DISSERTATION

Diagnostic and prognostic value of selenium biomarkers in severe burns

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Diagnostischer und prognostischer Wert von Selen-Biomarkern bei schweren Verbrennungen

zur Erlangung des akademischen Grades
Doctor medicinae (Dr. med.)

vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

von

Tabaël Lee Turan

Erstbetreuung: Prof. Dr. rer. nat. Lutz Schomburg

Datum der Promotion: 29. November 2024

Table of contents

List of tables

List of figures

List of abbreviations

ABSI	Abbreviated Burn Severity Index
AUROC	Area under the ROC curve
BMI	Body mass index
CI	Confidence interval
CRP	C-reactive protein
D	Day
EPIC	European Prospective Investigation into Cancer and Nutrition
ESPEN	European Society for Clinical Nutrition and Metabolism
GPx1	Glutathione peroxidase 1
GPx3	Glutathione peroxidase 3
GSH	Glutathione
HR	Hazard ratio
ICU	Intensive care unit
IQR	Interquartile range
LOS	Length of stay
M	Month
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
NPV	Negative predictive value
PCT	Procalcitonin
PPV	Positive predictive value
RLU	Relative light units
ROC	Receiver Operating Characteristic
ROS	Reactive oxygen species
SD	Standard deviation
Se	Selenium
Sec	Selenocysteine
SELENBP1	Selenium-binding protein 1
SELENOP	Selenoprotein P
SeMet	Selenomethionine
TBSA	Total body surface area
TXRF	Total reflection X-ray fluorescence
W	Week
WBC	White blood cells

Abstract

Severe burns are devastating injuries, estimated to account for 180,000 deaths annually. Skin barrier disruption and invasive procedures lead to an increased susceptibility to infections and sepsis, being the leading cause of death. Burn patients frequently exhibit an acute selenium (Se) depletion, associated with adverse clinical outcomes. As Se exerts essential antioxidant and anti-inflammatory properties by incorporation into selenoproteins, the serum Se status at admission may affect sepsis risk in these patients. Selenium-binding protein 1 (SELENBP1), an intracellular protein, was hypothesized to be elevated in serum of burn patients, with potential prognostic implications.

In this longitudinal observational study, adult patients admitted to the Burn Center of the University Hospital Zurich, Switzerland, were enrolled. As part of routine clinical care, high-dose intravenous Se supplementation was administered during the first week post-burn. Blood samples were drawn upon admission and at eight further time-points for up to six months after the injury. In addition to SELENBP1, three complementary biomarkers of Se status were assessed in patient sera, namely total Se, selenoprotein P (SELENOP), and glutathione peroxidase 3 (GPx3). The obtained data were correlated with clinical parameters, and the development of sepsis (Sepsis-3) was chosen as the primary outcome.

Of the 90 included patients, 73 (81%) were male. The median age was 48 years. A large proportion of patients developed sepsis during hospitalization (n = 55; 61%). The initial Se status was markedly depressed and inversely associated with burn severity. In contrast, SELENBP1 was initially elevated, directly related to burn severity, and declined within the first day. A transient normalization of Se status was observed as of week 1. Patients with low baseline levels of SELENOP (< 3.65 mg/L) were at significantly higher risk of sepsis than those showing a higher SELENOP at admission (adjusted HR, 1.94; 95% CI, 1.05–3.63; p = 0.035). Regarding sepsis risk prediction, a combination of the Abbreviated Burn Severity Index (ABSI) and baseline concentrations of SELENOP and white blood cells (WBC) achieved an area under the curve of 0.84 (95% CI, 0.75–0.93; p < 0.0001), thus outperforming the predictive power of the ABSI alone or the ABSI and WBC combined.

In conclusion, the clinical implementation of serum SELENOP assessment after severe burns may assist in Se supplementation monitoring and early sepsis risk stratification, facilitating an improved personalization of nutritional therapy and infection control.

Zusammenfassung

Schwere Verbrennungen stellen verheerende Verletzungen dar, die für schätzungsweise 180.000 Todesfälle jährlich verantwortlich sind. Die Störung der Hautbarriere und invasive Maßnahmen führen zu einer erhöhten Anfälligkeit für Infektionen und Sepsis, der Haupttodesursache. Verbrennungspatienten zeigen häufig einen akuten Mangel an Selen (Se), der sich nachteilig auf das klinische Outcome auswirkt. Da Se über Inkorporation in Selenoproteine essenzielle antioxidative und antiinflammatorische Eigenschaften aufweist, könnte der Se-Status bei Patientenaufnahme das Sepsisrisiko beeinflussen. Das Selen-bindende Protein 1 (SELENBP1), ein intrazelluläres Protein, könnte im Serum von Verbrennungspatienten erhöht sein und prognostische Bedeutung haben.

In die vorliegende Längsschnittstudie wurden erwachsene Patienten eingeschlossen, die im Verbrennungszentrum des Universitätsspitals Zürich aufgenommen wurden. Im Rahmen der Standardversorgung wurde eine hochdosierte intravenöse Se-Supplementation während der ersten Woche durchgeführt. Blutentnahmen erfolgten bei Aufnahme und an acht weiteren Zeitpunkten für bis zu sechs Monate nach der Verletzung. Zusätzlich zu SELENBP1 wurden drei Biomarker des Se-Status im Serum bestimmt, nämlich Gesamt-Se, Selenoprotein P (SELENOP) und Glutathionperoxidase 3 (GPx3). Die erhobenen Daten wurden mit den klinischen Parametern korreliert und Sepsis (Sepsis-3) wurde als primärer Endpunkt festgelegt.

Von 90 eingeschlossenen Patienten waren 73 (81 %) männlich. Das Medianalter lag bei 48 Jahren. Ein Großteil der Patienten erkrankte im Zuge des Klinikaufenthaltes an einer Sepsis (n = 55; 61 %). Der initiale Se-Status war deutlich vermindert und korrelierte invers mit der Verbrennungsschwere. Im Gegensatz dazu war SELENBP1 in direktem Zusammenhang mit der Verbrennungsschwere erhöht und fiel innerhalb des ersten Tages wieder ab. Ab Woche 1 war eine vorübergehende Normalisierung des Se-Status zu beobachten. Patienten mit niedrigen Serum SELENOP-Konzentrationen bei Aufnahme (< 3,65 mg/L) waren einem signifikant höheren Sepsisrisiko ausgesetzt als Patienten mit höheren SELENOP-Konzentrationen (adjustierte HR 1,94; 95 % CI 1,05–3,63; p = 0,035). Eine Kombination aus dem Abbreviated Burn Severity Index (ABSI) sowie SELENOP und Leukozytenzahl bei Aufnahme erreichte für die Vorhersage von Sepsis eine Fläche unter der Kurve von 0,84 (95 % CI 0,75–0,93; p < 0,0001) und übertraf somit die Vorhersagekraft des ABSI oder einer Kombination aus ABSI und Leukozytenzahl.

Schlussfolgernd könnte die Messung von SELENOP im Serum bei der Überwachung der Se-Supplementation und bei der Risikostratifizierung nach schweren Verbrennungen helfen und dadurch eine verbesserte Personalisierung der Ernährungstherapie und Infektionskontrolle ermöglichen.

1 Introduction

1.1 Burn injury

Burns are defined as traumatic injuries to the skin or other organic tissues caused by heat, radiation, electricity, certain chemicals, or other acute exposures. Depending on their cause, size, depth, and anatomic location, burn injuries can lead to severe and persistent physical and psychological alterations associated with impaired quality of life and substantial direct and indirect costs (1-4).

Burn incidence and burn-related mortality have declined over the last decades, mainly due to the successful implementation of burn prevention measures, as well as burn care advances (5). Still, the burden of burn morbidity and mortality remains disproportionately higher in low- and middle-income countries, where access to specialized burn care is limited and effective prevention programs may not be established (6, 7). The World Health Organization estimates that burns account for 180,000 deaths annually (8). Burn mortality is determined by a variety of factors, including age, burn size, and inhalation injury, i.e., damage to the respiratory system resulting from exposure to thermal or chemical irritants (9, 10).

1.1.1 Classification

Clinically, burn injuries need to be classified according to their severity. Burn depth and burn size constitute the main determinants of burn severity (11).

The depth of injury is commonly categorized into four degrees (**Figure 1**):

- Superficial (first-degree) burns involve only the epidermis. Since the epidermal barrier is intact, the affected area appears erythematous and dry. Pain is usually moderate and limited in duration; restitutio ad integrum within a few days.
- Partial-thickness (second-degree) burns can either involve the epidermis and the outer layer of the dermis (superficial partial-thickness) or penetrate further into the deep dermis (deep partial-thickness). In the former case, burns are erythematous, moist, blistered, and painful. The tissue typically blanches with pressure. On the other hand, deep partial-thickness burns appear drier, do not blanch, and are less painful because of partial destruction of the pain receptors. Partial-thickness burns are more likely to scar the deeper they are.

- Full-thickness (third-degree) burns involve all skin layers, i.e., the epidermis, dermis, and subcutis. They are characterized by a painless, leather-like eschar. The risk of (hypertrophic) scarring is high. In most cases, surgery is indicated.
- Fourth-degree burns extend through the skin into underlying tissues, such as fascia, muscle, or bone. These are life-threatening injuries that frequently require amputation of the burned parts.

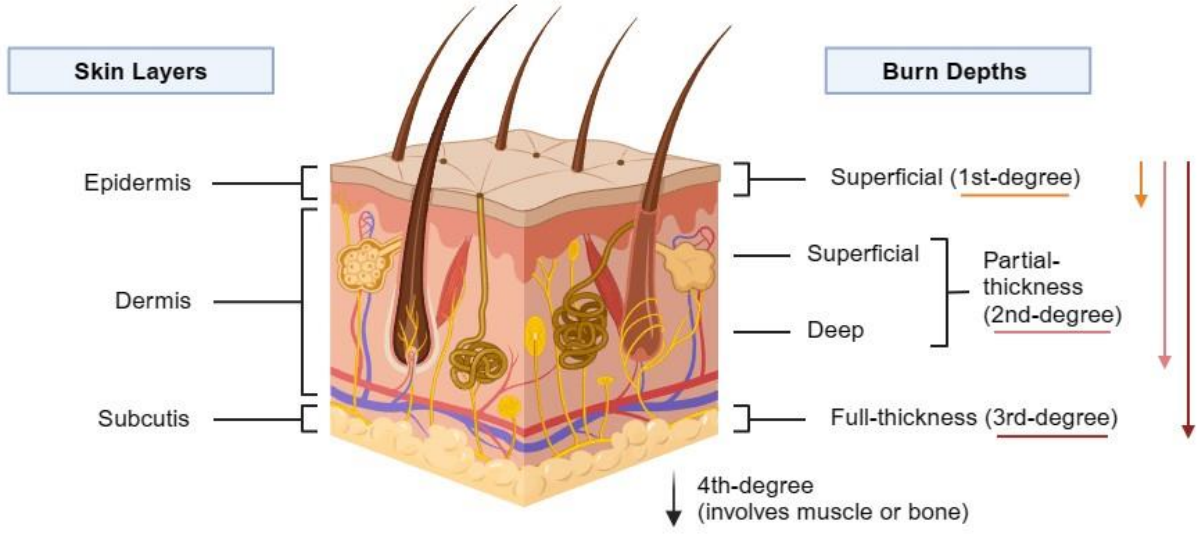

Figure 1: Burn depth. The precise assessment of burn depth is pivotal to selecting the most appropriate treatment in severely burned patients. Four degrees of burns can be differentiated by the skin layers involved. Burns affecting the epidermis (superficial/first-degree) or dermis (partial-thickness/second-degree) can heal without surgery, whereas injuries penetrating into the subcutis (full-thickness/third-degree) or even deeper tissues (fourth-degree) require surgical interventions. Own figure, created with BioRender.com.

Burn size is best described by the percentage of total body surface area (TBSA) burned. The *Wallace Rule of Nines* and, more accurately, *Lund-Browder charts* are well-established methods for estimating TBSA. Superficial burns must be excluded from the TBSA calculation. Besides mechanism, depth, and size, the anatomic location of a burn dictates its severity and therefore guides the treatment the patient will undergo. For instance, burn injuries on the face, hands, feet, or perineum require specialized management. Moreover, burns can be classified as either minor or major. According to the 2022 American Burn Association Guidelines for Burn Patient Referral, burns meeting the following criteria are considered severe and should be treated at a specialized burn center (12, 13):

- Full-thickness burns
- Partial-thickness burns ≥ 10% TBSA
- Deep partial- or full-thickness burns involving certain body regions such as the face, hands, feet, or genitalia
- Concomitant traumatic injuries
- Comorbidities which may lead to prolonged treatment
- Inhalation injury
- All burns in children (≤ 14 years or < 30 kg)
- Chemical burns
- Electrical burns

1.1.2 Pathophysiology

Burn injuries trigger a complex pathophysiologic response at both a local and a systemic level, as visualized in **Figure 2**. The magnitude of the host response is directly related to the burn severity (14).

In 1953, D. M. Jackson proposed a model in which the local changes in burn wounds are divided into three concentric zones: the peripheral *zone of hyperemia* with increased blood flow and inflammation, the intermediate *zone of stasis* which can either survive or progress to coagulative necrosis, and the central *zone of coagulation* with irreversibly damaged tissue (15). Alongside inflammatory vasodilation, the early burn wound microenvironment is characterized by microthrombosis and a considerable release of pro-inflammatory cytokines, chemokines, and reactive oxygen species (ROS). Early wound care aims at preventing horizontal and vertical burn wound expansion (16-18).

The inflammatory response during the initial stage after injury appears crucial for wound healing promotion and mitigation of secondary infection risk. Following severe burns, however, this process can become dysregulated (19, 20). The deleterious hypermetabolic and hyperinflammatory state involves all major organ systems and persists up to three years postburn. Excessively secreted catecholamines, glucocorticoids, and cytokines induce a cascade of systemic metabolic alterations, resulting in a profound catabolic state. Another hallmark of burn pathophysiology is the formation of local and generalized edema in consequence of increased vascular permeability and extravasation of intravascular fluid (*capillary leakage*). Hence, an early and adequate intravenous fluid resuscitation is essential to prevent progression to hypovolemic shock (21-24).

Due to metabolic and immunological derangements, impaired skin integrity, exposure to invasive procedures, and prolonged hospitalization, severely burned patients are at particular risk of developing infectious complications. Sepsis and subsequent multiple organ failure are the leading causes of mortality among these patients (25-27).

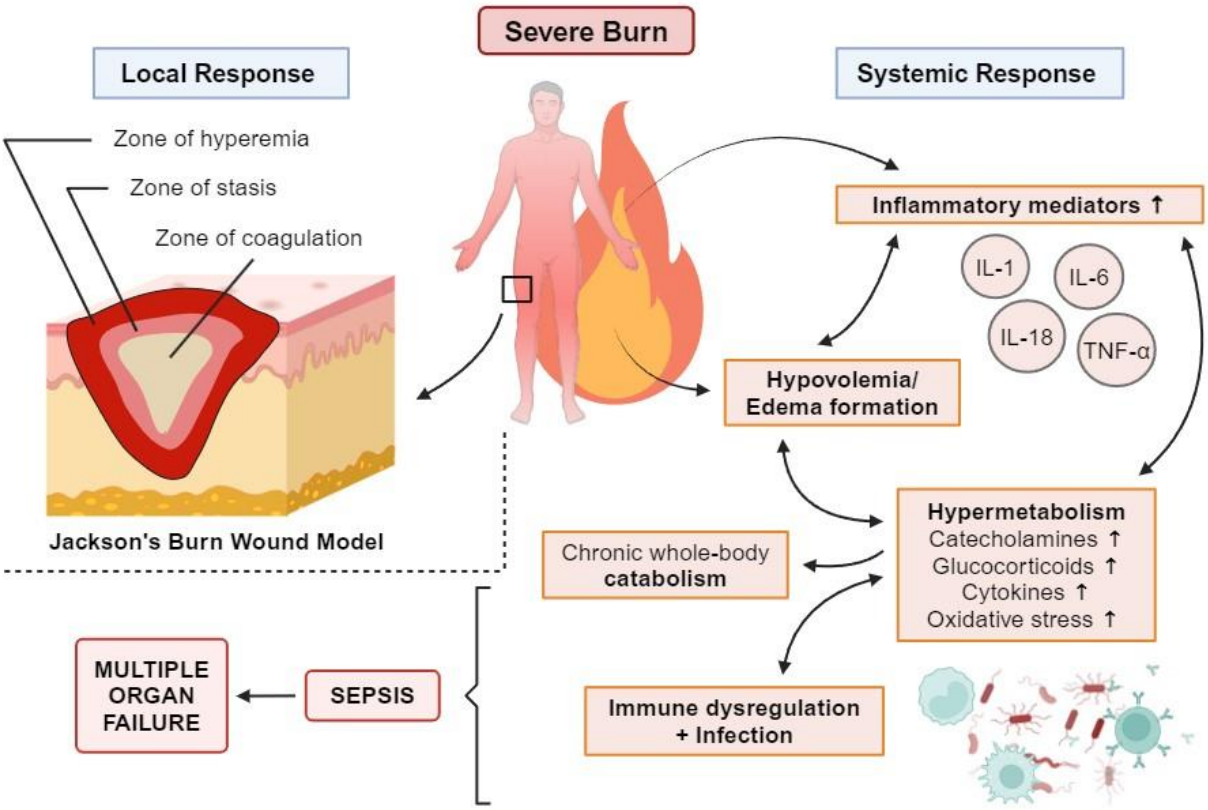

Figure 2: Local and systemic effects of severe burns. Severe burns not only affect the skin, but also induce a profound pro-inflammatory and hypermetabolic response, which may lead to infectious complications and multiple organ failure. Own figure, created with BioRender.com.

1.1.3 Management

The great etiological and clinical heterogeneity in severely burned patients necessitates a complex treatment regimen that addresses both the local burn wound as well as the long-term psychological consequences of the injury. As severe burns are potentially life-threatening, the initial assessment and management should prioritize the ABC (airway, breathing, circulation) approach (28). Upper or lower airway obstruction is a large contributor to early burn deaths. Endotracheal intubation is particularly indicated in patients with burns of more than 40% TBSA, full-thickness facial burns, and clinically significant smoke inhalation. In case of suspected carbon monoxide or cyanide poisoning, 100% high-flow oxygen should be administered (29, 30). Considering the risk of burn shock

development owing to intravascular volume depletion, burns greater than 20% TBSA require intravenous fluid resuscitation with balanced crystalloids. The Parkland formula (4 ml of Ringer's lactate solution/kg/%TBSA in adults) is the most widely used method for estimating resuscitative fluid needs for the first 24 hours postburn, with the first half given in the first eight hours (31-33).

Prevention and control of infectious complications and sepsis is another major component of burn management. In addition to topical and systemic antibiotics, early burn wound excision and skin grafting are accepted practice (11, 34). Furthermore, in view of the hypercatabolic state, proper nutritional support should be provided, preferably enterally within 24 hours of injury. A diet high in protein (1.5–2 g/kg/d in adults) and energy, based on regular indirect calorimetry measurements, is essential (35). As a result of increased metabolic requirements and extensive cutaneous exudative losses, severely burned patients frequently develop decreased serum levels of trace elements (36). Notwithstanding that the clinical significance of micronutrients in critical illness is controversial (37), early intravenous supplementation of essential trace elements is recommended in burn patients as it has been associated with a reduced incidence of secondary infections, improved wound healing, and a shortened hospitalization (38, 39).

1.2 Selenium-containing proteins

Selenium (Se) is an essential trace element, discovered by Swedish chemist Jöns Jacob Berzelius in 1817. Dietary Se is obtained mainly in organic forms such as selenocysteine (Sec) and selenomethionine (SeMet), whereas nutritional supplements commonly contain the inorganic Se compounds selenite or selenate (40, 41). Selenium exerts its numerous biological effects predominantly by incorporation into proteins in the form of genetically encoded Sec (42, 43). These proteins are designated as selenoproteins, a majority of which exerting enzymatic functions using Sec at their active site. In humans, 25 selenoprotein genes have been identified (44). The encoded proteins are grouped into multiple subfamilies, such as the glutathione peroxidases, the iodothyronine deiodinases, and the thioredoxin reductases (45). Notably, their synthesis is regulated hierarchically, i.e., certain selenoproteins are prioritized over others in case of insufficient Se supply (46, 47). In serum, extracellular GPx (GPx3) and selenoprotein P (SELENOP) are of particular functional relevance. They are directly related to total serum Se until optimized expression levels are achieved. Saturated expression of SELENOP and GPx3 has been reported at

serum Se concentrations of 125 and 90 µg/L or greater, respectively. Therefore, they are regarded as most suitable biomarkers for the assessment of serum Se status in Se-deficient subjects (48-50). As dietary-derived SeMet can replace methionine during translation, many proteins in blood contribute to the Se status, albeit to a small und unregulated extent, solely dependent on the fraction of SeMet that was taken up by the diet (51). The remaining group of Se-containing proteins comprises the poorly characterized Se-binding proteins, in which Se is directly bound to the molecules (42).

1.2.1 Selenoprotein P

Selenoprotein P is an extracellular glycoprotein with up to 10 Sec residues per molecule (52, 53). It accounts for most of the Se in plasma (54, 55). After its hepatic synthesis and release into the bloodstream, SELENOP primarily serves as the main Se transporter to essential target tissues such as the brain and the reproductive organs (56-60). Several studies have demonstrated the suitability of SELENOP as a reliable and meaningful biomarker of Se status, at least under conditions of Se deficiency (55, 61). As mentioned above, SELENOP requires larger Se intakes to reach full expression than GPx3, indicating that it constitutes a better marker of Se nutritional status (48, 49). Additionally, circulating SELENOP may be capable of detecting excessive Se intakes as it exceeded the intermediate plateau concentration of 6–7 mg/L in patients receiving daily doses of > 1 mg sodium selenite intravenously (62, 63). The expression of serum SELENOP is reported to be downregulated by inflammatory cytokines (64-66) and certain substances (67-69), among other things. Serum levels have been shown to significantly decline in critical illness (70-73).

1.2.2 Glutathione peroxidase 3

Glutathione peroxidases catalyze the reduction of a variety of hydroperoxides, typically using glutathione (GSH) as the reducing substrate. The extracellular isoform, GPx3, is a glycoprotein synthesized mostly in the kidney (74-76). It accounts for a major part of the antioxidant activity in plasma (77). The expression of GPx3 is transcriptionally upregulated by hypoxia through a HIF-1-binding site (78). Likewise, there has been evidence of GPx3 upregulation by ROS-mediated inflammation (79, 80). Early decreases in GPx3 activity have been measured in major burns (39) as well as in sepsis (81, 82).

1.2.3 Selenium-binding protein 1

Selenium-binding protein 1 (SELENBP1) is an intracellular, ubiquitously expressed 56-kDa protein that covalently binds Se (42, 83, 84). To date, the biological function of SELENBP1 under physiological and pathological conditions is largely unknown, even though it was already discovered in 1989 (85). However, it may be involved in cell differentiation (86, 87), proteasomal protein degradation (88), intra-Golgi transport (89), and redox modulation (90), including reciprocal functional interference with the antioxidant activity of the selenoenzyme glutathione peroxidase 1 (GPx1) (91). Moreover, it may act as a tumor suppressor, and reduced expression levels have been correlated with a poor clinical prognosis of various human malignancies (92). Through *SELENBP1* mutations in patients with extraoral halitosis, the encoded protein has been identified as the human methanethiol oxidase (90, 93). This enzymatic activity was found to be dependent on the trace element copper, a surprising notion and in some contrast to its initial perception and denomination (94). Subcellularly, SELENBP1 is localized in both the nucleus and the cytoplasm (95). Following major trauma, however, SELENBP1 can be released into the systemic circulation, e.g., after traumatic spinal cord injury (96) or acute coronary syndrome (97). Elevated serum concentrations have been associated with adverse clinical outcomes in these patients.

1.3 Research aims

Severe burns are accompanied by a persistently dysregulated pathophysiologic state. Selenium, an essential trace element for the biosynthesis of proteins with antioxidant and anti-inflammatory properties, rapidly declines in serum of burn patients. Previous trials found early Se supplementation to sufficiently counteract these losses. However, serum SELENOP and SELENBP1 dynamics have not yet been examined in burn injury and Se status assessment was only carried out during the first month postburn (98). Given that infectious complications and sepsis remain the primary causes of death following major burns, reliable biomarkers for both an early diagnosis and therapeutic monitoring are needed (99).

This investigation aimed at testing the following research hypotheses (**Figure 3**):

- Serum SELENBP1 is elevated in burn patients immediately after injury.
- Serum Se status (total Se, SELENOP, GPx3) and serum SELENBP1 at baseline are inversely and positively associated with burn severity, respectively.

- Early high-dose intravenous Se supplementation induces a stabilization of serum Se status during the first six months postburn.
- Burn patients with a lower serum Se status and higher serum SELENBP1 levels at baseline are more likely to develop sepsis during the clinical course, irrespective of trauma severity.

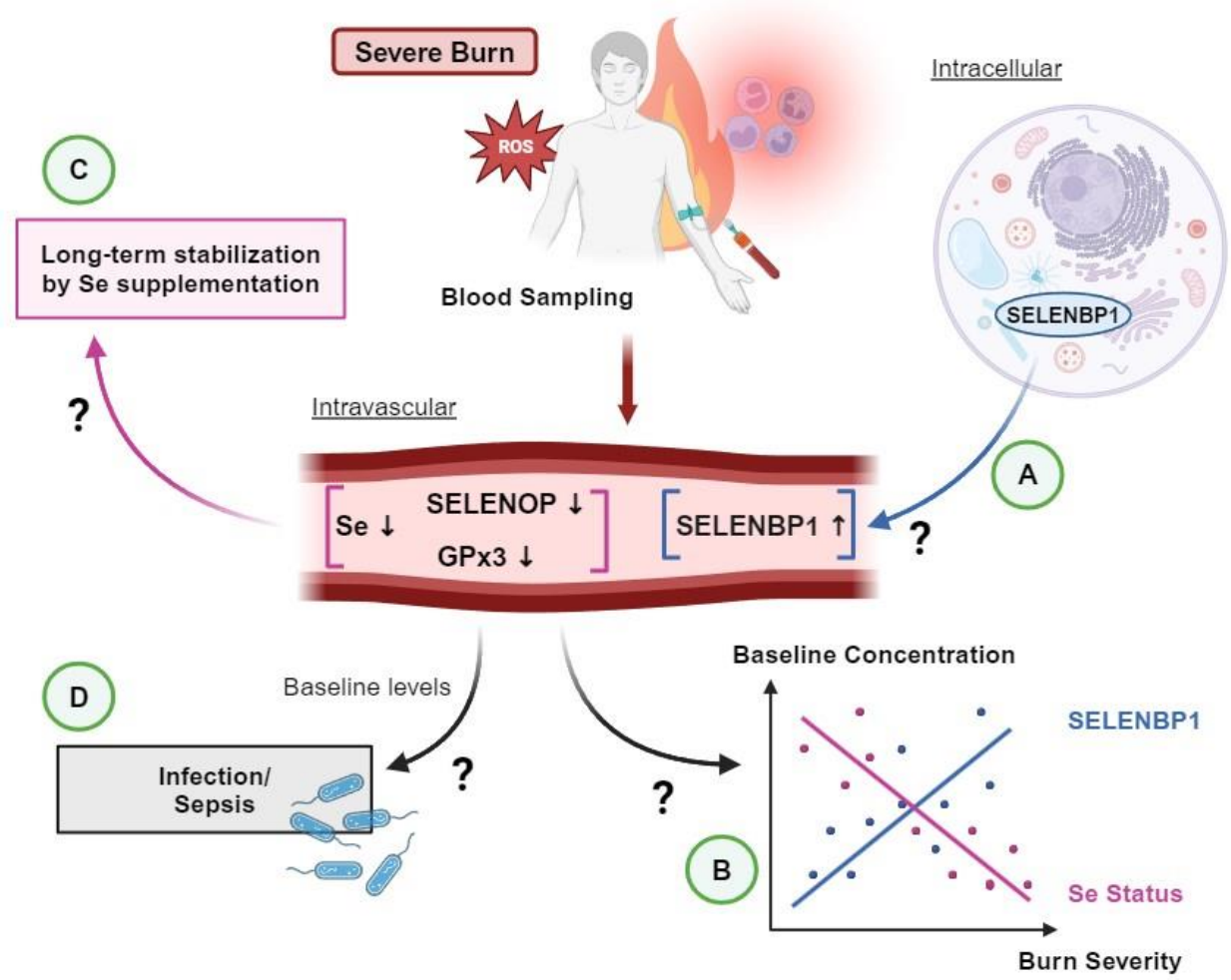

Figure 3: Research hypotheses. The present thesis aimed to assess (**A**) the serum SELENBP1 dynamics following major burns and (**B**) how baseline concentrations of Se status and SELENBP1 correlate with the burn severity. Furthermore, we hypothesized that (**C**) an early supplementation of Se may induce a sustained stabilization of serum Se status. Lastly, (**D**) burn patients with a lower serum Se status and higher serum SELENBP1 levels at baseline were assumed to be more likely to develop sepsis. Own figure, created with BioRender.com.

2 Methods

2.1 Study design

In this prospective cohort study, adult patients (≥ 18 years of age) admitted to the Burn Center of the University Hospital Zurich, Switzerland, between May 2015 and October 2018 were eligible for participation. Patients with current infection at admission, immunosuppressive medication, and burns older than 6 h were excluded. All patients or legally authorized representatives provided informed consent before enrollment. The trial was conducted in accordance with the principles of the Declaration of Helsinki. Ethics approval was obtained from the Ethics committee of the University of Zurich, Switzerland, on April 20th, 2015 (KEK-ZH-No.: 2014–0631).

Variables that were recorded at inclusion were age, sex, body mass index (BMI), and coexisting conditions. The initial burn severity assessment was based on the Abbreviated Burn Severity Index (ABSI), a widely used predictive scoring system that was developed by Tobiasen et al. in 1982. It consists of five parameters: sex (1 point for females), age (1 point for every 20-year increase in age), presence of inhalation injury (1 point), presence of full-thickness burn (1 point), and percentage of TBSA burned (1 point for every 10% increase in TBSA) (100). Affected TBSA was calculated using Lund-Browder charts. The patients received standard care according to local practice, which included an initial cardiovascular and respiratory stabilization, surgical interventions, fluid resuscitation, early enteral nutrition, and regular indirect calorimetry to estimate caloric requirements. Besides, all patients were supplemented with 1000 µg of Se daily from admission to day 7 inclusively, administered by continuous infusion of sodium selenite. Unstable patients continued receiving intravenous Se (500 µg/d) until clinical stability was reached.

As illustrated in **Figure 4**, venous blood sampling was first performed upon admission. Subsequent blood samples were longitudinally collected at eight additional time-points postburn: days (D) 1, 2, and 3; weeks (W) 1 and 2; and months (M) 1, 3, and 6. In total, *n* = 598 serum samples were prepared and stored at –80 °C. Follow-up sampling was also carried out after hospital discharge in a subset of patients. Within the first two weeks, less than 10% of the data were missing. However, the number of missing values increased over time, for example due to patient refusal to further participate in the study. At M6 after injury, blood samples had been successfully obtained in 37% of patients.

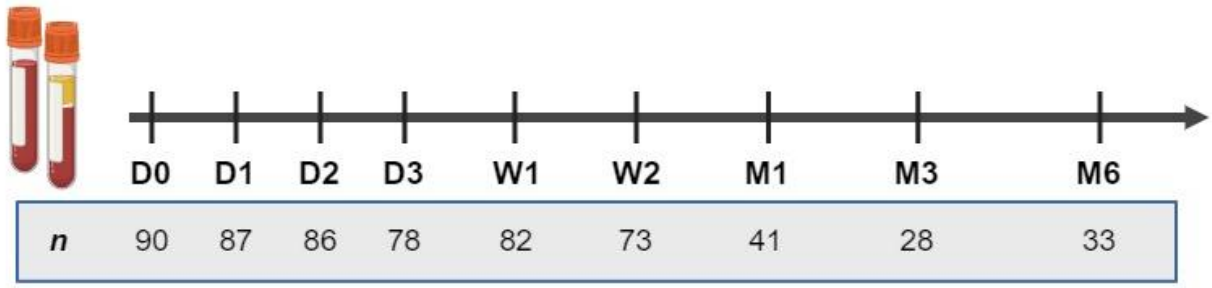

Figure 4: Timeline of blood sampling. Blood samples were drawn at nine time-points over a 6-month period, starting at admission to the burn center (D0). The number of patients (*n*) at each time-point is shown. D: day; W: week; M: month. Own figure, created with BioRender.com.

Pulmonary, catheter-related, urinary tract, cutaneous, and bloodstream infections were differentiated, with reference to the Centers for Disease Control and Prevention definition for nosocomial infections (101). Development of sepsis was chosen as the primary clinical outcome measure. Sepsis diagnosis was based on the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3), which define sepsis as a “life-threatening organ dysfunction caused by a dysregulated host response to infection” (102). Secondary clinical outcomes were nosocomial infections, in-hospital mortality, hospital length of stay (LOS), and intensive care unit (ICU) LOS. Pneumonia diagnosis required a new pulmonary infiltrate on the chest radiograph, accompanied by relevant clinical manifestations, e.g., fever, cough, purulent expectoration, or dyspnea.

2.2 Laboratory analyses

Measurements of serum Se status and SELENBP1 were conducted in the laboratories of the Institute for Experimental Endocrinology, Charité – Universitätsmedizin Berlin, by researchers and technicians blinded to the clinical data.

Total serum Se was quantified by total reflection X-ray fluorescence (TXRF) using a benchtop TXRF spectrometer (S4 T-STAR, Bruker Nano GmbH, Berlin, Germany) (103, 104). Briefly, serum was diluted 1:2 with a gallium standard (1000 µg/L), and 8 µL of the dilution was applied to polished quartz glass slides. Samples were dried overnight in a 37 °C incubator. Seronorm serum standard (Sero AS, Billingstad, Norway) served as control. Before each use, the quartz sample carriers were successively cleaned with distilled water, acetone, and nitric acid under a laboratory fume hood.

Serum SELENOP concentrations were measured by a validated commercial sandwich ELISA (selenOtest ELISA, selenOmed GmbH, Berlin, Germany) (105). In short, pre-diluted serum samples were applied to antibody pre-coated 96-well plates. According to the manufacturer's instructions, a biotin-labeled human SELENOP monoclonal antibody and, subsequently, horseradish peroxidase-conjugated streptavidin were added. After that, a 3,3',5,5'-tetramethylbenzidine-based peroxidase substrate was added, followed by diluted sulfuric acid to stop the enzymatic reaction. The color then changed from blue to yellow, and the light absorbance was measured at a wavelength of 450 nm. Standards and controls were included for calibration and quality control.

The activity of GPx3 was determined by monitoring nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) consumption at 340 nm in a coupled enzymatic test, as described earlier (106). In brief, serum samples were applied to 96-well plates and incubated with a test mixture containing 1 mmol/L NaN_3, 3.4 mmol/L reduced GSH, 0.3 U/mL glutathione reductase, and 0.27 mg/mL NADPH. After the plates were transferred to a microplate reader (Infinite M200 Pro, Tecan Group AG, Männedorf, Switzerland), measurements were started by adding 10 µL of 0.00375% H_2O_2. Consumption of NADPH was proportional to GPx3 activity in 5 µL serum. Quality of measurements was verified by including a standard serum sample into each assay run.

Serum levels of SELENBP1 were assessed by a recently established luminometric sandwich assay (97). A monoclonal anti-SELENBP1 antibody was coated onto 96-well plates. Following intermediate washing and blocking steps, the samples were diluted with phosphate-buffered saline and added to the plates. Thereafter, the detection antibody was added, and the plates were incubated for one hour at room temperature, washed, and finally placed into a luminometer (Mithras LB 940, Berthold Technologies GmbH & Co. KG, Bad Wildbad, Germany) to measure the relative light units (RLU) at 430 nm, after injecting 0.06% H_2O_2 and 0.2 mol/L NaOH. Calibration standards and a standard serum were included in each plate. Absolute concentrations of SELENBP1 were derived from the RLU values by regression analysis.

Inflammatory biomarkers, such as white blood cells (WBC), C-reactive protein (CRP), and procalcitonin (PCT), were measured at the Institute of Clinical Chemistry, University Hospital Zurich, as part of routine clinical procedures.

2.3 Statistical analyses

Statistical analyses were conducted using GraphPad Prism (Version 10.0.2; GraphPad Software Inc., San Diego, CA, USA). The D'Agostino-Pearson test was used to assess data normality. Normally and non-normally distributed continuous variables were reported as means ± standard deviations (SD) and medians with interquartile range (IQR, Q1–Q3), and further evaluated using the Student's *t*-test and the Mann-Whitney *U* test, respectively. Categorical variables were expressed as frequencies (percentages), and further analyzed using the Fisher's exact test. Kruskal-Wallis test was applied when comparing more than two groups. Correlations were examined using nonparametric Spearman's rank correlation test. Considering the number of missing values, biomarker time courses were compared between groups using a repeated measures mixed-effects model with Geisser-Greenhouse correction, followed by post hoc analysis through Šidák's test.

Cox proportional hazards regression was used to estimate hazard ratios (HR) and 95% confidence intervals (CI) for the association between Se biomarkers at baseline and sepsis occurrence, both crude and adjusted for age, sex, and parameters of burn severity. The assumption of proportional hazards was tested by a graphical approach prior to that. Given the limited sample size, further covariates such as inflammatory biomarkers were not included in multivariate analysis, as an increase in model complexity could have resulted in overfitting and multicollinearity. Instead, Receiver Operating Characteristic (ROC) analysis was conducted to compare the ability of clinical and laboratory parameters to predict the development of sepsis in burn patients. Points on the ROC curve closest to (0,1) were defined as cut-off values, and the area under the ROC curve (AUROC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were reported accordingly.

Reference values for serum Se and SELENOP were derived from a healthy subset (n = 2069) of the multicenter European Prospective Investigation into Cancer and Nutrition (EPIC) study (107). The 2.5th percentile of the reference population was set as threshold for deficiency, i.e., Se < 45.9 µg/L and SELENOP < 2.56 mg/L (108). No such threshold was available for GPx3 activity, as it was not measured in EPIC. Two-tailed p-values < 0.05 were considered significant; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

3 Results

3.1 Patient characteristics

Baseline characteristics and clinical outcomes of the study participants are presented in **Table 1**. A total of *n* = 90 burn patients with a median (IQR) age of 48 (31.0–57.8) years were enrolled to the study, comprising 73 (81.1%) males and 17 (18.9%) females. Female patients were at increased risk of death, as indicated by a considerably higher ABSI (9.4 ± 3.0 vs. 6.9 ± 2.2; $p = 0.001$). Within the study cohort, 5 (5.6%) patients with a mean (± SD) ABSI of 11.8 (± 2.2) died during the observation period.

Table 1: Demographic and clinical characteristics of the patients.

	Sepsis (*n* = 55)	**No Sepsis (*n* = 35)**	**Total (*n* = 90)**
Baseline characteristics			
Male sex	42 (76.4)	31 (88.6)	73 (81.1)
Age (years)	52.0 (31.5–60.0)	38.0 (31.0–54.0)	48.0 (31.0–57.8)
BMI (kg/m^2)	24.8 (22.4–29.2)	26.3 (23.0–30.3)	25.7 (22.6–29.3)
TBSA (%) ****	35.0 (25.5–45.5)	20.0 (14.3–29.5)	29.0 (20.0–37.0)
Full-thickness burn **	39 (70.9)	13 (37.1)	52 (57.8)
Inhalation injury *	18 (32.7)	4 (11.4)	22 (24.4)
ABSI ****	8.3 ± 2.4	5.9 ± 2.0	7.3 ± 2.5
Clinical outcomes			
Infection ****	55 (100.0)	4 (11.4)	59 (65.6)
Pulmonary ****	36 (65.5)	1 (2.9)	37 (41.1)
Cutaneous	8 (14.5)	1 (2.9)	9 (10.0)
Catheter-related *	7 (12.7)	0 (0.0)	7 (7.8)
Bacteremia	3 (5.5)	1 (2.9)	4 (4.4)
Urinary	1 (1.8)	1 (2.9)	2 (2.2)
Mortality	4 (7.3)	1 (2.9)	5 (5.6)
Length of stay (d) ****	46.0 (22.0–78.0)	20.0 (11.5–26.0)	27.0 (19.0–56.0)
Length of ICU stay (d) ****	27.0 (16.0–50.5)	8.0 (2.5–15.0)	17.5 (8.3–40.8)

N (%); mean ± SD; median (IQR). BMI: body mass index; TBSA: total body surface area; ABSI: abbreviated burn severity index; ICU: intensive care unit.

* $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$ (Student's *t*-test; Mann-Whitney *U* test; Fisher's exact test). Own table.

Of the 90 burn patients included, 55 (61.1%) developed sepsis at a median (IQR) of 6 (3–8) days after admission. The groups did not differ significantly regarding sex ratio, age, and BMI. However, septic patients have suffered more severe burns, as evidenced by a higher TBSA, a higher fraction of full-thickness burns and inhalation injuries, and, consequently, a higher ABSI (8.3 ± 2.4 vs. 5.9 ± 2.0; $p < 0.0001$). Sepsis resulted primarily from pulmonary infection (36/55; 65.5%). Four patients developed an infectious complication without accompanying sepsis. The median [IQR] LOS was significantly longer in septic patients (46 [22–78] d) than in those who did not develop sepsis (20 [11.5–26] d). Septic patients also experienced a longer median ICU LOS.

3.2 Correlation of selenium biomarkers

Three complementary biomarkers of serum Se status were evaluated, namely total Se, SELENOP, and GPx3 activity. Correlation analyses among these parameters revealed significant positive correlations across the full set of samples (**Figure 5**), with total Se and SELENOP exhibiting the most stringent correlation (**Fig. 5A**), followed by total Se and GPx3 (**Fig. 5B**), and SELENOP and GPx3 (**Fig. 5C**). The obtained results are indicative of the largely depressed Se status in burn patients and verify the high sample quality as well as the suitability of the analytical methods used.

Consistent with our understanding of optimized selenoprotein expression, Se concentrations exceeding the threshold for SELENOP saturation (125 µg/L) were less strongly correlated with SELENOP (R = 0.384; $p = 0.008$) than Se levels below 125 µg/L (R = 0.736; $p < 0.0001$) (**Fig. 5A**). Likewise, the correlation of total Se and GPx3 activity was less stringent above the GPx3 saturation threshold (90 µg/L), as compared to Se levels below 90 µg/L (R = 0.099; not significant vs. R = 0.518; $p < 0.0001$) (**Fig. 5B**). A similar pattern of more stringent correlation at lower Se status was observed for the interrelationship between serum SELENOP and GPx3 activity (**Fig. 5C**).

In time-dependent analyses, serum SELENBP1 showed significant inverse correlations with total Se at D1 (R = –0.282) and D2 (R = –0.227), and with SELENOP at admission (R = –0.382), D1 (R = –0.305), and D2 (R = –0.303). There was no significant association between SELENBP1 and GPx3 activity at any time-point.

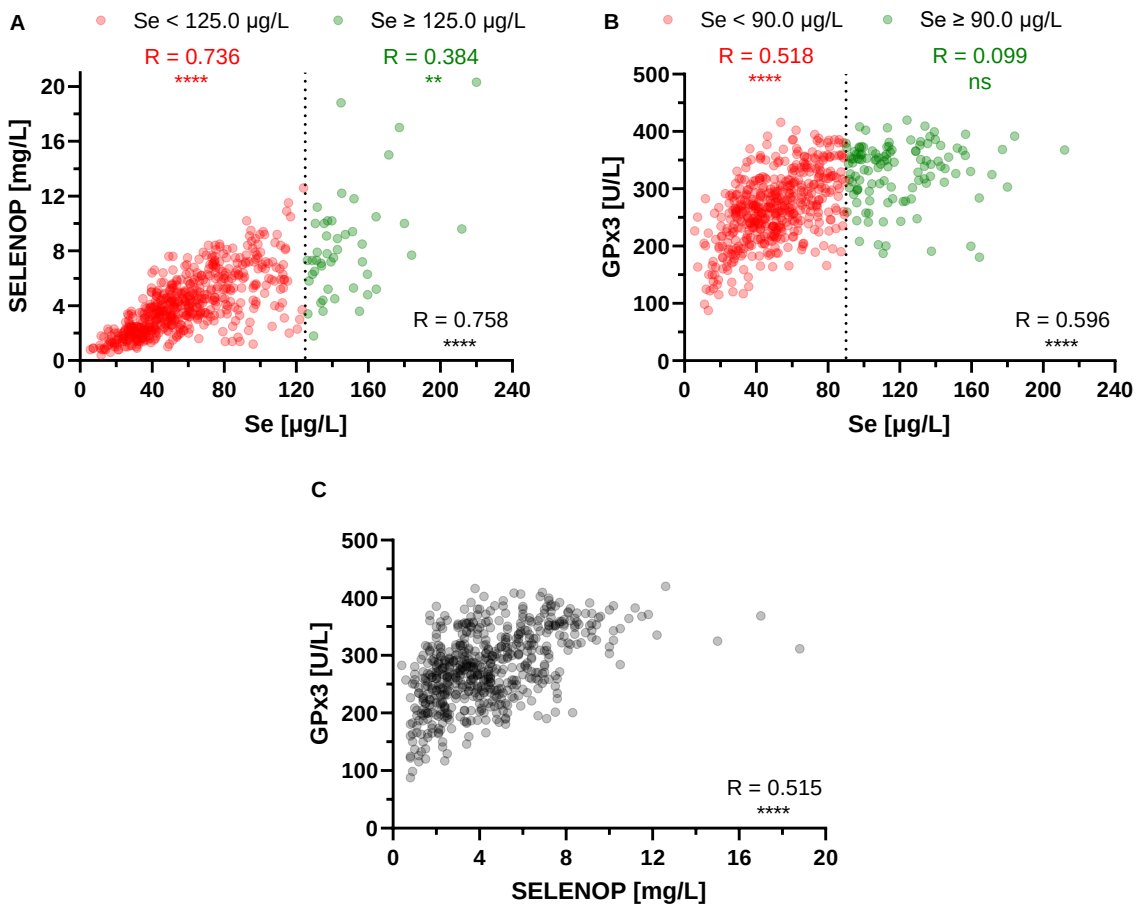

Figure 5: Interrelation between Se status biomarkers in burn patients. All parameter pairs displayed highly significant correlations, $p < 0.0001$. The most stringent correlation was observed between (**A**) total Se with SELENOP (R = 0.758), followed by (**B**) total Se with GPx3 (R = 0.596), and (**C**) SELENOP with GPx3 (R = 0.515). In agreement with the saturation kinetics of selenoprotein expression, (**A, B**) total serum Se and circulating selenoproteins were more strongly correlated at a lower Se status. The red vs. green dots separate the Se concentration ranges where suboptimal or saturated expression levels of SELENOP and GPx3, respectively, have been reported. R: Spearman's rank correlation coefficient, two-tailed. ** $p < 0.01$; **** $p < 0.0001$. Own figure.

3.3 Baseline selenium biomarkers in relation to burn severity

Immediately postburn, patients displayed a significantly decreased serum Se status with median (IQR) Se and SELENOP concentrations of 42.9 (34.3–53.4) µg/L and 3.65 (2.5–4.7) mg/L, respectively. Hence, median total Se was below the threshold for deficiency in healthy European subjects (45.9 µg/L), whereas baseline SELENOP did not fall below its preset threshold for deficiency (2.56 mg/L).

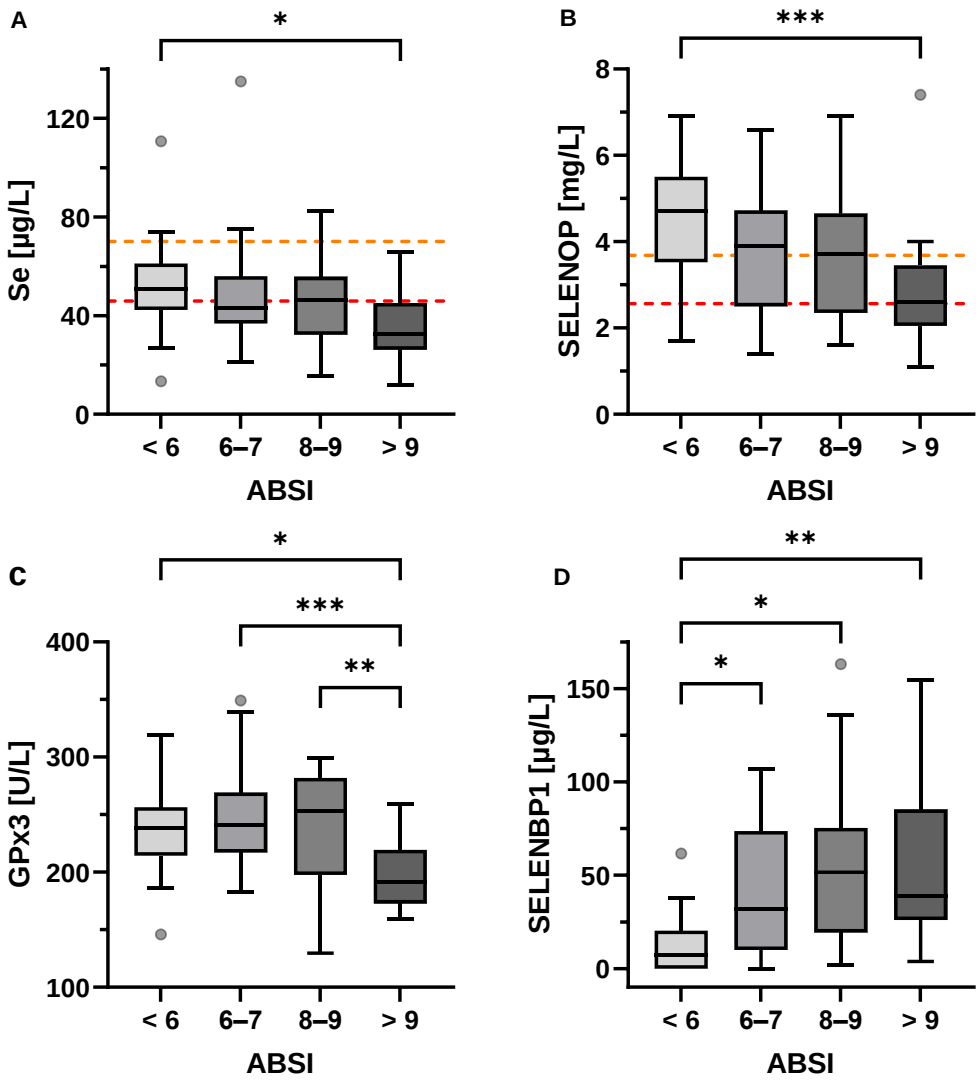

Figure 6: Baseline Se status biomarkers and SELENBP1 stratified by burn severity. Patients were stratified based on their Abbreviated Burn Severity Index (ABSI) into four subgroups of comparable sizes (ABSI < 6: *n* = 20; 6–7: *n* = 36; 8–9: *n* = 17; and > 9: *n* = 17), and Se biomarkers at admission were compared accordingly. Serum levels of (**A**) Se and (**B**) SELENOP declined from the least affected (ABSI < 6) to the most severely affected patients (ABSI > 9), yielding significant differences between ABSI < 6 vs. ABSI > 9 ($p = 0.014$ and $p = 0.0007$, respectively). Even though (**C**) GPx3 activity did not decrease steadily with increasing burn severity, significantly lower concentrations were observed in burn patients with an ABSI > 9. On the contrary, (**D**) SELENBP1 at baseline was positively associated with burn severity; lowest concentrations were measured in patients with an ABSI < 6. Results are presented as Tukey-style box plots. The dotted red and orange lines correspond to the 2.5th and 25th percentile of the reference cohort of healthy adult patients, respectively. The (**D**) y-axis limit was set at 175 µg/L for optimal visualization; six data points exceeding 175 µg/L are not shown. Comparisons were conducted by Kruskal-Wallis test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Own figure.

The ABSI was used to classify the patient cohort according to the degree of burn severity, and baseline concentrations of Se biomarkers were compared between the resulting groups (**Figure 6**). Total Se and SELENOP were inversely associated with burn severity, as both parameters continuously declined with increasing ABSI (**Fig. 6A, B**). Activity levels of GPx3 were similar across the ABSI subgroups < 6, 6–7, and 8–9, but, consistent with total Se and SELENOP, lowest GPx3 levels were found in burn patients with an ABSI > 9 (**Fig. 6C**). Median (IQR) serum levels of total Se, SELENOP, and GPx3 activity at admission among these most severely affected patients were 32.4 (26.3–42.2) µg/L, 2.6 (2.1–3.4) mg/L, and 191.2 (173.0–208.8) U/L, respectively. In contrast, SELENBP1 was positively associated with burn severity (**Fig. 6D**); median [IQR] baseline concentrations were significantly higher in patients scoring an ABSI > 9, as compared to patients with an ABSI < 6 (39.0 [26.1–82.7] vs. 7.4 [0–18.5] µg/L; $p = 0.002$).

Nonparametric correlation analyses between the burn extent (%TBSA) on the one hand, and serum Se, SELENOP, GPx3 activity, and SELENBP1 on the other hand, yielded Spearman coefficients of –0.258 ($p = 0.015$), –0.375 ($p = 0.0003$), –0.321 ($p = 0.002$), and 0.416 ($p < 0.0001$), respectively.

3.4 Serum dynamics of selenium biomarkers postburn

Burn patients experienced dynamic changes of serum Se biomarkers over time (**Figure 7**). Regardless of sepsis development, total serum Se remained low in the first days after admission, before reaching a median (IQR) peak of 88.2 (62.5–112.3) µg/L at W1, most likely in response to the early high-dose Se supplementation administered. At M3 and M6, total Se significantly declined and almost returned to the initial serum levels (**Fig. 7A**). Similar dynamics were observed for SELENOP (**Fig. 7B**) and GPx3 (**Fig. 7C**), even though median [IQR] peak concentrations were achieved later (6.7 [5.2–7.6] mg/L at M1 and 350.7 [312.9–364.5] U/L at W2, respectively). Serum SELENOP declined even further by about 30% during the early postburn period, reaching a median (IQR) nadir of 2.4 (1.9–3.2) mg/L at D2 (**Fig. 7B**). Serum SELENBP1 displayed a considerable elevation already at admission, with a median (IQR) concentration of 26.9 (10.0–61.0) µg/L. Thereafter, serum levels rapidly declined and remained at low levels during follow-up (**Fig. 7D**).

The assessment of serum kinetics after subdivision into septic and non-septic patients revealed a lower initial Se status in patients who subsequently developed sepsis, yielding significant differences for SELENOP at admission ($p = 0.004$) and D2 ($p = 0.025$) (**Fig.**

7B). After having achieved peak concentrations at W2, GPx3 activity decreased faster in non-septic patients, thus bearing a significant difference between the groups at M1 (p = 0.012) (**Fig. 7C**). No significant differences were found regarding serum SELENBP1 dynamics (**Fig. 7D**).

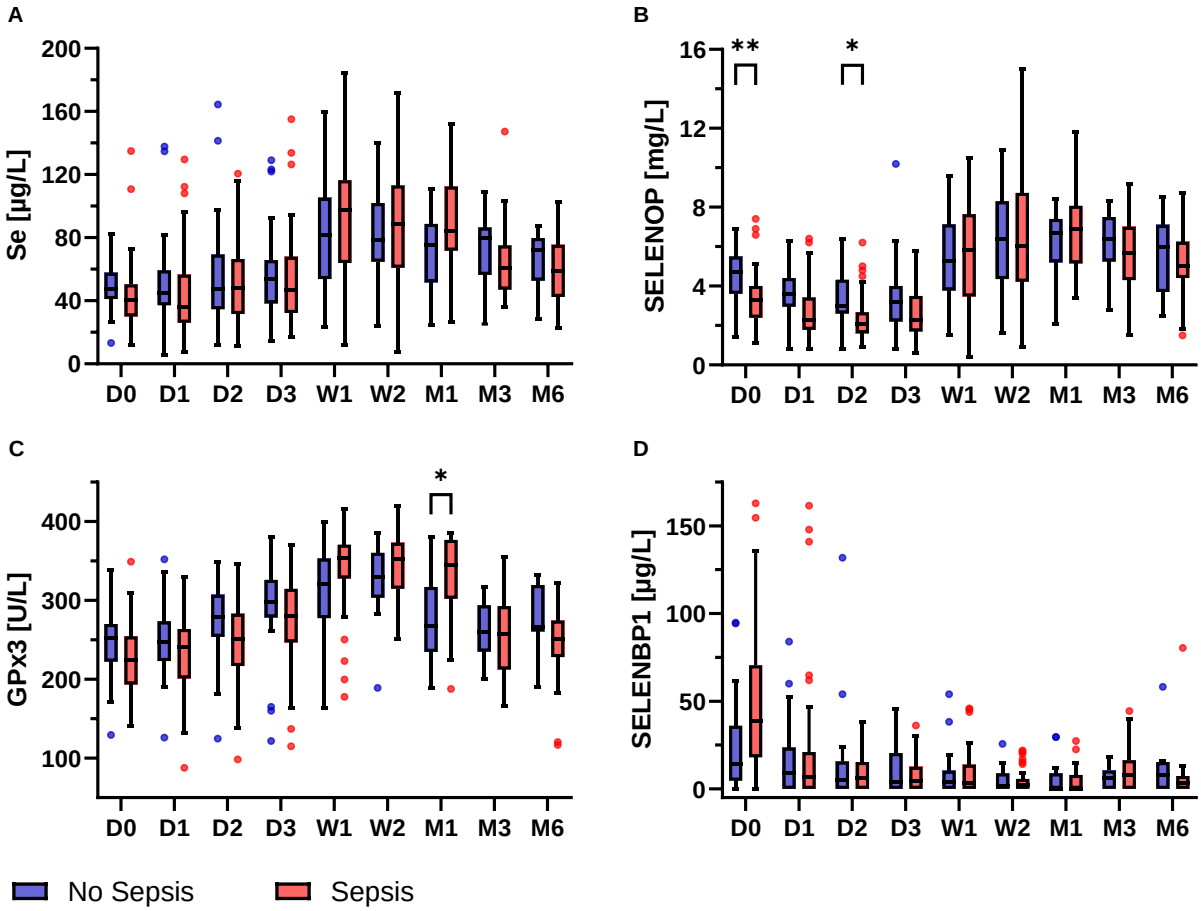

Figure 7: Time course of Se biomarkers postburn. In burn patients receiving early high-dose Se supplementation, serum concentrations of (**A**) total Se and (**C**) GPx3 activity remained low throughout the first posttraumatic days, and peaked on W1 and W2, respectively. An initial decline of serum levels was observed for (**B**) SELENOP, before reaching maximum levels at M1. Normalization of Se status did not last until the end of follow-up, as (**A–C**) serum levels markedly dropped as of M3. (**D**) Serum SELENBP1 was elevated upon admission, but rapidly declined within the first day. Results are presented as Tukey-style box plots for the groups of non-septic (blue) and septic (red) burn patients. The (**A, B, D**) y-axis limits were set at 200 µg/L (2 data points not shown), 16 mg/L (3 data points not shown), and 175 µg/L (12 data points not shown), respectively. Comparisons between groups were conducted by a repeated measures mixed-effects model, using Šidák's test for post hoc analyses. * $p < 0.05$; ** $p < 0.01$. D: day; W: week; M: month. Own figure.

3.5 Correlation of baseline selenium biomarkers with sepsis incidence

After the patients were grouped by median values of baseline Se (42.9 µg/L), SELENOP (3.65 mg/L), GPx3 activity (231.45 U/L), and SELENBP1 (26.9 µg/L), multiple Cox regression analyses were conducted (**Table 2**). Univariate models showed that patients exhibiting lower baseline levels of Se status biomarkers were at greater risk of sepsis in the further course. In addition, patients with higher SELENBP1 concentrations upon admission were more likely to develop sepsis. Adjustment for age and sex did not change these results. However, after full correction for parameters of burn severity, SELENOP remained the only Se biomarker with baseline concentrations that were significantly associated with sepsis incidence (adjusted HR, 1.94; 95% CI, 1.05–3.63; $p = 0.035$). Among the patients with baseline SELENOP < 3.65 mg/L, 80% (36/45) developed sepsis, whereas only 42% (19/45) with higher baseline SELENOP did.

Table 2: Cox regression analyses for sepsis in burn patients.

	At Risk	Sepsis	Univariate Analysis		Multivariate Analysis[a]	
	n	***n***	**HR (95% CI)**	***p***	**HR (95% CI)**	***p***
Se						
< 42.9 µg/L	44	34	2.03 (1.18–3.56)	**0.011**	1.63 (0.93–2.94)	0.095
≥ 42.9 µg/L	44	21	–	–	–	–
Total	88	55				
SELENOP						
< 3.65 mg/L	45	36	2.62 (1.51–4.68)	**0.001**	1.94 (1.05–3.63)	**0.035**
≥ 3.65 mg/L	45	19	–	–	–	–
Total	90	55				
GPx3						
< 231.45 U/L	45	33	2.07 (1.20–3.64)	**0.01**	1.43 (0.76–2.70)	0.272
≥ 231.45 U/L	44	21	–	–	–	–
Total	89	54				
SELENBP1						
< 26.9 µg/L	45	22	–	–	–	–
≥ 26.9 µg/L	45	33	1.92 (1.12–3.34)	**0.019**	1.38 (0.77–2.48)	0.279
Total	90	55				

HR: hazard ratio; CI: confidence interval.

[a] Adjusted for age, sex, total body surface area, full-thickness burn, and inhalation injury.

Own table.

Finally, to investigate the predictive value of SELENOP for sepsis in comparison with the ABSI and established inflammatory biomarkers, ROC analysis was performed (**Figure 8**) and the validity of predictive parameters was assessed (**Table 3**).

The ABSI displayed the best discriminative accuracy (AUROC, 0.78; 95% CI, 0.68–0.88; $p < 0.0001$), followed by SELENOP (AUROC, 0.74; 95% CI, 0.62–0.85; $p = 0.0002$) and WBC (AUROC, 0.70; 95% CI, 0.59–0.81; $p = 0.0019$). Baseline SELENOP < 4.25 mg/L predicted sepsis risk with a sensitivity of 80.0% and a specificity of 62.9%. Initial levels of PCT and CRP did not prove clinically useful in this regard (AUROCs, 0.59 and 0.52, respectively; **Fig. 4A**). Combining the ABSI and SELENOP improved the predictive value for distinguishing septic from non-septic patients with an AUROC of 0.81. With WBC taken into the analysis, the AUROC was further increased to 0.84 (**Fig. 4B**).

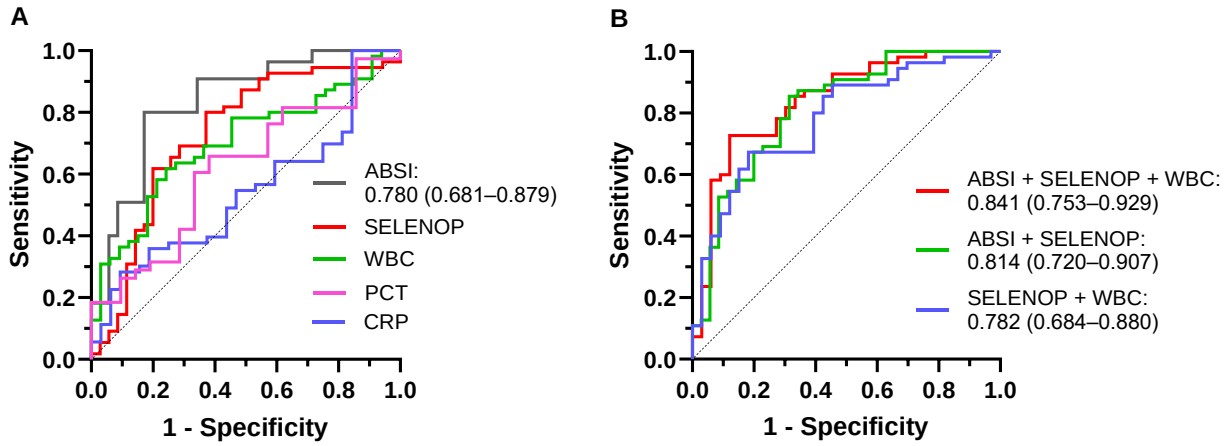

Figure 8: ROC analysis for sepsis prediction by burn severity and serum biomarkers. In (**A**) univariate assessments, the Abbreviated Burn Severity Index (ABSI) was most accurate, followed by SELENOP and white blood cells (WBC). Procalcitonin (PCT) and C-reactive protein (CRP) were not predictive of sepsis. (**B**) A final model including the ABSI, SELENOP, and WBC yielded an improved AUROC of 0.84. Own figure.

Table 3: Validity of the ABSI, SELENOP, and WBC for prediction of sepsis.

Biomarker	Cut-off value	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%)	NPV (%)
ABSI	> 6.5	80.0 (67.6–88.5)	65.7 (49.2–79.2)	78.6	67.6
SELENOP	< 4.25 mg/L	80.0 (67.6–88.5)	62.9 (46.3–76.8)	77.2	66.7
WBC	> 14.1 x 10^9/L	61.8 (48.6–73.5)	75.8 (59.0–87.2)	80.0	55.8

ABSI: abbreviated burn severity index; SELENOP: selenoprotein P; WBC: white blood cells; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value. Own table.

4 Discussion

4.1 Key results

The present work provides a comprehensive analysis of serum Se status and SELENBP1 dynamics in burn patients and explores the interrelations among initial serum concentrations, the severity of trauma, and sepsis development. The underlying hypotheses on an immediate and strong Se status deficit and SELENBP1 elevation have been substantiated. Upon admission to the burn center, biomarkers of serum Se status were inversely associated with burn severity, whereas SELENBP1 exhibited an upward trend towards higher ABSI values. Unexpectedly, adjuvant intravenous administration of high-dose sodium selenite did not induce a long-term normalization of serum Se status. In fact, we observed a re-deterioration of the three Se status biomarkers, starting three months post-burn. After adjustment for burn severity parameters, low SELENOP levels at admission were significantly associated with sepsis incidence. Baseline SELENOP showed a higher predictive validity for burn sepsis than established inflammatory biomarkers. In combination with the ABSI, baseline SELENOP achieved an even better value in this regard.

4.2 Interpretation of results

4.2.1 Serum selenium status in burn injury

As severe burns lead to a complex pathophysiologic response, a plethora of mechanisms may cause the acute Se depletion. Wound exudation owing to the skin damage and fluid losses through drains and surgical hemorrhage pose major contributing factors (109, 110), supported by the inverse correlation of burn extent and serum Se status. Insufficient Se intakes despite growing nutritional needs as well as hemodilution by fluid resuscitation (111) may aggravate the depletion. An increased urinary Se excretion has also been noticed in burn patients (109). Notably, urinary losses were not proportional to the intakes (112), reinforcing the concept of Se redistribution to prioritized tissues under conditions of Se deficiency (57, 113). The Se transporter SELENOP as a negative acute phase reactant is likely to play a key role in these critical disturbances of Se homeostasis (64, 114), bearing in mind the relevance of inflammatory cytokines after major burns (21, 115). Interestingly, serum SELENOP was the only Se status biomarker for which a further decline in the first days of hospitalization has been observed, potentially attributable to an impaired hepatic function (116, 117), an accelerated uptake by target cells (60), the time

delay between Se supply and completion of its biosynthesis (118), and downregulation by the ongoing inflammation and hypoxia (66, 119). Declining SELENOP expression can also be hypothesized as a mechanism of averting potentially fatal infections by depriving invading microbes of the essential trace element (53, 120).

The inverse correlation of injury severity and immediate serum changes of total Se and SELENOP has been shown after major trauma (72). However, trauma and burn patients mainly differ in the loss of the skin, the human body's largest organ that provides an immunological barrier against external pathogens, putting burn patients at particular risk of secondary infections and sepsis. Importantly, we identified SELENOP at admission as a prognostic biomarker of burn sepsis, yielding a higher accuracy than total Se, GPx3 activity, and established inflammatory biomarkers. The underlying causes may be as multifaceted as the characteristics of this unique glycoprotein. With a relatively short half-life of 3–4 h, serum SELENOP is turning over rapidly (121), and allows for a diagnostic and prognostic usability already upon admission. In contrast, CRP and PCT display slower serum kinetics due to the delayed onset of the acute phase response and, therefore, do not represent reliable biomarkers within the first few hours after major trauma (122, 123). Not only is SELENOP essential for maintaining Se homeostasis and providing Se for renal GPx3 expression, but it also possesses antioxidant properties by reducing phospholipid hydroperoxides, similar to GPx4 (63, 124, 125). On a related note, SELENOP was found to protect endothelial membranes against oxidative damage, thus mitigating endothelial dysfunction as a central hallmark of sepsis progression (126-128).

Nutritional support including the early provision of micronutrients is a decisive aspect of burn care (129). According to the European Society for Clinical Nutrition and Metabolism (ESPEN) recommendations, patients with burns greater than 20% TBSA should be given 350 µg Se/d for 2–3 weeks, with an extended duration of supplementation in particularly large burns (35, 130). The rationale behind this approach lies in the importance of Se for antioxidant defense and immune regulation. For example, Se has been suggested to enhance macrophage bactericidal activities as well as autophagy in infected macrophages (131, 132). In critically ill patients, low plasma Se was associated with infectious complications and increased mortality (133), and correction of the deficit improved clinical outcomes (38, 39). Yet, considering that ESPEN recommendations are based on lower quality of evidence, the endorsed doses are still under evaluation and guideline adherence in burn center ICUs is limited (134). Numerous studies indicated that early parenteral Se

supplementation induces a normalization of serum Se and GPx3 activity in burn patients (39, 112, 135, 136). In line with these trials, we detected a normalized serum Se status in supplemented patients. This work expands the existing body of evidence by the assessment of serum SELENOP, an established biomarker of Se status. Furthermore, it is the first to provide insights into long-term Se status changes in severely burned patients, as prior supplementation studies performed blood sampling for a maximum of 30 days postburn. Interestingly, we noticed a late decrease of total Se, SELENOP, and GPx3 activity, consistent with the knowledge about severe burns as chronic conditions with enduring inflammatory and metabolic alterations (3, 137). The significantly higher GPx3 activity in septic patients at M1 could be explained by the prolonged Se supply in clinically unstable patients.

Here, a 7-day intravenous Se supplementation with 1000 µg of sodium selenite daily as part of local standard of care proved feasible and efficient to transiently raise the expression of selenoproteins in blood. No side effects attributable to Se supply were noted. Still, the observed re-deterioration of serum Se status suggests the need for adjuvant high-dose Se beyond the acute phase of burn management. In a large cohort of unburned septic ICU patients, sodium selenite doses of 1000 µg/d were administered for several weeks without any apparent side effects (138). However, the risk of Se toxicity must not be underestimated (139). The inverse correlation with burn severity, the initial decline despite Se supplementation, and the ability to identify patients at high risk of sepsis, who potentially require more supplemental Se, render serum SELENOP a meaningful parameter when it comes to optimizing the dose und duration of Se supply in burn patients.

4.2.2 Serum selenium-binding protein 1 in burn injury

The rapid increase of SELENBP1 in serum of burn patients ran contrary to the changes in total Se, SELENOP, and GPx3 activity. While postburn alterations of serum Se status biomarkers are mostly subject to cutaneous exudative losses and the acute phase response, SELENBP1 is an intracellular protein, conceived of getting released into the bloodstream in response to tissue damage. This notion of a trauma-associated release is supported by the absence of relevant serum SELENBP1 amounts in healthy subjects (83, 97) and the positive correlation of serum concentrations at admission and the burn extent observed in our study. Indicative of the excessive damage, baseline levels were similar to those in patients with most severe traumatic spinal cord injury (96).

Even after trauma and burn patients survive the emergent phase, subsequent interventions such as surgical procedures and placement of intravenous lines can trigger a recurrent inflammatory activation, previously described as the “two-hit” model, an attempt at explaining the late occurrence of multiple organ failure (19, 140). Notably, SELENBP1 declined within the first day and remained low throughout the observation period. One possible reason could be the opposing regulation between SELENBP1 and GPx1, an intracellular selenoenzyme that modulates cellular oxidative stress by preventing the accumulation of hydrogen peroxides (91, 141-143). Higher susceptibility to diquat- and paraquat-induced, lethal oxidative stress has been detected in GPx1 knockout mice (144, 145). Besides, SELENBP1 has been found to interact with HIF-1α, a ROS-upregulated transcription factor (146, 147). Owing to its low rank in the hierarchy of selenoproteins, GPx1 responds sensitively to Se supplementation in a dose-dependent manner (46, 148). As all patients were supplemented with high-dose Se during the first week, the resulting stimulation of intracellular GPx1 expression might have prevented a second increase in serum SELENBP1. Additionally, in consideration of the indirect aggravation of oxidative stress by intracellular SELENBP1, the postburn shift to the intravascular space can be hypothesized as a protective mechanism (149). Still, these hypotheses concerning the interaction between SELENBP1 and GPx1 in the critically ill are yet to be evaluated.

Although serum SELENBP1 did not prove highly predictive for the main clinical outcome in the adjusted multivariate analysis, the acquired results may pave the way for a deeper understanding of the intricate interplay between inflammation, hypoxia, SELENBP1, and Se status in severely burned patients.

4.3 Strengths and limitations

This observational study is the first to assess serum concentrations of SELENOP and SELENBP1 in the context of severe burns. The longitudinal sampling over a considerable period allowed for a comprehensive evaluation of supplementation effects, based on three complementary biomarkers of serum Se status. Patient sera were of high quality, and established reliable methods were used for measurements, as reflected in the stringent correlations among serum Se biomarkers. Blinding of researchers and technicians who carried out the measurements further increased the internal validity of the results. Another noteworthy strength includes the well-characterized patient cohort, with provision of various prospectively collected clinical parameters by the treating physicians.

However, certain limitations need to be mentioned. A small sample size and male predominance are typical of burn studies. The present work is no exception, even though a total of 90 included patients can be considered relatively large, as compared to most other burn studies. Sex-specific differences regarding the responsiveness to Se supplementation and the susceptibility to sepsis were not explored due to the small percentage of female patients. It is also important to note that the increasing number of missing values diminished the statistical robustness of the analyses. The study was conducted at a single center in Switzerland, a geographical area with mostly adequate Se supply. Another factor limiting the generalizability of the findings was the enrollment of adult subjects only. Most importantly, serum samples of patients unexposed to supplemental Se were not accessible. Therefore, the concept of an additional, delayed increase of SELENBP1 in serum of non-supplemented burn patients remains hypothetical. Lastly, it cannot be ruled out that unmeasured confounding factors, e.g., nutritional status, immune status, timing of burn wound excision, and consumption of alcohol prior to burn admission, relevantly distorted the association between baseline Se biomarkers and sepsis development.

4.4 Implications and significance

Burn sepsis differs substantially from sepsis in the general population, both pathophysiologically and clinically (150). Distinguishing the hyperinflammatory and hypermetabolic state postburn from the onset of sepsis poses a particular challenge in routine clinical practice. As sepsis remains the leading cause of death in severely burned patients, an early recognition and timely treatment are paramount. Established biomarkers such as PCT and CRP have been characterized as poorly predictive in burn patients (99). This research identified serum SELENOP as a promising biomarker that could prove clinically useful in a threefold way. First, the continuous decrease of baseline levels with increasing burn severity implies the potential diagnostic value with regard to burn severity assessment and risk stratification. Second, it may assist in monitoring supplementation efficacy and detecting surplus supply, thus averting Se toxicity in burn patients requiring supraphysiological Se doses. Third, it could serve as a prognostic biomarker, as it demonstrated high discriminative ability to identify burn patients at risk of sepsis already at the time of admission. The latter point may contribute to a future personalization of infection prevention and control measures in the burn unit, aiming at reducing the huge burden of

sepsis in severely burned patients. In light of the recently identified SELENOP-autoantibodies that emerge in a subset of burn patients and potentially impair Se transport and expression of protective selenoproteins, new opportunities for an accurate Se status monitoring in critically ill patients gain in importance.

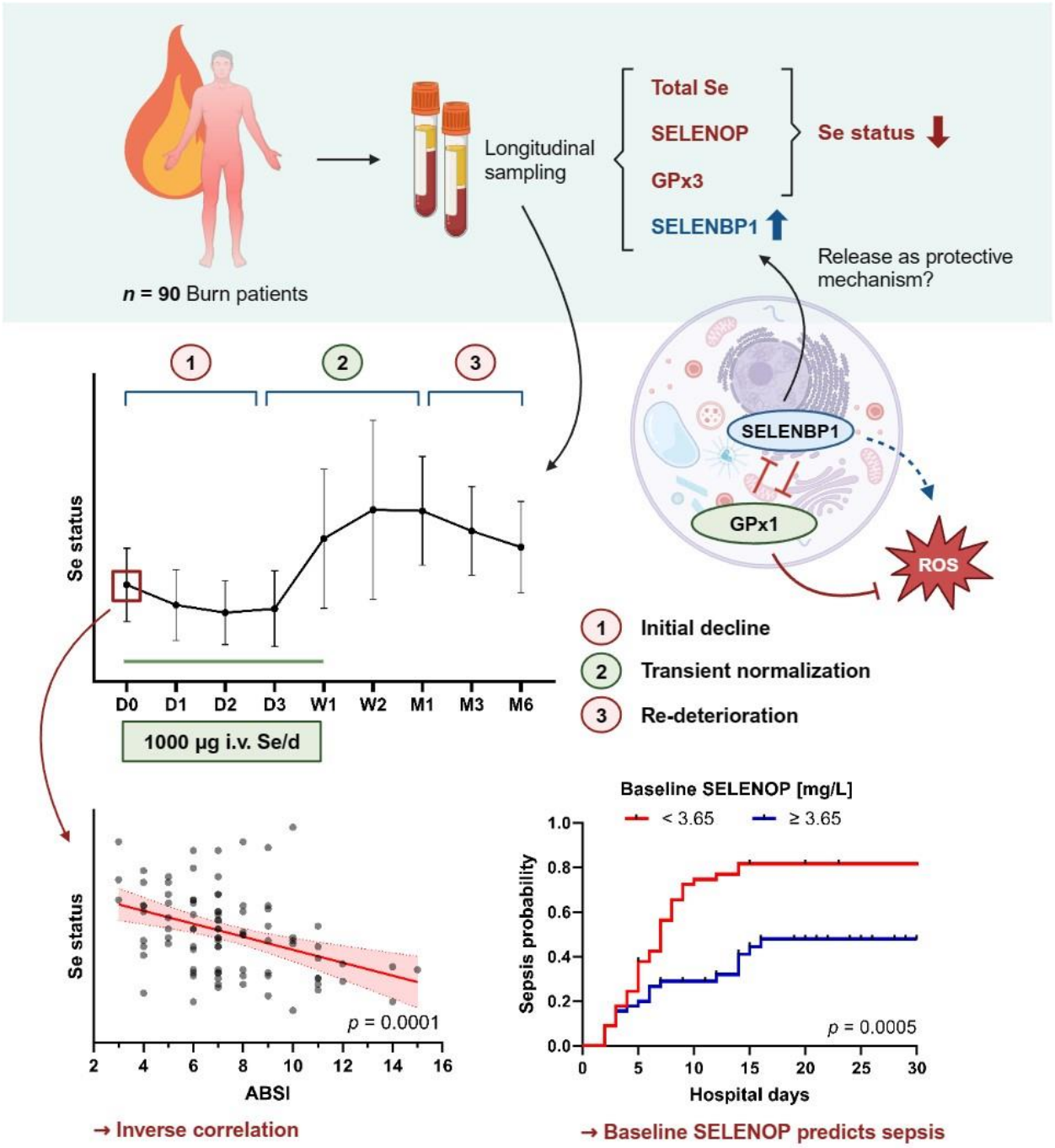

Figure 9: Visual abstract. ABSI: abbreviated burn severity index; D: day; i.v.: intravenous; GPx1: glutathione peroxidase 1; GPx3: glutathione peroxidase 3; M: month; ROS: reactive oxygen species; Se: selenium; SELENBP1: selenium-binding protein 1; SELENOP: selenoprotein P; W: week. Own figure, created with BioRender.com.

5 Conclusions

Overall, the data obtained throughout these analyses corroborated previous studies showing that severe burns induce an acute Se depletion, associated with adverse clinical outcomes. Serum SELENOP stood out as a novel promising biomarker with notable diagnostic, monitoring, and prognostic properties. Along with established clinical and laboratory parameters, the evaluation of Se status including SELENOP may bring about significant progress in burn care, with particular relevance to a personalized adjuvant nutritional therapy and sepsis prevention. Large multicenter trials are warranted to confirm these findings.

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Statutory Declaration

“I, Tabaël Lee Turan, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic “Diagnostic and prognostic value of selenium biomarkers in severe burns” – “Diagnostischer und prognostischer Wert von Selen-Biomarkern bei schweren Verbrennungen” independently and without the support of third parties, and that I used no other sources and aids than those stated.
All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; http://www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me.”

Date Signature

Declaration of my own contribution to the publication

Tabaël Lee Turan contributed the following to the below listed publication:

Turan TL, Klein HJ, Hackler J, Hoerner L, Rijntjes E, Graf TR, Plock JA, and Schomburg L. Serum Selenium-Binding Protein 1 (SELENBP1) in Burn Injury: A Potential Biomarker of Disease Severity and Clinical Course. *Antioxidants*. 2023; 12(11):1927.

The study was conceptualized by L. Schomburg, J. A. Plock, and me. During my literature review, I formulated research questions and designed the methodology. I performed the laboratory measurements of serum Se status biomarkers (total Se, SELENOP, GPx3). J. Hackler and L. Hoerner assessed serum SELENBP1. Serum samples, clinical data, and serum concentrations of WBC, CRP, and PCT were provided by J. A. Plock, H. J. Klein, and T. R. Graf (University Hospital Zurich, Switzerland). I created all figures and tables displayed in this dissertation and the publication based on my own statistical evaluation. I drafted the original manuscript; minor modifications were made by L. Schomburg, J. A. Plock, and E. Rijntjes. L. Schomburg and I responded to reviewer comments, and I revised the manuscript accordingly. Proofreading of the accepted manuscript prior to publication was conducted by L. Schomburg and me.

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Article

Serum Selenium-Binding Protein 1 (SELENBP1) in Burn Injury: A Potential Biomarker of Disease Severity and Clinical Course

Tabael L. Turan [1], Holger J. Klein [2,3], Julian Hackler [1], Livia Hoerner [1], Eddy Rijntjes [1], Theresia Reding Graf [4], Jan A. Plock [2,3] and Lutz Schomburg [1,*]

[1] Institute for Experimental Endocrinology, Max Rubner Center for Cardiovascular Metabolic Renal Research, Charité—Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, 10115 Berlin, Germany; tabael-lee.turan@charite.de (T.L.T.); livia.hoerner@charite.de (L.H.); eddy.rijntjes@charite.de (E.R.)
[2] Department of Plastic Surgery and Hand Surgery, University Hospital Zurich, 8091 Zurich, Switzerland; holger.klein@ksa.ch (H.J.K.); jan.plock@ksa.ch (J.A.P.)
[3] Department of Plastic Surgery and Hand Surgery, Cantonal Hospital Aarau, 5001 Aarau, Switzerland
[4] Department of Visceral Surgery and Transplantation, University Hospital Zurich, 8091 Zurich, Switzerland
* Correspondence: lutz.schomburg@charite.de; Tel.: +49-30-450-524-289

Abstract: Oxidative stress, systemic inflammation, and metabolic derangements are hallmarks of burn pathophysiology. Severely burned patients are highly susceptible to infectious complications. Selenium-binding protein 1 (SELENBP1) modulates intracellular redox homeostasis, and elevated serum concentrations have been associated with adverse clinical outcomes in trauma patients. We hypothesized that serum SELENBP1 at hospital admission and during hospitalization may constitute a meaningful biomarker of disease severity and the clinical course in burn injury, with pulmonary infection as primary endpoint. To this end, we conducted a prospective cohort study that included 90 adult patients admitted to the Burn Center of the University Hospital Zurich, Switzerland. Patients were treated according to the local standard of care, with high-dose selenium supplementation during the first week. Serum SELENBP1 was determined at nine time-points up to six months postburn and the data were correlated to clinical parameters. SELENBP1 was initially elevated and rapidly declined within the first day. Baseline SELENBP1 levels correlated positively with the Abbreviated Burn Severity Index (ABSI) ($R = 0.408$; $p < 0.0001$). In multiple logistic regression, a higher ABSI was significantly associated with increased pulmonary infection risk (OR, 14.4; 95% CI, 3.2–88.8; $p = 0.001$). Similarly, baseline SELENBP1 levels constituted a novel but less accurate predictor of pulmonary infection risk (OR, 2.5; 95% CI, 0.7–8.9; $p = 0.164$). Further studies are needed to explore the additional value of serum SELENBP1 when stratifying patients with respect to the clinical course following major burns and, potentially, for monitoring therapeutic measures aimed at reducing tissue damage and oxidative stress.

Keywords: selenium; trace element; critical disease; pneumonia; prognosis; critical care

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Citation: Turan, T.L.; Klein, H.J.; Hackler, J.; Hoerner, L.; Rijntjes, E.; Graf, T.R.; Plock, J.A.; Schomburg, L. Serum Selenium-Binding Protein 1 (SELENBP1) in Burn Injury: A Potential Biomarker of Disease Severity and Clinical Course. *Antioxidants* 2023, *12*, 1927. https://doi.org/10.3390/antiox12111927

Academic Editor: Maria Letizia Manca

Received: 4 October 2023
Revised: 24 October 2023
Accepted: 26 October 2023
Published: 29 October 2023

1. Introduction

Selenium-binding protein 1 (SELENBP1) is among the diverse group of selenium (Se)-containing proteins. Unlike the proteins containing selenocysteine (Sec) or selenomethionine, SELENBP1 directly binds Se [1]. While it was first discovered in 1989 [2], the role of SELENBP1 under physiological and pathological conditions still remains poorly understood [3]. However, it may participate in several physiological processes, such as cell differentiation [4,5], proteasomal protein degradation [6], intra-Golgi transport [7], and redox modulation by its oxidoreductase activity [8], with copper (Cu) as a required cofactor [9]. SELENBP1 does not appear to be induced by dietary Se exposure, as shown in rodents [10] and the nematode *C. elegans* [9]. It is ubiquitously expressed and subcellularly localized in both the nucleus and the cytoplasm [11]. Intracellularly, SELENBP1

affects redox homeostasis by reciprocal interference with the antioxidative activity of the selenoenzyme glutathione peroxidase 1 (GPx-1) [12,13]. Downregulation of SELENBP1 expression has been associated with carcinogenesis and the poor prognosis of various human malignancies [3]. Furthermore, SELENBP1 has been shown to be upregulated in the prefrontal cortex of patients with schizophrenia [14], even causing and augmenting negative symptoms [15]. Elevated serum levels of SELENBP1 have been detected and correlated with adverse clinical outcomes in patients with traumatic spinal cord injury [16], acute coronary syndrome [17], and following cardiac surgery [18].

Severe burns entail immediate inflammatory and metabolic alterations that persist for years after the injury [19]. Long-term inflammation is largely maintained by burn-induced systemic oxidative stress [20,21]. Acute Se depletion is frequent after major burns [22], and trace element supplementation is associated with an improved clinical outcome [23]. Due to metabolic derangements, skin barrier disruption, exposure to invasive procedures, and prolonged hospitalization, burn patients are particularly prone to infectious complications [24,25]. Sepsis and preceding infections, pulmonary infections in particular, are the leading cause of morbidity and mortality among burn patients [26–28]. With that in mind, the importance of expanding strategies to predict and prevent infections in the early postburn period comes into focus.

In the present trial, we report on the dynamics of serum SELENBP1 expression in burn patients and examine whether it relates to the severity of the injury. We also address our hypothesis that higher baseline levels of serum SELENBP1 may be predictive of an adverse clinical outcome in these patients, accentuating pulmonary infection as a main source of mortality.

2. Materials and Methods

2.1. Study Design

A total of 90 adult patients (≥18 years old) admitted to the Burn Center of the University Hospital Zurich, Switzerland, were prospectively recruited between May 2015 and October 2018. Exclusion criteria were current infection at admission, immunosuppressive medication, and burn injuries older than 6 h.

Burn severity was assessed using the Abbreviated Burn Severity Index (ABSI), a predictive score for burn mortality consisting of five variables: sex, age, presence of inhalation injury, presence of full-thickness burn, and percentage of the total body surface area (TBSA) burned. The patients can thereby be stratified into six groups with varying degrees of injury [29]. Inhalation injury refers to an acute respiratory tract involvement caused by exposure to thermal or chemical noxae. Full-thickness burns affect all layers of the skin, including the epidermis, dermis, and subcutis. Affected TBSA was determined using Lund–Browder charts. Patients were treated in accordance with the standard of care, which included surgical interventions, intravenous fluid resuscitation, early enteral nutrition, a high-protein diet, and regular indirect calorimetry to estimate caloric requirements. In addition, all patients received 1000 µg of Se per day from admission to day 7 inclusively, which was administered by the continuous infusion of sodium selenite. Continuation of intravenous Se supplementation with 500 µg per day was conducted in unstable patients until clinical stability was reached. Serum Cu and zinc (Zn) levels were determined once a week and supplemented based on personal need until discharge.

The primary clinical outcome measure was the development of pulmonary infection during hospitalization. A pneumonia diagnosis required a new pulmonary infiltrate to be detected on the chest radiograph, accompanied by relevant clinical manifestations, e.g., fever, cough, purulent expectoration, or dyspnea. Secondary clinical outcomes were in-hospital mortality, hospital length of stay (LOS), intensive care unit (ICU) LOS, and sepsis occurrence, based on Sepsis-3 [30]. We additionally recorded demographic characteristics, comorbidities, and trauma-related data.

The trial was registered at ClinicalTrials.gov (NCT02537821) on 2 September 2015.

2.2. Blood Sampling and Measurements

Blood samples were drawn upon admission (D0) and at the following time-points postburn: days (D) 1, 2, and 3; weeks (W) 1 and 2; and months (M) 1, 3, and 6. A set of $n = 598$ serum samples was prepared and stored at −80 °C until analyzed. In a subset of patients, post-discharge follow-up sampling was also carried out. Within the first two weeks, less than 10% of the values were missing; 6 months postburn, blood sampling had been successfully performed in 37% of patients.

Measurements were conducted in the laboratories of the Institute for Experimental Endocrinology, Charité—Universitätsmedizin Berlin, by technicians and researchers blinded to all the clinical data of the study population. Serum SELENBP1 concentrations were determined by a luminometric immunoassay as described earlier [17]. Moreover, the following serum parameters were evaluated by standardized procedures: trace elements (Se, Cu, Zn) by total reflection X-ray fluorescence (TXRF) [31], selenoprotein P (SELENOP) by a validated immunoluminometric sandwich assay (selenOtest ELISA; selenOmed GmbH, Berlin, Germany) [32], and GPx-3 activity by monitoring NADPH consumption in a coupled enzymatic test [33]. Inflammatory markers, such as white blood cells (WBCs), C-reactive protein (CRP), and procalcitonin (PCT), were measured by laboratories of the University Hospital Zurich as part of routine clinical procedures.

2.3. Statistical Analyses

Statistical analyses were performed using GraphPad Prism (Version 10.0.0; GraphPad Software, Inc., San Diego, CA, USA). Normality of the data was tested by the D'Agostino–Pearson test, and normally and non-normally distributed continuous data were expressed as means ± standard deviations (SD) and medians with interquartile range (Q1–Q3), respectively. Categorical variables were reported as absolute numbers and percentages (%). As appropriate, two groups were compared using the Student's *t*-test, the Mann–Whitney U test, or Fisher's exact test. Correlations were assessed by Spearman's rank correlation coefficient. SELENBP1 time courses were compared between groups using a repeated measures mixed-effects model. Post hoc analyses for differences between groups were carried out by the Šidák test. Receiver-Operator-Curve (ROC) analysis was conducted to determine the validity of parameters that potentially predicted the development of pulmonary infection in burn patients. Thereafter, multiple logistic regression was performed to estimate the odds of developing pulmonary infection by baseline SELENBP1 levels and the ABSI. Patients developing infections other than pneumonia were excluded from both ROC and regression analyses. Two-tailed *p*-values < 0.05 were considered statistically significant: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

3. Results

3.1. Patient Demographic and Clinical Characteristics

The demographic and clinical characteristics of the participants are reported in Table 1. A total of 90 patients were included in this study, out of which 37 developed a pulmonary infection at a median (IQR) of 4 (3–6) days after admission. Twenty-two patients developed other infectious complications, namely cutaneous infections ($n = 9$), catheter-related infections ($n = 7$), bloodstream infections ($n = 4$), or urinary tract infections ($n = 2$). The majority of patients were male (73/90, 81.1%). The mean (± SD) age was 45.7 (± 17.7) years. Female patients suffered more severe burns than male patients, as evidenced by a markedly higher ABSI (9.4 ± 3.0 vs. 6.9 ± 2.2; $p = 0.001$). Compared with patients without infections, those with an accompanying pulmonary infection displayed a higher TBSA and a larger fraction of full-thickness burns and inhalation injuries, resulting in a significantly higher median [IQR] ABSI (8.0 [7.0–10.0] vs. 6.0 [4.0–7.0]; $p < 0.0001$). Sepsis occurred in almost all pneumonia patients (36/37, 97.3%). The enrolled patients had a median LOS of 27 days, with an expected longer duration of hospitalization in burn patients with a pulmonary infection.

Table 1. Baseline characteristics and clinical outcomes.

Variables	Pulmonary Infection (n = 37)	Other Infections (n = 22)	No Infection (n = 31)	Total (n = 90)
Sex				
Female	8 (21.6)	6 (27.3)	3 (9.7)	17 (18.9)
Male	29 (78.4)	16 (72.7)	28 (90.3)	73 (81.1)
Age (yr)	51.4 ± 17.9	40.6 ± 17.4	42.4 ± 16.1	45.7 ± 17.7
BMI (kg/m^2)	24.9 (22.5–28.4)	27.2 (22.7–29.9)	26.2 (22.8–30.8)	25.7 (22.6–29.3)
TBSA (%)	35.0 (26.0–40.0)	30.0 (22.9–50.8)	20.0 (13.5–29.5)	29.0 (20.0–37.0)
Full-thickness burn	26 (70.3)	16 (72.7)	10 (32.3)	52 (57.8)
Inhalation injury	15 (40.5)	4 (18.2)	3 (9.7)	22 (24.4)
ABSI	8.0 (7.0–10.0)	7.0 (6.0–9.0)	6.0 (4.0–7.0)	7.0 (6.0–9.0)
SELENBP1 (µg/L)	45.6 (21.2–70.5)	35.8 (8.7–61.6)	12.5 (3.7–34.2)	26.9 (10.0–61.0)
Sepsis	36 (97.3)	19 (86.4)	0 (0.0)	55 (61.1)
Mortality	3 (8.1)	1 (4.5)	1 (3.2)	5 (5.6)
Length of stay (d)	54.0 (21.0–87.0)	31.5 (23.3–44.8)	20.0 (10.0–25.0)	27.0 (19.0–56.0)
Length of ICU stay (d)	32.0 (17.0–54.0)	18.5 (13.3–27.0)	8.0 (2.0–14.0)	17.5 (8.3–40.8)

TBSA, total body surface area; ABSI, Abbreviated Burn Severity Index.

The median [IQR] serum concentration of SELENBP1 at baseline was found to be considerably higher in the group of patients developing pulmonary infections than in burn patients without infections (45.6 [21.2–70.5] vs. 12.5 [3.7–34.2] µg/L; $p = 0.007$). Out of 31 patients without infections, 5 baseline samples were below the detection limit of the assay. In contrast, none of the patients who developed pneumonia were initially SELENBP1-negative.

3.2. SELENBP1 Is Elevated following a Severe Burn

On admission, serum SELENBP1 was considerably elevated in burn patients with a median (IQR) concentration of 26.9 (10.0–61.0) µg/L. Within the first posttraumatic day, serum levels declined significantly to a median (IQR) of 9.0 (0–22.5) µg/L. Thereafter, SELENBP1 remained at low levels throughout the observation period (Figure 1A). Stratified analysis by baseline characteristics revealed non-significantly higher initial SELENBP1 levels in females (Figure 1B) and burn patients with a TBSA ≥ 30% (Figure 1D) and full-thickness burns (Figure 1E). No such associations were found for stratification by age (Figure 1C) or the presence of inhalation injury (Figure 1F).

3.3. Baseline SELENBP1 Is Positively Associated with Burn Severity

A moderate positive correlation was found between serum SELENBP1 levels on admission and the ABSI ($R = 0.408$; $p < 0.0001$) (Figure 2A). The median (IQR) baseline SELENBP1 concentration was 11.7 (1.3–34.9) µg/L and 38.9 (22.0–87.2) µg/L in burn patients with an ABSI < 7 ($n = 34$) and ≥ 7 ($n = 56$), respectively ($p = 0.0002$) (Figure 2B). Interestingly, a groupwise Spearman's correlation between the baseline SELENBP1 concentration and the ABSI by clinical characteristics revealed stronger associations in less severely affected patients: $R = 0.339$ ($p = 0.021$) vs. $R = 0.198$ ($p = 0.198$) in patients with TBSA < 30% vs. ≥ 30%; $R = 0.444$ ($p = 0.005$) vs. $R = 0.138$ ($p = 0.329$) in patients without vs. with full-thickness burns; and $R = 0.390$ ($p = 0.001$) vs. $R = 0.311$ ($p = 0.159$) in patients without vs. with inhalation injury, respectively (not shown). Moreover, baseline SELENBP1 levels and the ABSI correlated more strongly in patients aged < 45 years than in patients aged ≥ 45 years: $R = 0.584$ ($p < 0.0001$) vs. $R = 0.157$ ($p = 0.292$), respectively. No significant difference was observed between men and women.

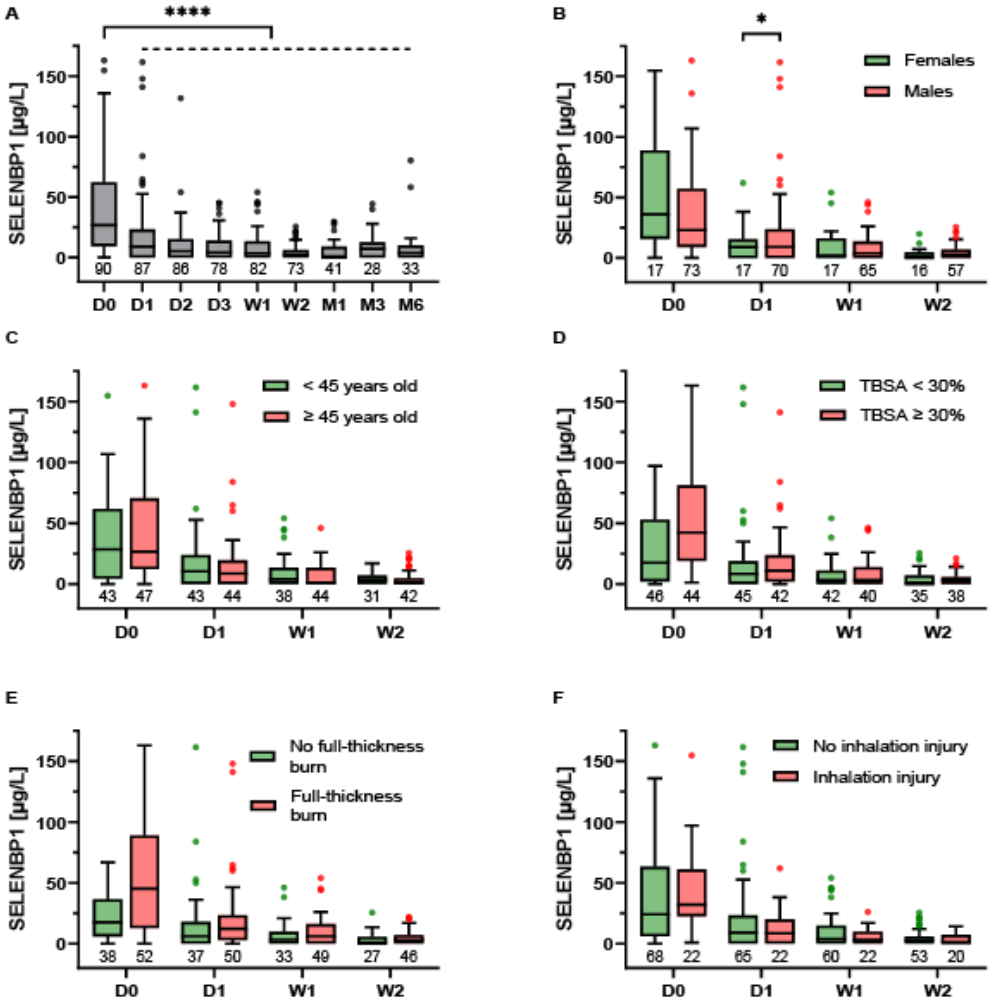

Figure 1. Time course of serum SELENBP1 in burn patients. (**A**) SELENBP1 levels were elevated upon admission (D0) but significantly dropped within the first day (D1) postburn and remained constant from then on. Patient stratification (**B**–**F**) revealed initially higher SELENBP1 levels in (**B**) females than in males, and in patients with (**D**) a total body surface area (TBSA) ≥ 30% vs. < 30% and (**E**) full-thickness burns vs. no full-thickness burns. Stratification by age (**C**) and the presence of inhalation injury (**F**) did not show considerable differences. SELENBP1 decreased more strongly in female patients compared with males, potentially explaining the significantly higher levels in males on day 1 ($p = 0.048$) (**B**). Results are presented as Tukey-style box plots. The numbers below the boxes indicate the number of patients in each group. The Y-axis-limit was set at 175 µg/L for optimal visualization. Statistical comparisons were conducted by a repeated measures mixed-effects model and the Šidák test for post hoc analyses. * $p < 0.05$, **** $p < 0.0001$.

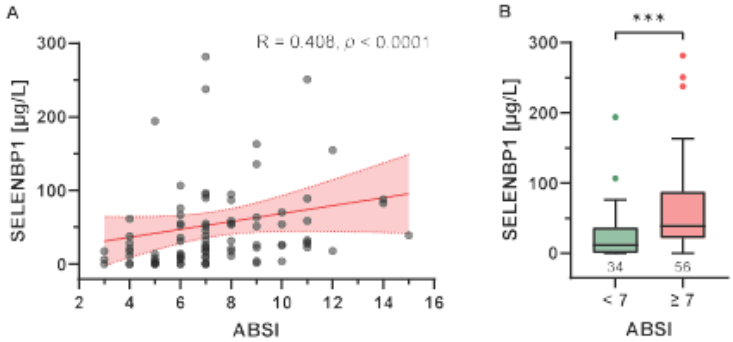

Figure 2. Correlation of baseline serum SELENBP1 concentration and burn severity. (**A**) Serum SELENBP1 concentration at baseline and the Abbreviated Burn Severity Index (ABSI) displayed a significant positive correlation, as visualized by a scatter plot and trend line (red) with 95% confidence intervals (red shadow); R: Spearman's correlation coefficient, two-tailed. (**B**) When grouped according to their ABSI, burn patients with an ABSI ≥ 7 exhibited significantly higher SELENBP1 on admission than patients with an ABSI < 7 ($p = 0.0002$). Results are presented as Tukey-style box plots. The numbers below the boxes indicate the number of patients in each group. The Y-axis limit was set at 300 µg/L for optimal visualization; 2 data points exceeding 300 µg/L are missing in the figures ([7, 425.17], [7, 451.68]). *** $p < 0.001$.

3.4. Correlation Analysis of SELENBP1

An exploratory correlation analysis was conducted between SELENBP1 and selenium status biomarkers, inflammatory parameters, and trace elements (Table 2). SELENBP1 showed a weak inverse correlation with parameters of Se status, including significant correlation coefficients over the first two days after admission for Se and SELENOP. No significant association with Cu or Zn was found, regardless of the time postburn. In line with the ABSI results, positive correlations of SELENBP1 with inflammatory markers were observed, e.g., WBCs and PCT on admission. SELENBP1 and CRP showed a weak positive correlation on D2.

Table 2. SELENBP1 correlations over time.

SELENBP1 Correlations ($p < 0.05$)							
	D0	D1	D2	D3	W1	W2	Full
Se		−0.282	−0.227				−0.230
SELENOP	−0.382	−0.305	−0.303				−0.210
GPx-3							−0.170
Cu							−0.127
Zn							−0.124
WBCs	0.421	0.249		−0.240			0.197
CRP			0.260				−0.243
PCT	0.340	0.253	0.391		0.228		

Se, selenium; SELENOP, selenoprotein P; GPx-3, glutathione peroxidase 3; Cu, copper; Zn, zinc; WBCs, white blood cells; CRP, C-reactive protein; PCT, procalcitonin. Significant Spearman correlation coefficients are presented (green: positive correlations; orange: negative correlations).

3.5. Baseline SELENBP1 and Burn Severity Are Associated with the Risk of Pulmonary Infection

To assess the potential value of baseline SELENBP1 concentration and the ABSI as predictors of pulmonary infection risk in burn patients, an ROC analysis was conducted, with an estimated area under the ROC curve (AUC) of 0.69 for SELENBP1 and 0.82 for the ABSI (Figure 3). Baseline levels of serum SELENBP1 > 21.2 µg/L predicted the risk of pulmonary infection with a sensitivity of 75.7% and a specificity of 64.5%. The ABSI has

proven to be a more accurate predictor, with a sensitivity of 86.5% and a specificity of 71.0% at a cut-off of 6.5 (Table 3). Multiple logistic regression confirmed both baseline SELENBP1 and the ABSI as predictors of pulmonary infection in burn patients (Table 4). Burn patients with an ABSI of 7 or higher had a 14.4-fold greater risk of pulmonary infection than patients with an ABSI equal to or less than 6 (OR, 14.37; 95% CI, 3.15–88.78; $p = 0.001$). Initial SELENBP1 levels > 21.2 µg/L were associated with a 2.5-fold greater risk of pulmonary infection, although this did not reach statistical significance (OR, 2.47; 95% CI, 0.68–8.88; $p = 0.164$).

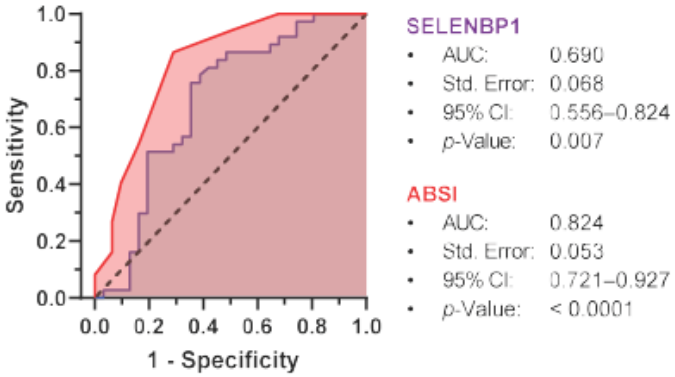

Figure 3. Receiver-Operator-Curve (ROC) analysis for the prediction of pulmonary infection by baseline SELENBP1 and ABSI. Both SELENBP1 (purple) and ABSI (red) are significantly associated with pulmonary infection risk, yielding areas under the ROC curve (AUC) of 0.690 and 0.824, respectively. Std. Error, standard error; CI, confidence interval.

Table 3. Validity of baseline SELENBP1 and the ABSI for the prediction of pulmonary infection.

	Cut-Off Point	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV
SELENBP1	>21.2 µg/L	75.7 (59.9–86.6)	64.5 (47.0–78.9)	59.8	79.2
ABSI	>6.5	86.5 (72.0–94.1)	71.0 (53.4–83.9)	67.5	88.3

ABSI, Abbreviated Burn Severity Index; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

Table 4. Multiple logistic regression.

	OR	95% CI	*p*-Value
Baseline SELENBP1 > 21.2 µg/L	2.47	0.68–8.88	0.164
ABSI > 6.5	14.37	3.15–88.78	0.001
Age	0.99	0.94–1.03	0.574
Sex (Female)	1.41	0.26–9.50	0.707
Intercept	0.25	0.03–1.66	0.161

ABSI, Abbreviated Burn Severity Index; OR, odds ratio; CI, confidence interval.

After adjusting for baseline SELENBP1 concentration and patient characteristics, changes in serum SELENBP1 concentrations within the first day after injury were not associated with an increased risk of pulmonary infection. Excluding female patients in multiple logistic regression did not yield significant changes regarding the predictive value of baseline SELENBP1 levels for pulmonary infection risk in burn patients.

4. Discussion

In this prospective cohort study, we observed an immediate systemic elevation of SELENBP1 after major burns. In line with previous studies reporting increased serum SELENBP1 levels following tissue destruction [16–18], baseline concentrations correlated with the degree of trauma and even exceeded those in patients with traumatic spinal cord injury [16], which is indicative of the excessive damage and systemic stress response to burn injury [34,35]. As shown in the subgroup analysis, initial SELENBP1 levels were particularly elevated in patients with a TBSA $\geq$ 30% and burns reaching the subcutaneous tissue (full-thickness burns). Considering the predominantly cytoplasmic localization of SELENBP1 in mature adipocytes under normal conditions [5], and its trauma-associated release into the systemic circulation, this interrelation becomes apparent and supports the notion of assessing serum SELENBP1 levels as a biomarker of burn severity. The greater elevation of baseline SELENBP1 among female patients is most probably attributable to their significantly higher burn severity as compared with male patients. Apart from that, no clear sex-specific differences concerning the dynamics and predictive value of SELENBP1 were found. This concurs with a previous trial in 75 patients undergoing cardiac surgery, which also reported only marginal sex-specific differences with regard to SELENBP1 kinetics [18].

After traumatic injury, the innate immune system induces a profound pro-inflammatory response, potentially aimed at promoting wound healing and mitigating the risk of microbe invasion and secondary infections. However, recurrent activation of the inflammatory cascade related to clinical procedures and complications can trigger a harmful "two-hit" response, which is common after major trauma and may predispose the host to opportunistic infections [36–38]. Acute burn injury leads to a similar release of pro-inflammatory cytokines, with peak levels persisting throughout the first week after the burn [39,40]. Likewise, inflammatory parameters, such as WBC counts, have been measured as being initially elevated in the serum of burn patients [41]. Consistent with these reports, we found serum SELENBP1 levels to positively correlate with inflammatory markers, i.e., WBCs and PCT, at admission. Baseline SELENBP1 concentration was not significantly associated with CRP, most likely due to the delayed onset of the acute phase response [42]. Unlike the previous research on circulating levels of SELENBP1, the present trial provided a prolonged follow-up period, allowing for an assessment of SELENBP1 dynamics up to six months after injury. We observed a rapid decrease in serum concentrations within the first day postburn. Interestingly, serum levels subsequently remained constantly low and did not show a "second hit", despite extensive surgical procedures and the development of secondary infections. The underlying mechanism may reflect the reciprocal functional interaction between SELENBP1 and GPx-1, as previously described [12]. GPx-1 is a most abundant and ubiquitously expressed Sec-containing selenoenzyme that reduces cellular oxidative stress by limiting hydrogen peroxide accumulation [43,44]. GPx-1 ranks low within the hierarchy of selenoproteins and thus responds sensitively to Se supply or Se decline [45]. Therefore, high-dose intravenous Se supplementation in severely Se-depleted burn patients may have supported intracellular GPx-1 expression and prevented a second increase in serum SELENBP1. However, tissue samples were not accessible for testing this hypothesis in the current study.

Aside from local inflammation, hypoxia is a common finding in the burn wound microenvironment. While short-term hypoxia contributes to the initiation of wound healing, chronic hypoxia exerts numerous detrimental effects at both a cellular and a systemic level. Hypoxia leads to the formation of reactive oxygen species (ROS), which, in turn, mediate the activation of the transcription factor HIF-1 [46,47]. The expression of GPx-3, a major extracellular ROS scavenger, is transcriptionally upregulated by hypoxia through a HIF-1-binding site [48]. Notably, SELENBP1 also seems to interact with HIF-1; the mouse homolog of SELENBP1 has been identified as a HIF-1 target gene [49], and SELENBP1 has been shown to negatively regulate the alpha subunit of HIF-1 in LNCaP prostate cancer cells [50]. Despite the immediately impaired Se status following burn injury, correlation analysis of

serum SELENBP1 did not reveal significant inverse associations with GPx-3 during the first days postburn, as opposed to Se and SELENOP. Since serum SELENBP1 levels and oxidative stress are both directly related to burn severity, the less pronounced decrease of GPx-3 in patients with higher SELENBP1 levels may result from hypoxia-induced GPx-3 expression. This finding appears to coincide with the results of a previous study where patients with septic shock exhibited a greater decline in serum SELENOP as compared with GPx-3 [51]. An analysis of SELENBP1 expression levels in relation to the local inflammatory response and the extent of hypoxia in the burn wound could provide useful information on the functional role of SELENBP1 in this context.

In line with our hypothesis, high concentrations of serum SELENBP1 at admission were predictive of pulmonary infection risk in burn patients, albeit not as accurately as overall burn severity, i.e., the ABSI. Infectious complications following major burns are largely driven by the enormous production of ROS and an impaired antioxidant defense [52]. GPx-1 deficiency in transgenic mice led to an increased susceptibility to ROS-induced cellular damage [53]. Accordingly, knockdown of SELENBP1 in HeLa cervical cancer cells decreased ROS levels and enhanced GPx-1 expression [54]. Based on these findings, one may speculate on an indirect aggravation of oxidative stress through intracellular SELENBP1 and that the shift of SELENBP1 to the intravascular space serves as a protective mechanism against oxidative damage and related post-acute sequelae. Still, this hypothesis and the physiological role and nature of the postulated SELENBP1-GPx-1 interaction in critically ill patients has yet to be elucidated.

Overall, this is the first study to assess the dynamics of serum SELENBP1 in severely burned patients and its linear correlation with the injury severity. Our study has certain strengths, including its relatively large and well-characterized patient cohort, the long-term follow-up, and the sensitive and robust immunoassay for SELENBP1 quantification. However, primarily as a consequence of its observational epidemiologic design, this study is not suitable for inferring mechanistic insights, such as the proposed SELENBP1-GPx-1 interaction, for which further molecular analyses are needed. Serum SELENBP1 kinetics in burn patients unexposed to supplemental Se and elevated GPx-1 activities cannot be deduced from this study, as supplemental Se was part of the routine clinical care of burn patients. The statistical power of this research is limited by the increasing number of missing values throughout the follow-up and the non-inclusion of additional, potentially confounding, variables in the multivariate analysis that are predictive of pulmonary infection risk in burn patients, e.g., pre-existing nutritional and immune status, comorbidities, timing of burn wound excision, and further supportive therapeutic measures. Furthermore, the analysis of sex-specific differences was limited by the small percentage of female patients.

Even though the association of serum SELENBP1 levels at admission with pulmonary infection risk did not reach statistical significance in our study, its immediate and strong elevation as well as its stringent positive correlation with burn severity indicate the potential relevance of this poorly characterized parameter in major trauma. Multicenter clinical trials and in vitro studies are warranted to allow greater insight into the regulation and function of SELENBP1 under conditions of hypoxia and inflammation. With a better understanding of the underlying cellular and molecular mechanisms, serum SELENBP1 may become a promising biomarker for estimating trauma severity and for identifying burn patients already at high risk of adverse clinical outcome at the time of hospital admission, who might benefit from intensified infection prevention measures.

5. Conclusions

Serum SELENBP1 concentration reflects the severity of trauma in burn patients and shows considerable associations with the risk of pulmonary infection postburn. Hereby, it complements the established parameters of injury like the ABSI and may contribute to a refined early assessment of damage severity and infection risk in severely burned patients. Further studies are required to elucidate the interplay between redox homeostasis, inflammatory response, serum and intracellular SELENBP1 changes, and clinical

outcome in burn injury in more detail, and to provide a better overview of the value of the different biomarkers.

Author Contributions: Conceptualization, T.L.T., J.A.P. and L.S.; methodology, T.L.T., H.J.K. and E.R.; software, T.L.T. and E.R.; formal analysis, T.L.T. and E.R.; investigation, T.L.T., H.J.K., J.A.P. and L.S.; resources, J.A.P. and L.S.; data curation, H.J.K., J.H., L.H. and T.R.G.; writing—original draft preparation, T.L.T. and L.S.; writing—review and editing, H.J.K., J.H., L.H., E.R., T.R.G. and J.A.P.; visualization, T.L.T.; supervision, J.A.P. and L.S.; project administration, J.A.P. and L.S.; funding acquisition, L.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Deutsche Forschungsgemeinschaft (DFG), Research Unit FOR-2558 "TraceAge" (Scho 849/6–2), and CRC/TR 296 "Local control of TH action" (LocoTact, P17).

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University of Zurich, Switzerland, on 20 April 2015 (KEK-ZH-No.: 2014-0631).

Informed Consent Statement: Informed consent was obtained from all the subjects involved in the study. In the case of incapacity to consent due to the extent of the injury, close relatives and legal representatives were asked for informed consent by proxy.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to containing patient confidential information.

Acknowledgments: We thank the patients for their participation, the clinicians involved in patient care and sample collection, and Vartitér Seher, Gabriele Boehm, and Anja Fischbach for excellent technical support in the laboratory analyses. The graphical abstract was created with BioRender.com, accessed on 27 October 2023.

Conflicts of Interest: L.S. holds shares of selenOmed GmbH, a company involved in selenium status assessment. No other relationships or activities that could appear to have influenced the submitted work are indicated. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Curriculum Vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Publication list

Turan TL, Klein HJ, Hackler J, Hoerner L, Rijntjes E, Graf TR, Plock JA, and Schomburg L. Serum Selenium-Binding Protein 1 (SELENBP1) in Burn Injury: A Potential Biomarker of Disease Severity and Clinical Course. *Antioxidants*. 2023; 12(11):1927. doi: 10.3390/antiox12111927.

Journal Impact Factor: 7.0 (2022)

Journal Rank: JCR – Q1 (46/285; Biochemistry and Molecular Biology)

Acknowledgments

First and foremost, my sincere gratitude goes to Prof. Dr. Lutz Schomburg, who gave me the opportunity to work on this highly interesting research topic. His enthusiasm for science and profound knowledge in the field of selenium metabolism guided me on this journey, and I could not have had a better supervisor than him. I would also like to thank my second supervisor, Dr. Eddy Rijntjes, for the many fruitful discussions.

I deeply appreciate the ongoing support from the members of the Schomburg working group. Vartitér Seher, Anja Fischbach, and Gabriele Boehm always provided excellent technical support and gladly offered a helping hand whenever needed. This work would also not have been possible without the scientific input and personal help of Dr. Waldemar Minich and my fellow (post-)doctoral researchers, especially Kamil Demircan, Dr. Qian Sun, Dr. Julian Hackler, Dr. Thilo Chillon, and Livia Hoerner. Moreover, I am grateful for the constructive cooperation with our Swiss colleagues. Importantly, many thanks go to the patients who took part in this research and therefore contributed to substantial progress in future burn care.

Lastly, I would like to thank my friends and family. In particular, I dedicate this work to my beloved parents. I am forever grateful for their encouragement and unlimited support.