteral and parenteral Se on biomarkers of Se status.

Due to impaired gastric motility intravenous (IV) supplements may be preferred during critical illness. In fact, IV Se was used in all clinical trials involving Se supplementation during critical illness (33). The dose of Se provided in critical illness trials among adults ranges from 250-1000ug per day (34). Data is available to show that 500 ug sodium selenite significantly increased plasma Gpx within 3 days (35), and that higher doses did not further increase Gpx (2).

Most studies continuously infused Se over 24 hours (2, 35-37) through a separate line. Large boluses over a short amount of time are not recommended due to a rapid rise in urinary Se excretion (2).

Selenium in Nutrition Support

Enteral and parenteral formulas also include selenium, usually in the form of sodium selenate (38-40). Enteral nutrition, commonly referred to as tube feeding, is administered via the digestive tract. All commercial brands of enteral tube feedings contain selenium (Table 2).

Table 2. Se content of tube feeding formulas at Shriners Hospital in Boston

Formula	Se Content (ug/L)
Pediasure	21.6
Peptamen	50
Peptamen AF	160
Replete	100
Jevity 1-Cal	52

Parenteral nutrition is administered directly into the blood, and is used when the intestinal tract is damaged or nonfunctional. The recommended amount of selenium via parenteral nutrition developed by the American Society for Parenteral and Enteral Nutrition (ASPEN) are featured in Table 3 (41). At SBH all patients on PN receive 20 ug of Se each day.

Table 3. ASPEN guidelines for Se content of parenteral nutrition.

Age Group	Se
Neonates 3-10 kg	2 ug/kg/day
Children 10 – 40 kg	1 – 2 ug/kg/day
Children > 40 kg	40 – 60 ug/day

Selenium Requirements

The Third National Health and Nutrition Examination Survey (NHANES III) reports that Americans consume 106 micrograms of Se per day (42). The

current Recommended Dietary Allowance (RDA) for Se is 20 µg/day for 1-3 year old children, 30 µg/day for 4-8 year old children, 40 µg/day for 9-13 year old children, and 55 µg for children > 13 and adults (31). Recommendations among adults are based on the amount of Se needed to achieve optimal GPx activity. Pediatric recommendations were determined by extrapolating from adult data. An exact recommendation for Se during an acute illness is not known.

Assessment of selenium status

Accurate determination of Se status during critical illness should include plasma Se, plasma Gpx, and urine Se (43). It is especially important to measure Gpx activity because it represents the functional marker of Se status. Gpx activity appears to plateau after a certain Se intake has been reached (44) and is not affected by the form of Se ingested(44).

B. Previous research to support proposed research

Preliminary study: Selenium status of pediatric patients with burns

A prospective study, designed to assess the longitudinal Se status of pediatric burn patients, was conducted by this research team. Twenty patients admitted to Shriners Hospital in Boston, MA with burns exceeding 15% of their total body surface area (TBSA) were studied longitudinally for 8 weeks following admission or until 95% wound closure was achieved (determined by the attending physician using a burn diagram). Dietary Se intake from all forms of nutrition (oral, enteral, parenteral) was calculated daily and plasma and urine samples were collected weekly. Plasma Se, plasma glutathione peroxidase (GPx) activity and urinary Se were determined. Demographic data is featured in Table 4.

Table 4. Demographics (N = 20)

	Mean \pm Standard Deviation	Range
Gender	15 males 5 females	
Age (years)	6.5 \pm 5.3	0.33 – 16
Length of Stay (days)	50 \pm 40	11 – 161
Initial %TBSA	42 \pm 21	15 - 93
% Full Thickness	27 \pm 27	0-90
Type of Burn	15 flame 5 scald	

The mean dietary Se intake throughout the 8 week study was 60 \pm 39 µg/day. Weekly selenium intake did not change significantly over the eight-week study

(Figure 1). All subjects consumed greater than the RDA for Se (Table 5). They also consumed more Se than recommended by the ASPEN (27).

Table 5. Dietary Se Intake and % AI According to Age

Age (years)	Number of Subjects	Mean Se Intake (ug/day)	Mean Se Intake (ug/kg/day)	% RDA
1-3	8	40	2.9	200
5-8	2	62	2.5	201
9-13	7	75	1.6	183
14-18	2	83	1.4	151

Plasma Se (mean = $1.08 \pm 0.34 \text{ } \mu\text{mol/L}$) did not change throughout the entire study. However, 82% of the total weekly samples were below the $1.35 \text{ } \mu\text{mol/L}$ plasma selenium level reported in healthy American children ages 1-18yrs (Figure 2). Higher plasma selenium was also correlated with a lower incidence of infection ($p = 0.043$).

The mean plasma glutathione peroxidase (GPx) level over the eight weeks was $3.29 \text{ Units/mg protein} \pm 1.42$. Furthermore, 40% of all weekly plasma GPx samples were below the $2.8-3.4 \text{ U/g protein}$ range established in healthy American girls ages 11-14 (34) . No statistical differences were found throughout the eight weeks.

There was no significant change in urinary selenium levels between weeks. The mean urinary selenium level throughout the eight-week study was $65.9 \text{ } \mu\text{g/L} \pm 50$, which is higher than the mean selenium intake from diet $60 \text{ } \mu\text{g/L} \pm 39$. A majority of the observed urinary selenium levels were higher than those reported ($28.7 \text{ } \mu\text{g/L} \pm 8.3$) in healthy American children ages 5-18 years (35).

In summary, these results indicate that selenium status in pediatric burn patients, as measured by plasma and urine selenium concentrations and plasma GPx activity, was inadequate throughout the eight weeks following thermal injury. Additionally, these results suggest that maintaining normal blood concentrations of selenium and GPx activity following burn injury reduce the incidence of infection. This data was presented at the American Burn Association Annual meeting in March 2007 and published in the Journal of Trauma in 2010.

Figure 1. Dietary Selenium. Selenium intake did not change significantly over the eight week study. Intake ranged from 100-400% the AI/RDA (based on age).

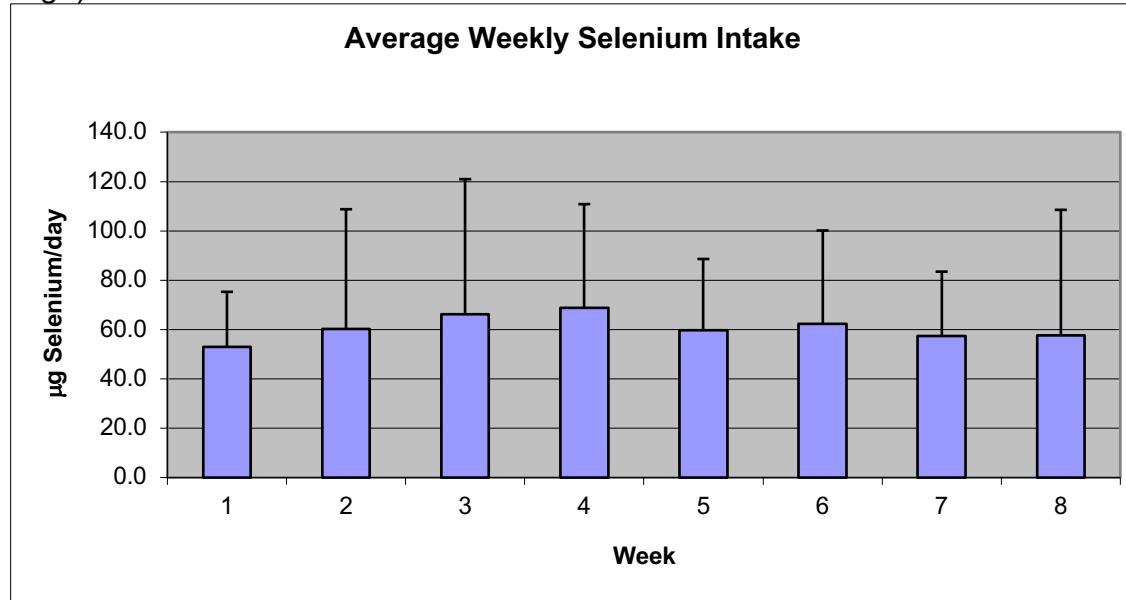
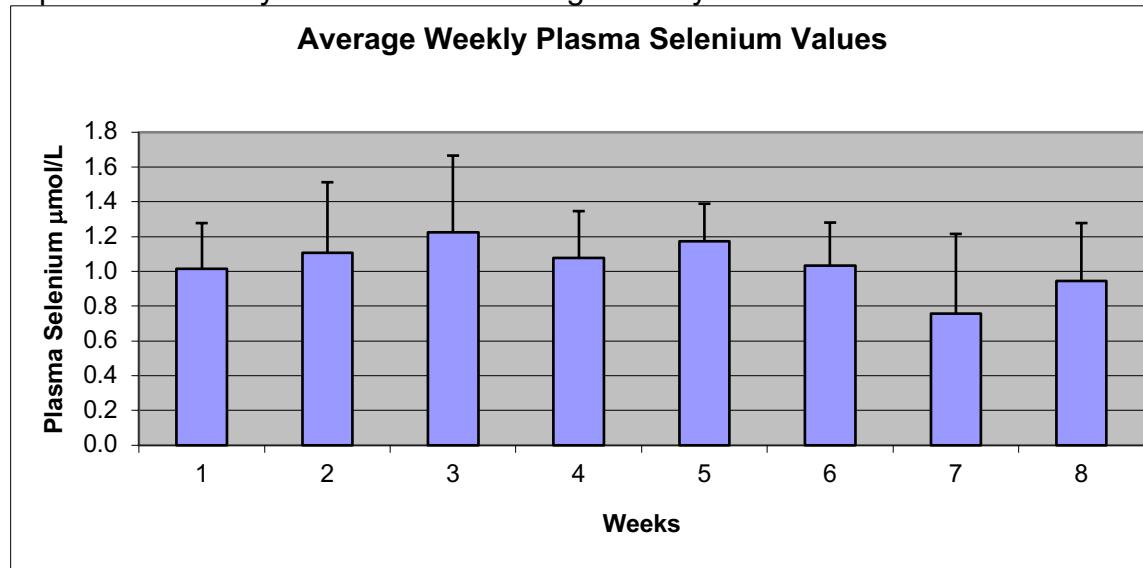


Figure 2. Plasma Selenium. There was no significant difference in weekly plasma selenium levels throughout the eight weeks of the study. However, 82% of the total weekly samples were below the 1.35 µmol/L plasma selenium level reported in healthy American children ages 1-18yrs.



Selenium supplementation in children

Few clinical trials have studied the impact of Se among sick children. The few studies available include various populations and methodologies (Table 6).

Table 6. Selenium supplementation trials among non-critically ill children.

Reference	Region	Subjects	Se supplement	Se dose	Outcome
(45)	South Malawi	2372 children (1-4 years) with malnutrition	Sodium selenate (MVI powder)	55 ug/day for 20 weeks	Se not related to risk of Kwashiorkor
(46)	USA	94 Latino children (6mos – 5 years)	Sodium selenate (MVI tablet)	1 tablet for 5-6 months	Se decreased Upper respiratory tract visits to pediatrician
(47)	Tibet	324 children (5-15 years) with Kashin-Beck disease	Sodium selenate (oral)	100 ug/day for 12 months	Se not related to Kashin-Beck disease
(48)	France	27 children (7-20 years) with cystic fibrosis	Sodium selenite (oral)	2.8 ug/kg for 2 months	Se not related to lipid peroxidation markers

Only one study was found in the literature pertaining to pediatric Se supplementation during burn injuries. Borner et al. (49) studied 65 children aged 1 to 16 years with severe inflammatory surgical diseases as well as widespread burns. All subjects received either a Se supplement (n =34) or no selenium supplement (n = 31). Selenium was administered intravenously for the first seven days of the maintenance period, and orally for an additional seven to 14 days. The initial Se dose was based on body weight. A one-time dose of 200 µg was administered to children weighing less than 15 kg. Likewise, a 500 µg dose was given to subjects weighing 15 – 30 kg, and a 1000 µg dose was given to subjects weighing greater than 30 kg. Subsequently the experimental group received 3 doses of 2 ug/kg Se everyday for 7-14 days. Blood samples were drawn on study days 1, 2, 3, 6, and on the final day of the trial. Results showed that the control group had lower plasma Se and GPx activity compared to the experimental group. The authors also reported that lipid peroxidation declined following Se supplementation, indicating increased cell protection.

Selenium supplementation during adult burn injuries

There is more data regarding Se supplementation among critically ill adults. A meta-analysis completed several years ago showed that Se supplementation alone or in combination with other antioxidants was associated with a reduced trend in mortality (p=0.09) (33). Although this data was not significant, high dose Se (500 or 1000 ug/day IV sodium selenite) had the best results.

In 2006 Angstwurm et al. (2) published the first trial on high-dose Se supplementation among adults in the intensive care unit. Patients (n=249) in this study were randomized to treatment (1000 ug IV sodium-selenite continuous infusion over 24 hours for 14 days) or placebo. Results showed that high dose Se supplementation reduced mortality among patients with severe sepsis.

In a slightly smaller study (n = 150) published in 2011, Valenta et al. (50) reported contradictory results. This study was a prospective, randomized, open-label, trial that investigated the impact of Se supplementation (1500 ug/day x 14 days) on patients with SIRS/sepsis. Although plasma Se and GPx activity were higher among the supplemented patients, mortality was similar among groups.

There is data among critically ill patients that suggests that Se reduces infections. In 2011, Andrews et al. (51) studied 502 adult ICU patients with gastrointestinal failure. In this randomized, double-blind study, subjects received parenteral glutamine, selenium (500 ug/day), or both for up to 7 days. Patients who received IV Se for \geq 5 days had less new infections compared to other groups (OR: 0.53(0.30-0.93). This data was supported by a prospective, randomized, placebo-control, single-blinded study published in 2011 (52). Thirty-one ICU patients were included in this study. The authors showed that a daily infusion of 1600 ug Se, following an initial bolus of 2000 ug, lowered the incidence of hospital-acquired pneumonia among patients in the intensive care unit.

Over the past 15 years, a research team led by Berger et al. conducted several studies that examined Se supplementation combined with other trace minerals during adult burn injuries (Table 7). In general, studies showed a reduction in infection rates among supplemented groups. However, it is not known if the results are due to selenium, another trace mineral, or a combination of the 3 trace minerals provided.

Table 7. Selenium supplementation trials among adult burn patients

Reference	Study Design	Subjects	Intervention	Duration	Results
Berger et al. 2006 (34)	Combination of 2 randomised, double-blind trial	41 burn patients with mean TBSA of 46%	IV trace element cocktail vs Placebo. Cu: 2.5-3.1 mg/d Se: 315-380 ug/d Zn: 26.2-31.4 mg/d	8-21 days	Plasma Se levels increased with supplementation; Reduced number of infections in supplemented pts (3.5 vs 2 episodes per pt)
Berger et al. 2007 (53)	Prospective, randomized, placebo-controlled trial	21 burn patients with mean TBSA of 45%	IV trace element cocktail vs Placebo. Cu: 3.75 mg/d Se: 375 ug/d Zn: 37.5 mg/d	14-21 days	Plasma Se sig higher in supplement group; Number of infect in first 30 days was sig lower in supplement group (2 vs 4 infections per

					pt); Supplement group had fewer regrafting episodes.
Berger et al. 1998 (36)	Prospective, randomized, placebo-controlled trial	20 burn patients with mean TBSA of 48%	IV trace element cocktail vs Placebo. Cu: 3.75 mg/d Se: 375 ug/d Zn: 37.5 mg/d	8 days	The number of infections were lower in supplement group

Overall the data among adults is promising, but does not identify a specific guideline for selenium supplementation during critical illness. The data among children is scarce and less impressive.

C. Rationale behind proposed research/ benefits to society

The Se status of children with major burns is suboptimal which may increase the incidence of infection. Se requirements during critical illness are not known. Results from this investigation may provide a tool for recommending Se supplements during burn injury.

II. Specific Aims

The hypothesis of this research is that Se supplementation will restore the depressed Se status among children with burn injuries. The secondary hypothesis is that Se status is related to the incidence of infection among pediatric patients with burns.

The specific aims of this research are

1. to determine the impact of supplemental Se needed on plasma Se, Gpx activity, and urine selenium among pediatric patients with burns $\geq 20\%$ TBSA.
2. to determine the association between Se supplementation, biomarkers of Se status and indicators of stress and infection.

III. Subject Selection

All subjects (n = 75) will be recruited from the Shriners Hospital for Children in Boston.

Inclusion Criteria:

- Between 1 and 18 years of age admitted to Shriners Burns Hospital
- TBSA burn of $\geq 20\%$
- Existing catheter
- Enrolled into study within 3 weeks of burn injury

Exclusion Criteria:

- < 1 year or > 18 years of age
- $< 20\%$ TBSA burn

- No existing catheter
- Renal disease (eGFR < 60)
- Liver disease (bilirubin greater than 3)
- Thyroid disorders (thyroid disease which currently requires treatment)
- Cancer
- HIV*
- Pregnancy (as determined by routine admission labs)

* Previously known HIV+ status. An HIV test will not be administered for the purpose of this study.

To ensure the safety of all patients, once every 2 weeks, blood samples will also be sent to the Massachusetts General Hospital Chemistry lab for analysis of selenium levels. If the selenium level is greater than 100 ug/dL the patient will be immediately removed from the study.

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If a patient decided to withdraw from the study or meets the exclusion criteria during the study, he/she will be removed from the study immediately. No further study procedures, including supplementation and blood and urine collection, will be completed. However, these subjects will be followed for 2 weeks for any adverse events related to the study. Neurological status, per routine care from the medical team, will be assessed. Additionally, lab values will be followed (if available from routine care) (complete blood count, serum creatinine, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, coagulation studies). Ongoing adverse events thought to be study drug related will be followed until resolution or the condition stabilizes.

IV. Subject Enrollment

All eligible subjects will be screened using the inclusion/exclusion criteria by the Clinical Research Nurse or Clinical Research Specialist, who will serve as the primary investigator. Assessment of potential eligibility of subjects will be provided by the primary physician. Subsequently, only members of the study staff can initiate the recruitment process. The consent and assent will only be obtained by members of the study staff who are a physician, a PhD, or an RN.

Following patient admission and stabilization, if the patient meets the inclusion criteria, a member of the clinical staff will approach the subjects/parents/guardian/ to gain permission for the study staff to discuss the study. If permission is granted, an appropriate member of the study staff will approach

subjects/parents/ guardians at the bedside, who meet the inclusion/exclusion criteria, with a brief explanation of the study. A more detailed explanation will be offered in a private room to those interested in participation. The alternative to participation is “not to participate”. Care will not be altered for patients who do not participate. Informed consent will be obtained if the patient and/or parent/legal guardian agrees to participate. Parents/legal guardians will be allowed an indefinite amount of time for questions and participation consideration. Parents/legal guardian will provide consent for 0-18 year old children (documented in writing using the PHRC approved consent form). The permission of both parents is required unless one parent is deceased, unknown, incompetent, or not reasonably available or if only one parent has legal responsibility for the care and custody of the child. Any child 14 years or older, who is able, will consent along with parent/guardian. The assent of each child (age 7 and older) who is capable of providing assent based on medical condition, age, maturity, and psychological state will be sought. According to a power analysis calculation, 75 children will be included in this study.

B. Treatment Assignment and Randomization

This is a randomized, double-blind, placebo-controlled trial. Patients will be randomly assigned to receive Selenium supplementation (either 2, or 4 ug/kg/day) or a placebo. 25 subjects will be in each group (total of 75 subjects). Randomization will occur by using a computerized randomization list. A pharmacist, who is not a member of the study staff, will prepare the supplements and label them accordingly prior to distribution.

V. Study Procedures

A. Study Parameters

- B. Lab studies conducted solely for the purpose of this study include plasma and urinary Se, plasma Gpx and dietary Se intake. Other parameters that will be included are demographics, medications, wound debridement procedures, invasive devices, mode of nutrition support, kilocalorie and protein intake, weekly weights, neurological status (as routinely assessed by the clinical team) and routine laboratory studies.
- C. Another parameter that will be recorded is the occurrence of pneumonia or infection (bacterial or fungal) in the wound, blood, or urine, as defined by The National Nosocomial Infection Surveillance System (A localized or systemic condition that results from adverse reaction to the presence of an infectious agent(s) or toxin(s)) and clarified by the Infection Control Nurse. Specific definitions are as follows:

Urinary Tract Infections

a) Patient has at least 1 of the following signs or symptoms with no other recognized cause: fever ($>38^{\circ}\text{C}$), urgency, frequency, dysuria, or suprapubic tenderness
and
patient has a positive urine culture that is $\geq 10^5$ microorganisms per cm^3 of urine with no more than 2 species of microorganisms.

b) Patient has at least 2 of the following signs or symptoms with no other recognized cause: fever ($>38^{\circ}\text{C}$), urgency, frequency, dysuria, or suprapubic tenderness
and at least 1 of the following

- Positive dipstick for leukocyte esterase and/or nitrate
- Pyuria (urine specimen with $\geq 10 \text{ WBC/mm}^3$ or $\geq \text{WBC/high power field of unspun urine}$)
- Organisms seen on Gram stain of unspun urine
- At least 2 urine cultures with repeated isolation of the same uropathogen (gram-negative bacteria or *S. saprophyticu*) with $\geq 10^2$ colonies/mL in nonvoided specimens
- $\leq 10^5$ colonies/mL of a single uropathogen (gram-negative bacteria or *S. saprophyticu*) in a patient being treated with an effective antimicrobial agent for a urinary tract infection
- Physician diagnosis of a urinary tract infection
- Physician institutes appropriate therapy for a urinary tract infection

c) Patient 1 year of age has at least 1 of the following signs or symptoms with no other recognized cause: fever ($>38^{\circ}\text{C}$), hypothermia ($<37^{\circ}\text{C}$), apnea, bradycardia, dysuria, lethargy, or vomiting
and
patient has a positive urine culture that is $\geq 10^5$ microorganisms per cm^3 of urine with no more than 2 species of microorganisms

d) Patient 1 year of age has at least 1 of the following signs or symptoms with no other recognized cause: fever ($>38^{\circ}\text{C}$), hypothermia ($<37^{\circ}\text{C}$), apnea, bradycardia, dysuria, lethargy, or vomiting
and at least 1 of the following:

- Positive dipstick for leukocyte esterase and/or nitrate
- Pyuria (urine specimen with $\geq 10 \text{ WBC/mm}^3$ or $\geq \text{WBC/high power field of unspun urine}$)
- Organisms seen on Gram stain of unspun urine
- At least 2 urine cultures with repeated isolation of the same uropathogen (gram-negative bacteria or *S. saprophyticu*) with $\geq 10^2$ colonies/mL in nonvoided specimens
- $\leq 10^5$ colonies/mL of a single uropathogen (gram-negative

bacteria or *S. saprophyticu*) in a patient being treated with an effective antimicrobial agent for a urinary tract infection

- Physician diagnosis of a urinary tract infection
- Physician institutes appropriate therapy for a urinary tract infection

Pneumonia

- a) 2 or more serial chest radiographs with at least 1 of the following: new or progressive and persistent infiltrate, consolidation, cavitation, pneumatoceles
- b) At least 1 of the following:
 - Fever ($>38^{\circ}\text{C}$) with no other recognized cause
 - Leukopenia ($< 4000 \text{ WBC/mm}^3$) or leukocytosis ($\geq 12000 \text{ WBC/mm}^3$)

and at least 2 of the following:

 - New onset of purulent sputum, or change in character of sputum, or increased respiratory secretions, or increased suctioning requirements
 - New onset or worsening cough, dyspnea, or tachypnea
 - Rales or bronchial breath sounds
 - Worsening gas exchange (e.g. O_2 desaturations, increased oxygen requirements, or increased ventilation demand

Burn Infection

- a) Patient has a change in burn wound appearance or character, such as rapid eschar separation; dark brown, black, or violaceous discoloration of the char; or edema at wound margin
and
histologic examination of burn biopsy shows invasion of organisms into adjacent viable tissue.
- b) Patient has a change in burn wound appearance or character, such as rapid eschar separation; dark brown, black, or violaceous discoloration of the char; or edema at wound margin
and at least 1 of the following:
 - Organism cultured from blood in the absence of other identifiable infection
 - Isolation of herpes simplex virus, histologic identification of inclusions by light or electron microscopy or visualization of viral particles by electron microscopy in biopsies or lesion scrapings

c) Patient with a burn has at least 2 of the following signs or symptoms with no other recognized cause: fever ($>38^{\circ}\text{C}$), hypothermia ($<37^{\circ}\text{C}$), hypotension, oliguria ($< 20 \text{ cm}^3/\text{hr}$), hyperglycemia at previously tolerated level of dietary carbohydrate, or mental confusion and at least 1 of the following:

- Histologic examination of burn biopsy shows invasion of organisms into adjacent viable tissue.
- Organism cultured from blood
- Isolation of herpes simplex virus, histologic identification of inclusions by light or electron microscopy, or visualization of viral particles electron microscopy in biopsies or lesion scrapings

Bloodstream Infections

a) Patient has a recognized pathogen cultured from one or more blood cultures and organism cultured from blood is not related to an infection at another site.

i. Patient 1 year of age has at least 1 of the following signs or symptoms with no other recognized cause: fever ($>38^{\circ}\text{C}$), chills, or hypotension and at least 1 of the following

- Common skin contaminant (e.g. diphtheroids, *Bacillus* sp. *Propionibacterium* sp, coagulase-negative staphylococci, or micrococci) is cultured from 2 or more blood cultures drawn on separate occasions
- Common skin contaminant (e.g. diphtheroids, *Bacillus* sp. *Propionibacterium* sp, coagulase-negative staphylococci, or micrococci) is cultured from at least 1 blood culture from a patient with an intravascular line, and the physician institutes appropriate antimicrobial therapy
- Positive antigen test on blood (e.g. *Haemophilus influenza*, *Streptococcus pneumoniae*, *Neisseria meningitidis*, or group B *Streptococcus*) and signs and symptoms and positive laboratory results are not related to an infection at another site.

c) Patient 1 year of age has at least 1 of the following signs or symptoms with no other recognized cause: fever ($>38^{\circ}\text{C}$), hypothermia ($<37^{\circ}\text{C}$), apnea, or bradycardia and at least 1 of the following:

- Common skin contaminant (e.g. diphtheroids, *Bacillus* sp. *Propionibacterium* sp, coagulase-negative staphylococci, or micrococci) is cultured from 2 or more blood cultures drawn on separate occasions
- Common skin contaminant (e.g. diphtheroids, *Bacillus* sp. *Propionibacterium* sp, coagulase-negative staphylococci, or micrococci) is cultured from at least 1 blood culture from a patient with an intravascular line, and the physician institutes appropriate antimicrobial therapy
- Positive antigen test on blood (e.g. *Haemophilus influenza*, *Streptococcus pneumoniae*, *Neisseria meningitidis*, or group B *Streptococcus*) and signs and symptoms and positive laboratory results are not related to an infection at another site.

B. Supplements to be used

All patients will receive routine nutrition support that includes oral, enteral, or parenteral nutrition or a combination of the 3 sources. All enteral nutrition products contain Se in the form of sodium selenate (Table 2). All patients who require parenteral nutrition receive 20 ug of IV selenious acid per day (this is standard therapy at SBH). According to our previous study, despite various routes of nutrition support within and among subjects, Se intake remained stable throughout the 8 week time period and cover approximately 150 – 200% of the RDA for Se. Therefore standard nutrition support, including Se, should not interfere with this proposed investigation (Figure 1).

In addition to standard nutrition support, all patients will be randomized to receive IV Se supplementation or placebo. The placebo (0.9% sodium chloride or 5% Dextrose solution, depending on the clinical situation) will look identical to the Se supplements. Patients in the experimental groups will receive parenteral Se as selenous acid (added to 25 ml of 5% Dextrose solution or 0.9% sodium chloride) (carrier solution will depend on clinical situation) as a 24-hour continuous infusion in doses 2 ug/kg/day (n = 25), or 4 ug/kg/day (n = 25). The placebo group (n = 25) will receive sodium chloride 0.9% in the same regimen. These Se supplement amounts were determined taking into account previous studies in the literature, current RDAs, UTLs, ASPEN guidelines for parenteral Se, and dietary data recorded from our previous study. According to reference weights adapted from NHANES III data, supplement doses (2 ug/kg/day and 4 ug/kg/day) do not exceed the upper tolerable limits for children.

The Se/Placebo will be provided as a 24-hour continuous infusion. The continuous infusion of Se/Placebo may be temporarily interrupted, if necessary, for reasons including, but not limited to, surgical procedures and drug compatibility issues. These temporary interruptions will not reduce the integrity of

the study. However, if the Se/Placebo infusion is held for longer than 48 consecutive hours or 72 hours in 7 days then the subject will be removed from the study. At this time no further study drug will be given or biological samples taken. All subjects will be monitored for any treatment-related adverse events for 2 weeks following discontinuation of the study therapy.

Prior to initiation of the study, the research nurse will hold an in-service for clinical staff to alert them of the study. A specific research sticker will be placed in the chart to alert clinical staff that the patient is enrolled in this research study. The MD responsible for the care of the patient will write the orders for the supplements. The Pharmacy will prepare all supplements and the nurses will administer them.

C. Data Collection

Plasma and urine Se and plasma Gpx will be measured weekly from the time of admission for a maximum of 8 weeks or until the patient achieves 95% definitive wound closure.

The research nurse will obtain 4 ml or 8ml (8 ml will be drawn instead of 4 ml every other week starting with week 1 for real time analysis) of whole blood off an existing catheter and place it into a trace-element free blood collection tube.

Every other week, when 8 ml of blood is needed, 4 ml will be transferred by the unit assistant to the MGH laboratory for immediate analyses (the MGH lab uses Quest Labs for analysis of selenium). If in the unlikely event that the MGH lab rejects the sample for any reason, another 4 mL blood draw will be collected and sent to the MGH lab for real-time analyses.

Weekly, the 4 ml sample will be transported by the nurse to the clinical research lab (in the same building). In the lab the nurse will use a centrifuge to separate the blood and then pipette the plasma and whole blood into collection vials to be frozen prior to analyses. A 24 hour urine will be initiated the day prior to blood sampling for analysis of urinary Se. Urine will be collected via an existing catheter. If no catheter exists patient will be asked to urinate into a container. If the patient is unable to urinate into a container, the urine sample will not be collected. Infant urine will be collected via diapers. The research nurse will then bring the urine container down to the clinical research lab and aliquot it into 50 ml vials prior to being frozen. Frozen samples are eventually mailed to a commercial laboratory for lab analyses. The PI is certified to ship biological products and all appropriate regulations will be followed.

Culture Data

Wound cultures will be obtained for all patients admitted to the acute care unit at Shriners Burns Hospital, per routine care, on admission. Urine cultures will also be obtained upon admission if one or more of the following criteria are met:

- Symptoms of urinary tract infection (UTI)
- History of recurrent UTI
- Structural abnormalities of the GU tract
- Patient requires urinary catheterization
- Fever of unknown origin
- Urinalysis testing indicates possible infection

Subsequently wound and urine (if meets criteria above) will be obtained at least once a week. Wound, urine, blood, and sputum cultures may also be obtained during a fever spike or when clinically appropriate per the medical team. If bacteremia or fungemia is suspected, 2 sets of blood cultures will be assessed.

All cultures are obtained by a trained registered nurse or a medical doctor and are transported to the bacteriology lab (accredited by CLSI) at Shriners Burns Hospital within 2 hours. According to infection control policies at Shriners Burns Hospital all specimens must be labeled with patient name and medical record number and placed in sealed plastic bags or other suitable containers when being transported. If a specimen will be cultured for fungus, the specimen will be labeled "fungal culture". Methods of culture collection are described below:

Blood: Venipuncture is the recommended means of obtaining blood for cultures. However, when this is not possible blood from catheters and other intravascular devices may be used.

Blood cultures are placed in a sealed plastic bag and brought to the laboratory as soon as possible.

Blood is cultured in our laboratory using the Bactec automated system consisting of a single blood culture bottle.

- Blood culture bottles: Plastic cap is removed from the bottle. Rubber stopper on top of bottle is cleaned with an alcohol pad.

Isolators are processed within 16 hours. Bottles are held for 5 days. A separate plate is routinely used to assess for fungus. Please note that regarding fungal cultures, many of the opportunistic fungi (Aspergillus, Penicillium and Mucor) are rapid growers and colonies will be seen within the first 24-48 hours (mature within 4-5 days) and identified down to the species level when possible. Candida will usually grow in 24-48 hours and can be identified at this time. Speciation requires 72 hours.

The pathogenic fungi (Histoplasma and Blastomyces) cultures will be referred to the MGH laboratory for speciation. Results are confidentially reported to the medical team at Shriners Burns Hospital.

Contaminates may be found among blood cultures. The decision on whether or not it is a contaminant is based on the clinical judgment of the medical doctors and clinical team. If a culture is deemed a “contaminant” it will be reported as such in the data collection sheet.

Burn: Wound cultures for patients include the following anatomical areas: head, chest, back, buttocks, groin, upper extremities, hands, and lower extremities (approximately 1 culture for every 10% burn surface injury). Open wound areas as well as donor sites are cultured. These cultures are usually obtained by swab. Occasionally, wound cultures will be obtained by biopsy if requested by the surgical physician. Wound site is cleaned and then gently rubbed in a circular motion to swab areas most likely to harbor pathogenic organisms (under eschar, purulent drainage etc.) Areas of normal tissue are avoided to reduce contamination with skin flora. Ampule at the bottom of the culture tube is crushed and the specimen is placed in a plastic bag and sealed.

The culture is brought to the laboratory as soon as possible, but no longer than 2 hours after collection. Wounds are plated on two plates: TSA with 5% sheep blood and Levine EMB. The swab is then put into a thioglycolate broth which will support the growth of most anaerobes. Plates are incubated for 24 hours. If there is no growth they are reincubated for another 24 hours. Tubes are kept until growth is seen or 7 days, which ever comes first.

A separate plate is routinely used to assess for fungus. Please note that regarding fungal cultures, many of the opportunistic fungi (Aspergillus, Penicillium and Mucor) are rapid growers and colonies will be seen within the first 24-48 hours (mature within 4-5 days) and identified down to the species level when possible. Candida will usually grow in 24-48 hours and can be identified at this time. Speciation requires 72 hours. .

The pathogenic fungi (Histoplasma and Blastomyces) cultures will be referred to the MGH laboratory for speciation. Results are confidentially reported to the medical team at Shriners Burns Hospital.

Histological exams are not routinely completed, but may be ordered by the physician.

Urine: Urine is collected in a sterile leak proof container. If the patient has a foley catheter, the injection port at junction of drainage tubing is cleaned with betadine. Needle is inserted and urine aspirated into the syringe. Urine is then transferred into the urine container. If a urine collection bag is used, the urinary meatus is cleansed and the urine bag is applied to collect the urine.

Urine specimen is sealed and transported to the laboratory as soon as possible, no longer than 1 hr. Samples are inoculated on BAP and EMB plates with a calibrated loop and incubated for 24 hours.

A separate plate is routinely used to assess for fungus. Please note that regarding fungal cultures, many of the opportunistic fungi (Aspergillus, Penicillium and Mucor) are rapid growers and colonies will be seen within the first 24-48 hours (mature within 4-5 days) and identified down to the species level when possible. Candida will usually grow in 24-48 hours and can be identified at this time. Speciation requires 72 hours. .

The pathogenic fungi (Histoplasma and Blastomyces) cultures will be referred to the MGH laboratory for speciation. Results are confidentially reported to the medical team at Shriners Burns Hospital.

Contaminates may be found among urine cultures. The decision on whether or not it is a contaminant is based on the clinical judgment of the medical doctors and clinical team. . If a culture is deemed a “contaminant” it will be reported as such in the data collection sheet.

Sputum: Sputum and aspirates are collected in a sterile cup or suction trap. Non-bacteriostatic normal saline may be instilled into the airway to aid in procurement of culture. Specimen container is placed in a plastic bag, sealed and brought to the laboratory as soon as possible. Sputum is plated onto 3 different agar plates: TSA with 5%sheep blood, Levine EMB and Chocolate plates. Plates are incubated in a CO₂ incubator for 24 hours and if no organisms are seen (or scant growth) they are reincubated for another 24 hours.

A separate plate is routinely used to assess for fungus. Please note that regarding fungal cultures, many of the opportunistic fungi (Aspergillus, Penicillium and Mucor) are rapid growers and colonies will be seen within the first 24-48 hours (mature within 4-5 days) and identified down to the species level when possible. Candida will usually grow in 24-48 hours and can be identified at this time. Speciation requires 72 hours. .

The pathogenic fungi (Histoplasma and Blastomyces) cultures will be referred to the MGH laboratory for speciation. Results are confidentially reported to the medical team at Shriners Burns Hospital.

Normal flora cultured from the sputum (viridians strep Neisseria species, coagulase negative Staphylococcus and rare amounts of yeast, enteric gram negatives, Staph. aureus.) will be documented as normal flora)

Dietary Data

Dietary data (enteral nutrition, parenteral nutrition, oral intake) will be collected daily from the medical record. Total Se intake via supplements and/or enteral feedings will be calculated based on daily intake and output sheets as recorded by the bedside nurse. Dietary Se intake from food will be determined using the Nutrient Database System for Research version 2005.

Other Data

Weights will be obtained as part of routine care by the bedside nurse, and recorded twice a week. Demographic data will be obtained from the medical record. Please see data collection sheet for details.

As part of routine care by the medical team neurological status will be assessed and several laboratory values will be monitored closely (including total protein, albumin, prealbumin and C-reactive protein complete blood count, serum creatinine, AST, ALT, alkaline phosphatase, total bilirubin, and coagulation studies) will be recorded from the medical record.

D. Selenium Analysis

Blood (4 ml or 8 ml) and urine samples will be obtained weekly. De-identified samples will be sent to a commercial laboratory for analyses. Results will be sent to Shriners Burns Hospital in Boston. Additionally, to ensure the safety of all patients, once every 2 weeks, blood samples (4 ml) will also be sent to the Massachusetts General Hospital Chemistry lab for analysis. These results are typically available after 24-48 hours.

The study endpoint will be whichever of the following factors happens first:

- 8 weeks
- discontinuation of central venous catheter
- a stop in Se/Placebo infusion lasting longer than 48 consecutive hours or 72 hours in 1 week,
- when the patient achieves 95% definitive wound closure determined by the attending physician.

No interim analyses are planned. Data obtained from our previous investigation on Se and pediatric burns was used for the power analysis calculation:

$$n = (2 \times [Z_{(1-\alpha/2)} + Z_{(1-\beta)}]^2) / \Delta^2$$

where $Z_{(1-\alpha/2)}$ and $Z_{(1-\beta)}$ represent percentage points of the normal distribution for statistical significance level and power, respectively. The values used were 1.96 for a significance level of 5% and 0.8416 for a power of 80%. The Δ represents the standardized difference (the treatment difference divided by its standard deviation). This was calculated by subtracting the mean plasma Se determined in our pilot study (1.08 $\mu\text{mol/L}$) from the average plasma Se of healthy American children 1.35 ($\mu\text{mol/L}$) and dividing by the standard deviation (0.34). This calculation revealed that 25 subjects are needed per group to obtain 80% power and a significance level of 5%.

All subjects enrolled will be included in the statistical analyses (intent-to treat). A 2-sided statistical test will be used for all analyses and the overall type-I error rate will be controlled at the 5% level. For each dependent variable (plasma selenium, urinary selenium, plasma GPx activity) a 2-way repeated measures ANOVA will be used to examine the difference among groups and over time (primary hypothesis). Tukey's will be utilized as the post-hoc test.

An ordinal logistic regression will be performed to determine the relationship between the incidence of infection (categorized by burn, bloodstream, urinary tract infection, and PNA) and plasma selenium, urinary selenium, plasma GPX, age, initial percent TBSA burned, and treatment group (secondary hypothesis).

Appropriate confounding factors (including but not limited to dietary Se intake, age, sex, race, initial %TBSA, and length of time from burn injury to study enrollment) will be included in the statistical models. All statistics will be completed using SAS software.

VI. Risks and Discomforts

Phlebotomy will be minimal (4 or 8 ml/wk) and will be obtained painlessly through existing catheters or during operative procedures.

Se toxicity is rare. Symptoms include:

- memory loss
- gastrointestinal upset,
- hair loss,
- weakened nails,
- white blotchy nails,
- garlic breath odor,
- metallic taste,
- vomiting,

- sweat
- dermatitis,
- dental defects,
- nervousness,
- mental depression,
- fatigue,
- irritability,
- mild nerve damage,
- Peripheral vascular collapse,
- Internal vascular congestion,
- Hemorrhage,
- Congested and edematus lungs,
- Brick-red color gastric mucosa
- Coma
- Death

Some symptoms of Se toxicity are not specific to Se toxicity and would be difficult to monitor in a pediatric burn patient. Additionally, blood and urine Se values will not be immediately available (being sent to an outside facility for analyses). To assess for toxicity, hair and nails will be monitored weekly for loss, change of appearance, and brittleness. Additionally, to ensure the safety of all patients, once every 2 weeks, blood samples will also be sent to the Massachusetts General Hospital Chemistry lab for analysis of selenium levels. If the selenium level is greater than 100 ug/dL the patient will be immediately removed from the study.

It is important to note that the IOM (31) state in their report that the UL does not apply to patients under medical supervision and that doses of Se above the UL should not be discouraged in clinical trials. Se toxicity has not been reported in trials administering Se supplements to adults with doses up to 1500 ug per day (approximately 25 times the RDA) for short periods of time (1-3 weeks).

VII. Potential Benefits

This study will provide enhanced understanding of Se requirements during burn injury.

VIII. Monitoring and Quality Assurance

All subjects will be monitored for any treatment-related adverse events for 2 weeks following discontinuation of the study therapy. Ongoing adverse events thought to be study drug related will be followed until resolution or the condition stabilizes. Any adverse events will be reported to the Partners Human Research Committee per the guidelines.

Per FDA reporting requirements, serious and unexpected adverse experiences, as well as, unexpected fatal and life-threatening experiences related to this study will be reported to the FDA no later than 7 days after the initial receipt of the information. A complete written report of the unexpected fatal or life-threatening experience will be provided to the FDA within 15 calendar days.

According to the FDA, all “Expected” and “Unexpected” adverse events related to this study will be: (1) classified by relation to study treatment, severity, action taken in response to the adverse event, and clinical outcome; (2) collected and recorded in patient case report forms and adverse event datasets; and (3) reported to the Food and Drug Administration (FDA)

An independent Data Safety Monitoring Board, consisting of 3 knowledgeable staff members, will meet 2 times per year to monitor the data for safety. Members include Carol Scott RN, MS; Derrick Hursey PharmD; Yong Ming Yu, MD, PhD. This board will perform the following duties:

- Verify that the investigators have adequate qualifications.
- Verify that the facilities are adequate to safely and properly conduct the trial.
- Verify that the IV selenium is properly stored and expired drugs are not used.
- Verify that the utilization of the IV selenium is properly documented.
- Verify that all subjects enrolled in the study meet the inclusion criteria throughout the study.
- Verify that written informed consent was obtained before each subject's participation in the trial.
- Verify that the approved protocol was followed.
- Data safety monitoring (review accumulated data to ensure all current and future subjects are safe and to make sure that the study still has scientific merit). All Adverse Events, and Serious Adverse Event reports will be discussed.
- Verify that the study staff is adequately informed about the trial.
- Verify that unauthorized individuals are not participating in the trial.
- Verify that all research records and essential documents are accurate complete, up-to-date, and maintained.

- Verify that the PI provides all required reports, notifications, applications, and submissions to the FDA and the IRB (including adverse events, withdrawals)
- The Board will provide a written report to the PI after each meeting.

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