

Article

Serum selenium status as diagnostic marker for prognosis in liver transplantation

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Abstract

The trace element selenium (Se) is taken up from the diet and becomes metabolized mainly by hepatocytes. Selenoprotein P (SELENOP) constitutes the liver-derived Se transporter. Biosynthesis of extracellular glutathione peroxidase (GPx3) in kidney depends on SELENOP-mediated Se supply. We hypothesized that Se status may serve as a useful prognostic marker for outcome in patients undergoing liver transplantation. Serum samples from patients were routinely collected before and after transplantation. Concentration of serum SELENOP and total Se as well as GPx3 activity were determined by standardized tests and related to survival, aetiology and pre-operative Child-Pugh and Model for End-Stage Liver Disease Scores. A total of 314 serum samples from 78 transplanted patients were available for analysis. The Se and SELENOP concentrations were on average below the reference ranges of healthy subjects. Patients with ethanol toxicity-dependent aetiology showed particularly low SELENOP and Se concentrations and GPx3 activity. Longitudinal analysis indicated declining Se concentrations in non-survivors. We conclude that severe liver disease necessitating organ replacement is characterized by a pronounced Se deficit before, during and after transplantation. A recovering Se status after surgery is associated with positive prognosis, and an adjuvant Se supplementation may thus support convalescence.

Keywords: trace element; liver transplantation; selenoprotein P; glutathione peroxidase; hepatitis C virus.

1. Introduction

The liver is of central relevance for trace element metabolism, in particular for controlling copper, iron, selenium (Se) and zinc homoeostasis [1-3]. Under inflammatory conditions, several converging pathways contribute to declining serum Se status, in part via reduced biosynthesis of the Se transport protein selenoprotein P (SELENOP) in hepatocytes as part of the negative acute phase response (APR) [4-7]. Studies in lipopolysaccharide (LPS)-injected mice as a rodent model of APR have indicated that the inflammatory stimulus reduces the transcription of central genes controlling Se metabolism, selenocysteine (Sec) formation and Sec insertion into growing selenoproteins [8-10]. Among the rate-limiting hepatic factors are the intracellular Se-binding protein 1 (Selenbp1), the phosphorseryl-tRNA-kinase (Pstk) and the Sec-specific elongation factor (eEFsec) [5,11]. Collectively, an impaired hepatic Se metabolism results during APR that is associated with decreased SELENOP biosynthesis and secretion causing a declining Se status in the circulation and in target tissues, as observed e.g. in sepsis [12], severe trauma [13], inflammatory disease [14] or in COVID-19 [15].

The central role of the liver for an undisturbed Se metabolism and Se status within normal reference ranges is supported by analyses of patients with liver disease [1,16-18]. Cirrhotic or fibrotic liver tissue causes reduced serum Se concentrations, and a recent report indicated a gradual decrease of serum SELENOP concentrations in non-alcoholic fatty liver disease (NAFLD) and steatohepatitis (NASH) [19]. The essential nature of Se for liver health was first proven in a rodent model of tissue necrosis in 1957 by Schwarz and Foltz, where supplemental Se proved essential for preventing organ destruction [20,21]. The direct interplay of Se and vitamin E in relation to iron-mediated oxidative stress and damage is of central importance for ferroptosis, a general mechanism of cell death [22,23]. The liver is constantly exposed to high oxidative stress, as it decisively contributes to the safe removal of endogenous and exogenous toxicants. Consequently, it was postulated that insufficient Se supply and low expression of relevant selenoproteins implicated in antioxidative protection might contribute to higher risk of malignant transformation of hepatocyte and hence higher incidences of liver tumours [24,25]. This notion was supported by a focused epidemiological case-control study nested within the European Prospective Investigation into Cancer and Nutrition (EPCI) cohort, where Se and SELENOP deficiency, respectively, was identified as most relevant risk factor for hepatocellular carcinoma (HCC) [26]. A Se or SELENOP concentration within the lowest tertile of the European population was associated with a 5- to 10-times increased risk for HCC [27].

A similarly close interrelation is described for Se deficits in viral infection and autoimmune disease. Patients suffering from hepatitis C virus (HCV) or hepatitis B virus (HBV) infection are characterized by relatively low Se status [28-30]. Another direct connection between Se and liver disease is given by the O-phosphoseryl-tRNA:selenocysteinyl-tRNA synthase (SEPSECS) [31], which has initially been described as soluble liver antigen SLA/LP in a distinct form of hepatic autoimmune condition [32,33].

From all of the above, we hypothesized that patients with severe liver disease undergoing liver transplantation are characterized by severe dysregulation of Se metabolism, and that one or all of the Se status biomarkers accessible from serum samples provide relevant and useful information for tissue functioning, transplantation success and prognosis.

2. Materials and Methods

2.1 Study design

A longitudinal study of patients with end stage liver disease, cirrhosis and hepatocellular carcinoma (HCC) selected for liver transplantation (LT) was conducted at the Department of Surgery at Charité - Universitätsmedizin Berlin. All patients analysed were transplanted by cadaveric organs for HCC in liver cirrhosis. They underwent LT primarily and all had provided written informed consent. The samples were collected between 05/2008 and 12/2015 and were deposited in a local biobank according to a predefined scheme (Fig. 1). A follow-up over a time span of 5-years was performed. The study was conducted in accordance with the declaration of Helsinki. Approval was granted by the Board of Ethics of Charité Medical School Berlin (EA2/092/19). The samples had been stored at -80°C until analysis. All measurements were conducted by scientists blinded to any clinical information.

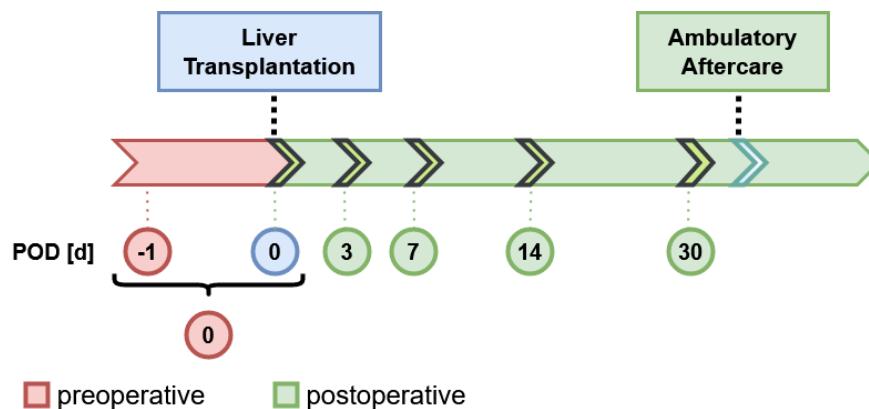


Figure 1: Sampling scheme for patients undergoing liver transplantation (LT). First serum sample was taken at day of LT, including a time frame of 24 h before, designated as postoperative day 0 (POD 0). Consecutive samples post-LT were collected on the 3rd, 7th, 14th and 30th day post-LT (POD 3, 7, 14, 30).

2.3 Selenium status analysis

Concentration of total serum Se was determined by total reflection X-ray fluorescence (TXRF) analysis using a benchtop TXRF analyser (T-Star, Bruker Nano GmbH, Berlin, Germany) as described [34]. Seronorm serum standard (Sero AS, Billingstad, Norway) served as control, and inter- and intra-assay coefficient of variation (CV) of the measurements were < 5%. Concentrations of selenoprotein P (SELENOP) were determined by a commercial immunometric sandwich assay (selenOtest ELISA, selenOmed GmbH, Berlin, Germany). Internal assay kit controls were measured in duplicate in concentration steps of 18.2 to 72.9 and up to 291.7

µg/mL, yielding inter- and intra-assay CV ≤ 12.8%. Serum glutathione peroxidase (GPx3) activity was measured using tert-butyl-hydroperoxide as substrate as described recently [35]. A pool of 25 human serum samples (commercial samples, provided by invent Diagnostica GmbH, Hennigsdorf, Germany) served as a control for GPx3 measurements, yielding inter- and intra-assay CV ≤ 11.0%.

Table 1. Anthropometric and clinical characteristics and Se biomarkers of the patients

parameter	data
patients [n], females (%)	78, (18.0)
serum samples [n]	314
Age, median (Q1 – Q3) [years]	59.0 (54 - 64)
BMI, median (Q1 – Q3) [kg/m ²]	27.0 (24 - 33)
MELD-score, mean ± SD	12.3 ± 4.9
Child-Pugh-Score	
A [n] (%)	47 (60.3)
B [n] (%)	23 (29.5)
C [n] (%)	8 (10.3)
transplantation indication, survival rate	
ethanol toxicity [n] (%)	35 (44.9)
HCV [n] (%)	26 (33.3)
other* [n] (%)	17 (21.8)
death [n] (%)	17 (21.8)
initial serum selenium status**	
<P2.5*** (<45.7 µg/L) [n] (%)	18 (23.4)
<Q1 (< 70.1 µg/L) [n] (%)	61 (79.2)
<Q2 (< 82.5 µg/L) [n] (%)	70 (90.9)
>Q2 (>82.5 µg/L) [n] (%)	7 (9.0)
initial serum SELENOP status**	
<P2.5*** (<2.5 mg/L) [n] (%)	60 (77.9)
<Q1 (< 3.7 mg/L) [n] (%)	74 (96.1)
<Q2 (< 4.3 mg/L) [n] (%)	76 (98.7)
>Q2 (>4.3 mg/L) [n] (%)	1 (1.3)

* including HBV, NASH, cryptic, heamatochromatosis, other; ** patients serum status at POD 0 (no serum available from Patient LTX 45 at POD 0, therefore n = 77) in comparison to cross-sectional data from healthy adult European subjects participating in the EPIC study [36]; *** 2.5th percentile, equal to the threshold for deficiency of the biomarker; Q1: 25th percentile; Q2: 50th percentile; MELD, Model for End-Stage Liver Disease.

2.4 Statistical analysis

Statistical analysis was performed with SPSS Statistics ® (version 25, IBM, Chicago, IL, USA) and GraphPad Prism (Version 9.0.0, GraphPad Software Inc., San Diego, CA, USA), respectively. Normal distribution of values was tested by the Shapiro-Wilk test. Comparisons between two groups were conducted by unpaired t test using Welch's correction. Comparisons of the characteristics between more than

two groups were conducted with ANOVA, assuming unequal variance and using Games-Howell correction. Correlations were tested by Pearson's correlation analysis. All statistical tests were two-sided and P-values < 0.05 were considered statistically significant; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. All group figures were plotted as mean \pm standard deviation, unless indicated differently.

3. Results

3.1. Selenium Status

Se status of patient samples was evaluated by different biomarkers, including total serum Se and serum SELENOP concentrations, for which reference data are available from a large cross-sectional study [36], as well as GPx3 activity. A high fraction of the serum samples from the LT patients displayed a considerable Se deficit, as judged by total serum Se (79.2% below the 25th percentile of 70.1 $\mu\text{g/L}$) or SELENOP (77.9% below the 2.5th percentile of 2.5 mg/L) (**Table 1**).

The biomarkers of Se status showed some consistent linear correlations. A total set of data from 314 serum samples was available for the correlation analysis of total serum Se, SELENOP as well as GPx3 activity (**Fig. 2A-C**). The results obtained support the notion on a general deficiency in the trace element Se as stringent correlations are observed between all of the three biomarkers, highlighting at the same time the high quality and integrity of the clinical samples available for analysis. As known from similar clinical analyses, the most stringent correlation is observed between total serum Se and SELENOP concentrations (**Fig. 2A**).

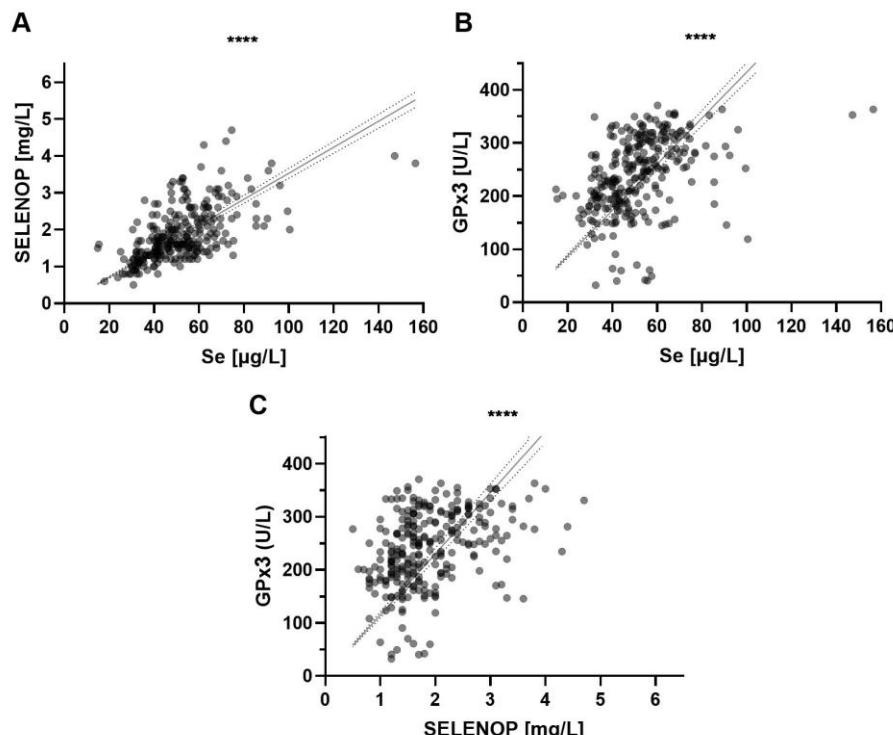


Figure 2: Interrelation of biomarkers of Se status in the samples from patients undergoing LT. The majority of samples displayed a relatively low Se status, averaging at about 52 $\mu\text{g/L}$. (A) Total serum

Se and SELENOP concentrations showed a tight and linear correlation over the full range of data (Pearson $r = 0.6081$). (B) Similarly, extracellular GPx3 activity correlated positively and linearly to serum Se concentrations (Pearson $r = 0.4068$). (C) The two circulating protein biomarkers of Se status, i.e., SELENOP and GPx3 showed a moderate positive correlation in the samples analysed (Pearson $r = 0.3769$). ****, $P < 0.0001$; $n = 314$ sets of data.

3.2. Comparison of Se status in relation to reference ranges and HCC aetiology

The Se status determined by total serum Se and circulating SELENOP concentrations was compared to reference ranges of a large cohort of healthy European subjects analysed by the same technology in the analytical lab. The reference data were derived from subjects participating in the European Prospective Investigation into Cancer (EPIC) cohort, where GPx3 activity had not been determined [36]. Mean serum Se concentrations of the patients undergoing LT differed significantly from reference values (Fig. 3A). The average Se concentration observed ($51.8 \mu\text{g/L}$) was in the range of the 6th percentile of healthy subjects ($53.2 \mu\text{g/L}$). SELENOP concentrations were also significantly lower than the reference group, and mean SELENOP concentrations in LT samples ($1.9 \pm 0.7 \text{ mg/L}$) were even below the 1st percentile of the EPIC cohort (2.3 mg/L) (Fig. 3B). Next, the patients were divided into three groups in relation to aetiology of liver cirrhosis, i.e., patients with ethanol-induced (EtOH) cirrhosis ($n=35$), HCV ($n=26$) or other aetiology ($n=17$). A direct comparison revealed particularly low Se status in patients with EtOH-dependent aetiology, both in relation to serum Se (Fig. 3C) and SELENOP concentrations (Fig. 3D) as well as in relation to GPx3 activities (Fig. 3E).

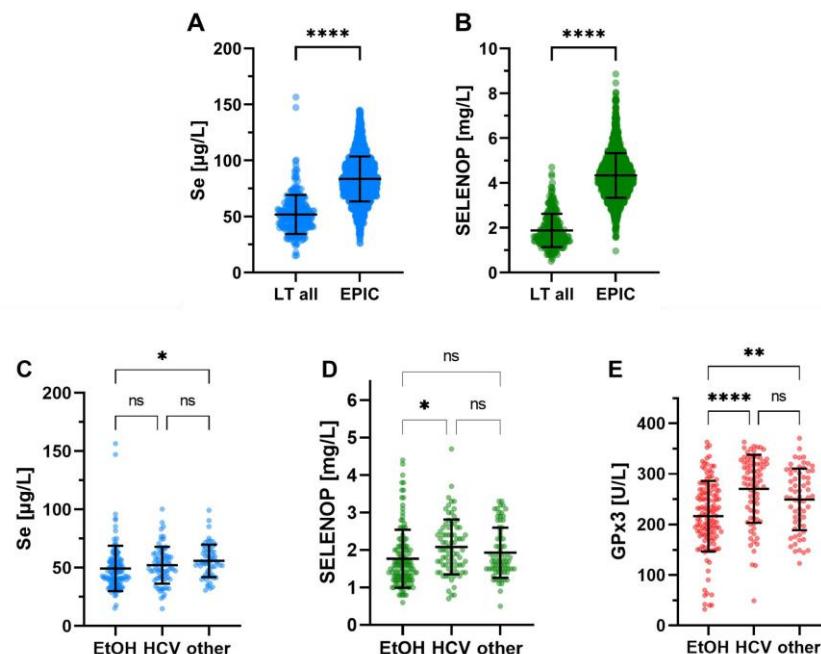


Figure 3: Depressed Se status in patients undergoing liver transplantation (LT). The set of serum samples from patients undergoing LT indicated a general Se deficit, with very low concentrations of (A) total Se and (B) SELENOP in comparison to a reference cohort of healthy subjects (EPIC). The samples were subdivided according to the aetiology of hepatocellular carcinoma (HCC) in liver cirrhosis. The largest group represented the patients with cirrhosis due to an ethanol toxicity (EtOH, $n=129$, 35 patients), followed by patients with hepatitis C virus cirrhosis (HCV, $n=79$, 26 patients), and

a group with other aetiologies (n=63, 17 patients). The patients with EtOH-dependent aetiology showed particularly low (C) total serum Se and (D) SELENOP concentrations as well as (E) relatively low GPx3 activities. Comparisons by ANOVA; * p<0.05, ** p<0.01, and *** p<0.0001.

3.3. Predictive value of the Se status biomarkers in relation to survival

Liver transplantation (LT) bears a certain risk of death, and 17 (21.8%) out of the 78 patients did not survive the intervention during a follow-up time of 5 years. The Se status biomarkers were tested for their predictive value in relation to survival. The direct comparison of total serum Se and SELENOP concentrations along with GPx3 activities indicated a significant difference between patients who died post-LT (group of non-survivors) and patients surviving LT (Fig. 4A-C). All three markers were significant higher in the group of survivors as compared to the group of non-survivors (mean±SD; Se [$\mu\text{g/L}$]: 53.3±18.0 vs. 45.9±13.4; SELENOP [mg/L]: 1.9±0.7 vs. 1.6±0.7; GPx3 [U/L]: 245.4±72.8 vs. 218.9±59.0).

Time-resolved analyses indicated noteworthy differences between the survivors and non-survivors in the time span around LT in all three biomarkers (Fig. 4D-F). Total serum Se concentrations declined initially to a similar degree in both groups of patients, but tended to recover in survivors only, beginning several days post-LT (Fig. 4D). In comparison, circulating SELENOP concentration was similar in both groups, with non-survivors displaying a tendency of stronger deficits (Fig. 4E). Serum GPx3 activities showed the strongest difference at POD 3 to POD 7, where the group of surviving patients displayed higher enzyme levels (Fig. 4F).

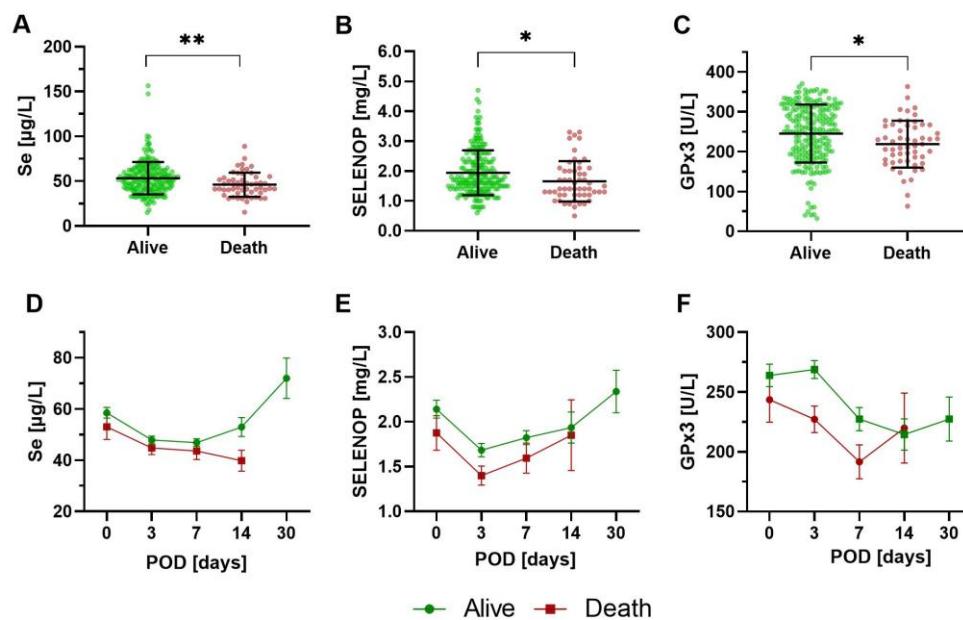


Figure 4: Comparison of Se status biomarkers in relation to survival. Patients undergoing liver transplantation (LT) were separated in survivors (Alive) and non-survivors (Death). Non-survivors displayed significantly reduced (A) serum Se, (B) SELENOP and (C) GPx3 levels. A time resolved analysis (D-F) revealed consistently reduced concentrations of all three biomarkers from postoperative day 0 (POD 0) to POD 7. Values in D-F are plotted as mean ± SEM. Comparisons by t test; * p<0.05, and ** p<0.01.

3.4 Comparison of Se status biomarkers with established indices of chronic liver disease

In order to assess the potential value of the Se status biomarkers as additional diagnostic parameters of liver disease, the initial values around the time of surgery (POD 0) were compared to the established indices of chronic liver disease (**Fig. 5**). Total serum Se concentrations correlated to liver disease severity as assessed by the Child-Pugh-Score (CPS), and patients with the most severe diagnosis (CPS class C) displayed the lowest Se levels (**Fig. 5A**). Neither SELENOP concentrations (**Fig. 5B**) nor GPx3 activities (**Fig. 5C**) showed a significant difference between the groups of mild, moderate or severe chronic liver disease as judged by the CPS classes A (mild), B (moderate) or C (severe disease).

In a second analysis, the Se status biomarkers were compared to the lab MELD-Score (**Fig. 5D-F**). Total serum Se determined at time of surgery (POD 0) showed a moderate negative correlation (Pearson's $r = -0.3726$) with the MELD-Score (**Fig. 5D**). Serum SELENOP concentrations displayed the same trend (Pearson's $r = -0.2368$) (**Fig. 5E**), whereas no significant interrelation of GPx3 activities with the lab MELD-Score (Pearson's $r = -0.1655$) was observed (**Fig. 5F**).

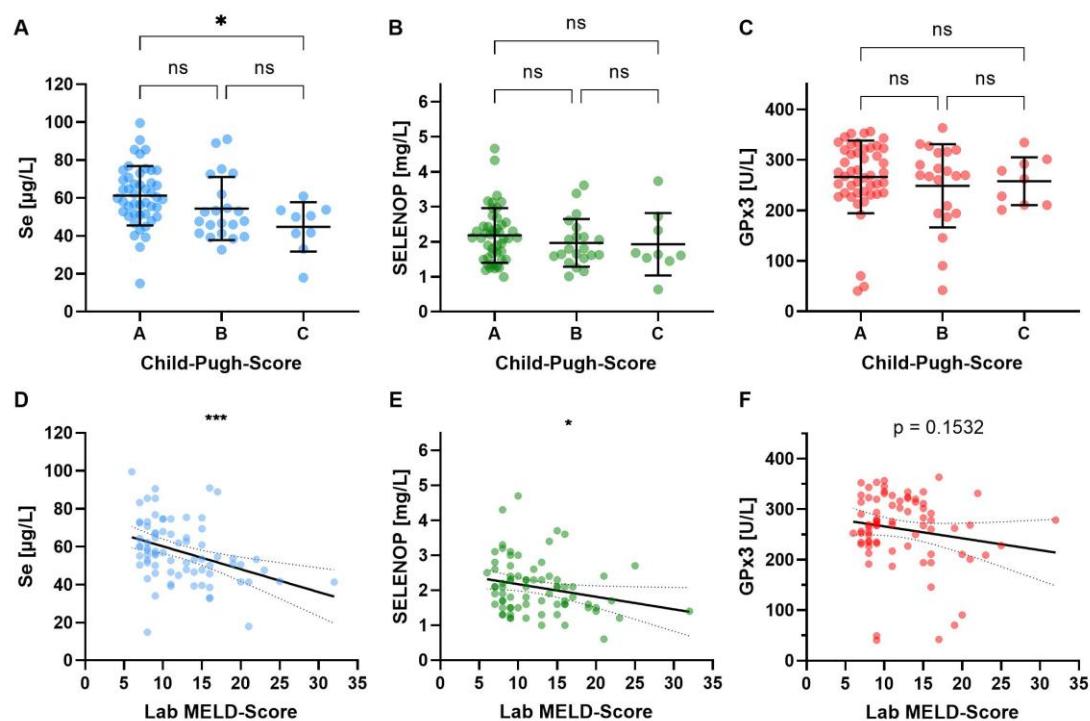


Figure 5: Serum biomarkers of Se status from patients undergoing liver transplantation (LT) at the time of LT (POD 0) in comparison to established diagnostic scoring systems. Total serum Se displayed the highest overlap to chronic liver disease diagnosis by (A) the Child-Pugh-Score, or (D) the Model for End-Stage Liver Disease (MELD)-Score. In comparison, (B) SELENOP concentrations, and (C) GPx3 activities showed no significant relation to the Child-Pugh-Score at POD 0. Using the MELD score, a negative correlation of (E) serum SELENOP levels (correlation coefficient; -0.3726), but not of (F) GPx3 activity was observed. Comparisons by ANOVA; * $p < 0.05$, and *** $p < 0.001$.

4. Discussion

In this manuscript, we report the dynamic changes observed in Se status biomarkers in patients undergoing LT. The rationale for our analysis was based on the notion that hepatocytes are the key cell type for converting dietary Se into organic forms in the mammalian organism and for systemic transport of the trace element via SELENOP as the major selenoprotein in the circulation [37]. Transgenic mouse experiments have indicated that mice devoid of hepatic Selenop biosynthesis develop severe symptoms of Se deficiency, including growth defects, altered bone quality, impaired motor control and even epileptic seizures [6,38]. Especially the brain phenotype was unexpected, as the central nervous system constitutes a most preferentially supplied target organ in the hierarchy of Se distribution within the organism [39-41]. Notably, transgenic cell-specific expression of human SELENOP in hepatocytes only was capable of supplying the trace element into brain and thereby protecting the neurons from damage and death [42]. Based on these insights from model systems, the hypothesis on a strongly disturbed Se status in patients undergoing LT was tested and verified.

The results obtained support the findings from previous analyses in relation to a suppressed Se status in severe liver disease [16,43]. Both chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) are characterized by a depressed Se status [44]. Two underlying reasons may account for these strong and unequivocal findings; firstly the loss of differentiated hepatocytes, and thereby an impaired uptake and conversion of the dietary trace element into circulating SELENOP [6,42], and secondly a profound and intense local and systemic inflammation, known to negatively affect hepatic selenoprotein biosynthesis and depressed Se status in blood and likely in target tissues [42,45]. On top of these direct interrelation of impaired liver function and depressed Se status, a predisposition for severe liver disease including HCC may also contribute to the observed Se deficit in HCC, as shown in longitudinal and nested case-control studies [27,46].

All three biomarkers of Se status analysed showed some stringent correlations, supporting the assumption that a profound Se deficit is present in most if not all of the patients. Subjects with sufficiently high Se status are characterized by saturated circulating selenoprotein levels, with GPx3 activities reaching their plateau at around 80-90 µg Se per litre serum, and SELENOP showing a slightly higher requirement and reaching a plateau at 120-130 µg/L [47-49]. Under such Se replete conditions, a stringent correlation between total serum Se and GPx3 or SELENOP concentrations is not observed, indicating sufficient supply for maximized selenoprotein expression. The Se concentrations in our patients undergoing LT are far below these saturating concentrations, clearly supporting a classification as severely Se deficient, as also mirrored in the majority of samples residing below the consented threshold for Se deficiency (total serum Se < 70 µg/L).

The central role of the liver for Se status is further supported by the kinetic analyses of the Se status biomarkers. From the first postoperative day and during aftercare, our patients started with a low-germ diet without any specific Se-containing supplements. The Se status was low, remained low and even declined further during the first days after surgery. From the data obtained, recovery of

SELENOP biosynthesis and secretion into the circulation seems to resume approximately one week post-LT. This time span may be needed for an initial recovery of liver function and hepatocyte metabolism, as blood supply improves and inflammatory tonus declines during the convalescence stage in surviving patients.

The kidneys are the major source of circulating GPx3, synthesized by renal tubular cells [50]. In transgenic mice, it was shown that renal GPx3 biosynthesis depends on hepatocyte-derived Selenop as source of the limiting trace element, mediated by a specific expression of the Selenop-receptor megalin (lipoprotein-related protein 2, Lrp2) [51,52]. This strict dependence of renal GPx3 on hepatic Selenop may have contributed to the distinct but overlapping patterns of both circulating selenoproteins in the kinetic analysis of changes in Se status in the weeks after LT. In how far a compromised kidney function with impaired GPx3 biosynthesis contributed to survival and a positive prognosis remains to be studied in future analyses.

A universal, constant and massive Se deficit in patients with HCC undergoing LT remains as the major insight from this study. The deficit extents from the time before surgery, to the intervention and to the first days and weeks post-LT. It is known from a large number of other conditions that a profound Se deficit constitutes a highly relevant but addressable risk factor for poor prognosis, e.g. in relation to cardiovascular disease, cancer at different sites, infections including sepsis and the current COVID pandemic, or major trauma or other severe disease [53-55]. However, clinical experiences with supplemental Se are sparse, and only few trials have reported consistent and convincing results, e.g. in relation to long-term survival, avoiding endemic Se-related diseases like Kashin-Beck and Keshan disease, endocrine Graves' orbitopathy or improving vaccination success [55,56]. In light of the profoundly low Se status observed in the LT patients, an adjuvant Se supplementation, potentially along with other essential micronutrients, should be considered both before and after LT. From all experiences with large scale Se supplementation studies, daily dosages of around 200 µg can be considered as safe and free of unintended side-effects, whereas 800 µg per day would constitute the upper tolerable limit, obviously chosen carefully as first toxic symptoms were observed at higher intakes of several mg Se per day in the clinical setting [57].

Among the particular strengths of our study are the relatively high number of patients undergoing LT, and the consequent longitudinal monitoring of Se status during the intervention and the immediate time span around surgery and thereafter. Moreover, the full assessment of all three established biomarkers of Se status by validated tests provides some solid information on Se status and its metabolism and dynamic alterations. Among the limitations are the nature of the study as a purely observational analysis, constricting our insights to associations only, and the lack of tissue biomarkers from biopsies which would allow some insights into the cellular processes and intracellular Se status and hepatic Se metabolism.

5. Conclusions

Patients undergoing LT display considerable deficits in Se and selenoproteins both before, during and after organ replacement. Biomarkers of Se status show some correlation to liver pathology scores and prognosis, and may be monitored in order to guiding adjuvant Se supplementation which should be considered in order to improving overall health, convalescence and prognosis. However, respective supplementation studies are missing at present, and the notion – albeit well founded on scientific grounds – lacks experimental prove from clinical trials at present.

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Conflicts of Interest. L.S. holds shares in selenOmed GmbH, a company involved in Se status assessment and supplementation. The other authors declare no competing interest with respect to this study.

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