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Epidemiology

Relation of selenium status to neuro-regeneration after traumatic spinal cord injury

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ABSTRACT

Introduction: The trace element selenium (Se) is crucial for the biosynthesis of selenoproteins. Both neurodevelopment and the survival of neurons that are subject to stress depend on a regular selenoprotein biosynthesis and sufficient Se supply by selenoprotein P (SELENOP).
Hypothesis: Neuro-regeneration after traumatic spinal cord injury (TSCI) is related to the Se status.
Study design: Single-centre prospective observational study.
Patients and methods: Three groups of patients with comparable injuries were studied; vertebral fractures without neurological impairment (n = 10, group C), patients with TSCI showing no remission (n = 9, group G0), and patients with remission developing positive abbreviated injury score (AIS) conversion within 3 months (n = 10, group G1). Serum samples were available from different time points (upon admission, and after 4, 9 and 12 h, 1 and 3 days, 1 and 2 weeks, and 1, 2 and 3 months). Serum trace element concentrations were determined by total reflection X-ray fluorescence, SELENOP by ELISA, and further parameters by laboratory routine.
Results: Serum Se and SELENOP concentrations were higher on admission in the remission group (G1) as compared to G0. During the first week, both parameters remained constant in C and G0, whereas they declined significantly in the remission group. Similarly, the concentration changes between admission and 24 h were most pronounced in this group of recovering patients (G1). Binary logistic regression analysis including the delta of Se and SELENOP within the first 24 h indicated an AUC of 90.0% (CI: 67.4%–100.0%) with regards to predicting the outcome after TSCI.
Conclusion: A Se deficit might constitute a risk factor for poor outcome after TSCI. A dynamic decline of serum Se and SELENOP concentrations after admission may reflect ongoing repair processes that are associated with higher odds for a positive clinical outcome.

1. Introduction

Traumatic spinal cord injury (TSCI) is a devastating and life-changing event that affects both the local site of injury and the entire body. TSCI causes severe medical, psychological, social and economic challenges for concerned patients, their families and the health care system [1]. Despite palliative treatment having improved considerably during recent years [2], causal therapy options and valid monitoring techniques are either missing or lacking sufficient clinical evidence to implement their routine use [3–5]. TSCI is characterized by different adjacent phases; initially by the mechanical trauma, the spinal damage and shock (early phase), and finally by a more complex local and systemic inflammatory response during the second phase after injury.

The initial immune response involves a variety of migrating and infiltrating inflammatory cells, such as neutrophils and monocytes, and their immediate activities. The second phase is characterised by a distinct release of cytokines, chemokines, matrix-metalloproteinases and specific growth-factors [6–9]. The subsequent tissue remodelling necessitates anabolic, proteolytic and protective pathways acting in concert and timely at the site of injury. In addition, the release of endogenous signaling substances, such as cortisol, glucagon and epinephrine is paramount during tissue healing, thereby dynamics of

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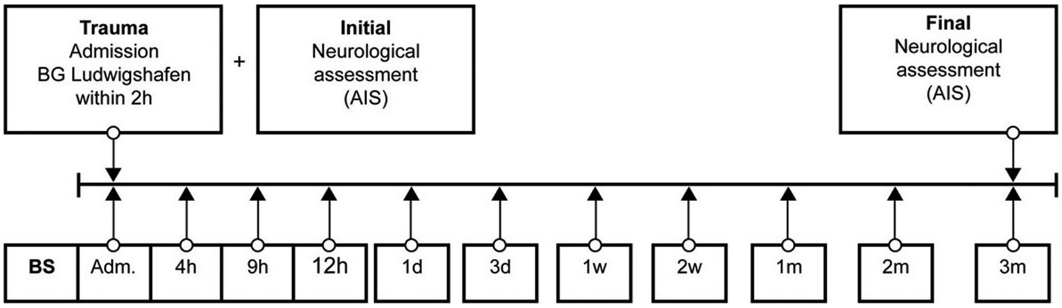

Fig. 1. Blood sampling (BS) scheme. Eleven blood samples per patient were taken at admission (0 h), 4 h, 9 h, 12 h, 1 day (1 d), 3 d, 1 week (1 w), 2 w, 1 month (1 m), 2 m and 3 m after TSCI.

Table 1
ASIA Impairment Scale (AIS) classification in relation to the clinical state.

AIS Grade	Clinical State
A	Complete—No motor or sensory function is preserved in the sacral segments S4-S5
B	Incomplete—Sensory but not motor function is preserved below the NLI and includes the sacral segments S4-S5
C	Incomplete—Motor function is preserved below the NLI, and more than half of key muscles below the NLI have a muscle grade less than 3
D	Incomplete—Motor function is preserved below the NLI, and at least half of key muscles below the NLI have a muscle grade of 3 or more
E	Normal—Motor and sensory function is normal

disease-related factors are traceable in peripheral blood of patients after TSCI [10,11].

Next to hormones, certain trace elements are involved in developmental and regenerative processes. Zinc (Zn) is present as cofactor in more than 300 enzymes and is linked to the remodeling mechanisms after injury of numerous types of tissues via e.g. Zn-dependent proteases, transcription factors and RNA-polymerase [12,13]. In particular, the Cu-/Zn-dependent superoxide dismutase (SOD1) decreases the oxidative stress and attenuates the MMP-9 mediated blood-brain barrier (BBB) disruption as shown in a rodent model of spinal cord injury (SCI) [14]. This is noteworthy, due to ischemic and damaged neuronal tissue being affected by lipid peroxidation of the cell membrane resulting in local edema and inflammation [15–18].

The group of selenocysteine (Sec)-containing selenoproteins contributes to protecting cells from oxidative damage, especially via the families of Se-dependent glutathione peroxidases (GPX) and thioredoxin reductases (TXNRD). In particular, the phospholipid hydroperoxide glutathione peroxidase (PHGPx, GPX4) as well as both the cytosolic TXNRD1 and mitochondrial TXNRD2 have proven to be essential for survival [19,20]. GPX4 plays an important role in neuroprotection, as it degrades oxidized lipids, prevents peroxidation of polyunsaturated fatty acids and constitutes the central regulatory element in ferroptosis [21]. Cell death from ferroptosis results in the release of immunogenic cell components and lipid metabolites that cause an increased local and systemic inflammatory response. Notably, a missing Gpx4 expression in adult mice triggered a ferroptosis-related loss of spinal motor neurons [22].

The expression of neuronal selenoenzymes depends on a sufficiently high Se supply, maintained by the hepatically-derived plasma protein selenoprotein P (SELENOP). Mice with genetically inactivated Selenop expression exhibit growth and developmental aberrations [23], and may develop neurological dysfunction and epileptic seizures [24]. Under physiological conditions, brain selenoprotein protein expression is hierarchically maintained through a prioritized Se supply [25]. However, in severe diseases the SELENOP-dependent Se transport can be disrupted, as observed in sepsis and septic shock [26]. Under these conditions, survival of patients strongly correlates to their Se status

Table 2
Patients' clinical characteristics.

Variables	All cases (n = 29)	Control C (n = 10)	No remission G0 (n = 9)	Remission G1 (n = 10)	P-value
Sex					
Female	10 (34)	5 (50)	2 (22)	3 (30)	$P = 1.000$
Male	19 (66)	5 (50)	7 (78)	7 (70)	
Age					
min	15	27	22	15	$P = 0.898$
max	75	71	65	75	
median (IQR)	48.0 (32.0, 59.0)	41.0 (34.0, 57.3)	49.0 (41.0, 60.0)	49.5 (27.5, 59.0)	
Etiology					
Fall	9 (31)	3 (30)	2 (22)	4 (40)	$P = 0.695$
Other	4 (14)	0 (0)	2 (22)	2 (20)	
Traffic	16 (55)	7 (70)	5 (56)	4 (40)	
AO					
A	21 (72)	8 (80)	5 (56)	8 (80)	$P = 0.266$
B	6 (21)	2 (20)	2 (22)	2 (20)	
C	2 (7)	0 (0)	2 (22)	0 (0)	
NLI					
Cervical	7 (24)	0 (0)	2 (22)	5 (50)	$P = 0.276$
Lumbar	3 (10)	0 (0)	1 (11)	2 (20)	
Thoracical	9 (31)	0 (0)	6 (67)	3 (30)	
None	10 (34)	10 (100)	0 (0)	0 (0)	
Paralysis					
Complete Paraplegia	11 (38)	0 (0)	7 (78)	4 (40)	$P = 0.375$
Complete Tetraplegia	1 (3)	0 (0)	0 (0)	1 (10)	
Incomplete Paraplegia	4 (14)	0 (0)	1 (11)	3 (30)	
Incomplete Tetraplegia	3 (10)	0 (0)	1 (11)	2 (20)	
No paralysis	10 (34)	10 (100)	0 (0)	0 (0)	
Initial AIS					
A	10 (34)	0 (0)	7 (78)	3 (30)	$P = 0.056$
B	5 (17)	0 (0)	2 (22)	3 (30)	
C	4 (14)	0 (0)	0 (0)	4 (40)	
E	10 (34)	10 (100)	0 (0)	0 (0)	
Final AIS					
A	7 (24)	0 (0)	7 (78)	0 (0)	$\mathbf{P = 0.002}$
B	4 (14)	0 (0)	2 (22)	2 (20)	
C	2 (7)	0 (0)	0 (0)	2 (20)	
D	6 (21)	0 (0)	0 (0)	6 (60)	
E	10 (34)	10 (100)	0 (0)	0 (0)	

Abbreviations: AO, AO classification; AIS, American Spinal Injury Association (ASIA) Impairment Scale; NLI, neurological level of injury. Age is expressed as mean years ± sd. Neurological remission was defined as improvement in AIS. Comparing G0 and G1 P-values were analysed with the Mann-Whitney-U test and show differences between G0 and G1.

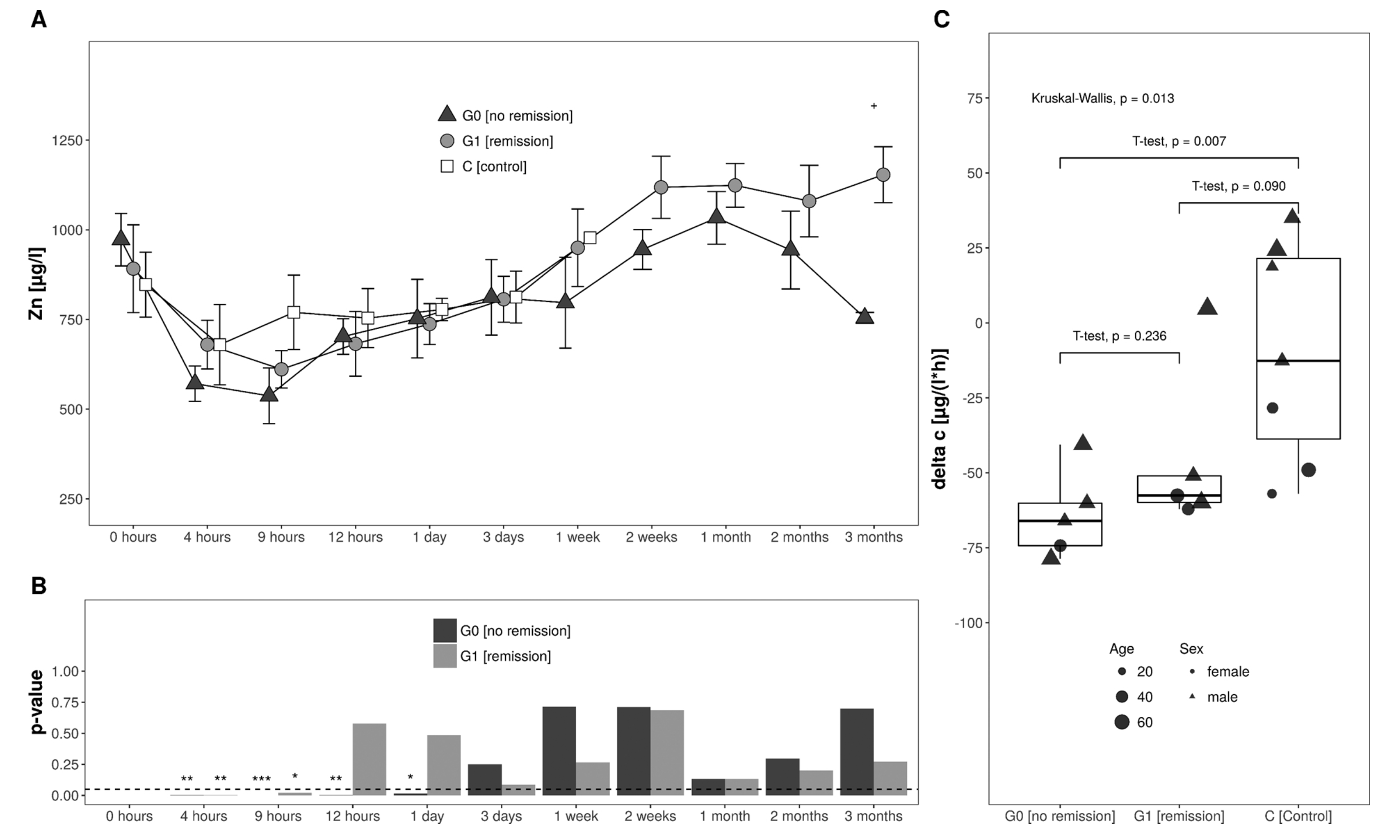

Fig. 2. Dynamic changes in serum Zn concentrations. Average Zn concentrations (mean ± SE) in the groups of patients with no remission (G0), remission (G1) and control show a dynamic pattern (A). In the first hours after admission, the groups display significant differences of Zn concentrations as compared to the initial value (B, p-values). Strongest differences in Zn concentrations are observed between the initial value and 9 h after admission (C). Test and p-values are indicated as follows: $*$, $p < 0.05$; $**$, $p < 0.01$; and $***$, $p < 0.001$. Normal distributions were assessed by Shapiro-Wilks Test followed by *t*-test analyses; (+) indicates Wilcoxon signed rank test analysis.

[27]. Whether the declining Se concentrations in blood are secondary to an impaired hepatic biosynthesis and secretion of SELENOP [28] or due to an increased demand by SELENOP-target tissues [29] remains to be determined.

Serum analyses of cytokines [9,30–32], chemokines [6,32], growth factors [9,33] and trace elements [34] may mirror neuro-regenerative processes during recovery. Therefore, we sought to investigate into the correlation of Se and SELENOP concentrations during TSCI and subsequent treatment and neurological remission. The findings of our study are intended to improve clinical risk assessment as a basis for discussing on whether a supplemental Se supply should be considered as a meaningful adjuvant treatment option in TSCI or not.

2. Patients, materials and methods

2.1. Study design

The clinical study was performed at the BG Trauma Centre Ludwigshafen, Department of Paraplegiology, Rhineland-Palatinate (Rheinland-Pfalz), Germany, according to the principles expressed in the Declaration of Helsinki in its current form. Patients were included after giving informed consent and willingness to participate in the study. Exclusion criteria were non-traumatic SCI, traumatic brain injury, severe abdominal trauma, traumatic amputation of extremities, coma and all patients with an additional major trauma apart from SCI. Participants were not given methylprednisolone sodium succinate during study participation. Collectively, blood samples were available from 28 patients (10 female, 18 male) affected by TSCI, between 2011 and 2016. Upon admission after injury (t1; t1 = 0 h after admission to the clinic) to the end of follow up after 3 months (t11; t11 = 3 m after admission), 11 blood samples per patient were taken at specific time points based on our standardized protocol [8,10] (Fig. 1). Immediately after drawing blood, samples were allowed to coagulate for 20 min and were then centrifuged at 3000 rmp, separated and stored at −80 °C. Our prospective observational study had been approved by the ethics committee of the University of Heidelberg (#S-514/2011) and the Landesärztekammer Rheinland-Pfalz (#837.188.12 / 8289-F), Germany. The study was registered in the German Clinical Trials Register DRKS (Study-ID: DRKS00009917/ Date of Registration: 23.03.2016/ Universal Trial Number (UTN): U1111-1179-1620). The database encompasses a total of 144 patients. Some samples are missing due to the complexity of the critical early interventions and due to loss during follow up.

Neurological remission was defined as positive conversion of AIS (Table 1) grades within 12 weeks after trauma. All ISNCSCI examinations were performed by the head physical therapist at the BG Trauma Center. Initial ISNCSCI examinations were performed within the first 72 h after admission [10]. The patients' demographics are given in Table 2.

Trace element analysis was carried out at an analytical lab remote from the study site, and by personnel blinded to the clinical characteristics of the serum samples. The measurements were performed by total reflection X-ray fluorescence (TXRF) using a benchtop TXRF device (PicoFox S2, Bruker nano, Berlin, Germany), with the protocol and assay characteristics described recently [35]. SELENOP concentrations were analyzed by an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (selenOtest™, ICI immunochemical intelligence GmbH, Berlin, Germany).

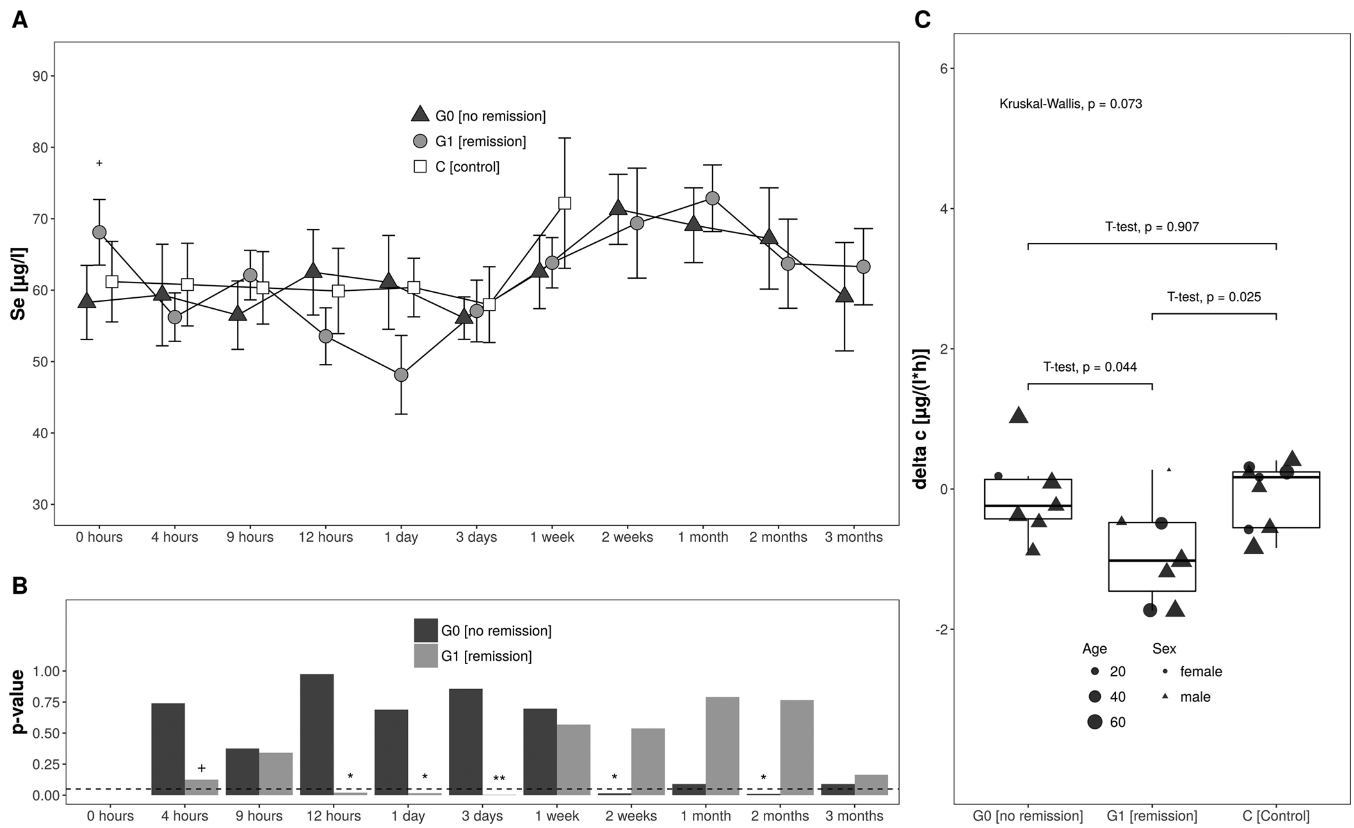

Fig. 3. Dynamic changes in serum Se concentrations. Average Se concentrations (mean ± SE) in the groups of patients with no remission (G0), remission (G1) and control show a dynamic pattern (A). Significant differences to the initial value do particularly develop in the remission group G1 (B). The differences become strongest 24 h after admission (C). Test and p-values are indicated as follows: ∗, $p < 0.05$; ∗∗, $p < 0.01$; and ∗∗∗, $p < 0.001$. Normal distributions were assessed by Shapiro-Wilks Test followed by *t*-test analyses; (+) indicates Wilcoxon signed rank test analysis.

2.2. Matching

Matching took place between the 144 patients based on the criteria age, sex, aetiology and AO classification as well as based on the neurological level of injury. In cases where more than one match was found for a case of non-remission, patients with the most comparable clinical data profile were chosen. According to an established protocol [8,10], we matched 10 patients with SCI and neurological remission (Group 1 = G1) with 9 patients without neurological remission (Group 0 = G0). Furthermore, 10 patients with an isolated vertebral fracture without neurological impairment served as a suitable and comparable control group (Group C=C).

2.3. Related work

The register has already been used to address certain scientific issues [6,30]. This study was performed based on the status of the register, and therefore different numbers of patients as well as different matched pairs of patients according to the inclusion and matching criteria were available. All samples were collected in a prospective approach, and some cases were enrolled in more than one study.

2.4. Statistical analysis

Location shifts between groups were assessed via parametric (T-Test) or non-parametric testing (Kruskal-Wallis Test, Man-Whitney-U Test and Wilcoxon signed-rank Test) at all timepoints according to the presence or absence of normal distribution assessed via Shapiro Wilks test. Categorial variables were evaluated using the Chi-square test.

Group differences between all subgroups were assessed via the non-parametric Kruskal-Wallis Test, whereas the non-parametric Man-Whitney-U Test assessed differences between two unpaired samples. Location shifts between paired samples were assessed using the Wilcoxon signed-rank Test if normal-distribution was not given. Otherwise the parametric *t*-test was used. Test and p-value are indicated. As this is an exploratory post-hoc analysis all p-values are to be interpreted descriptively. They are not adjusted for multiple testing. Statistical calculations were performed with R version 3.2.3 [36,37]. Figures were created using the package" ggplot2″ [38].

3. Results

The current study was designed as a prospective and explorative clinical observation study. Data analysis was performed as a matched pairs analysis. Matching criteria included age, sex, aetiology and AO-classification [39].

3.1. Demographics

A total of 29 patients were enrolled in the study consisting of 10 females and 19 males. The median age was 48.0 (IQR: 32.0, 59.0) years. Table 2 provides an overview on the demographics of the patients. No significant differences were present regarding age, gender, aetiology, AO classification or NLI between patients with and without neurological remission (referred to as G1 and G0, respectively).

All patients enrolled in G0 and G1 were initially treated surgically within 24 h after admission and underwent laminectomy. All 29 patients in G0, G1 and C were affected by vertebral fractures. The distribution of AIS grades at discharge ($p = 0.002$) differed significantly between G0 and G1 (Table 2).

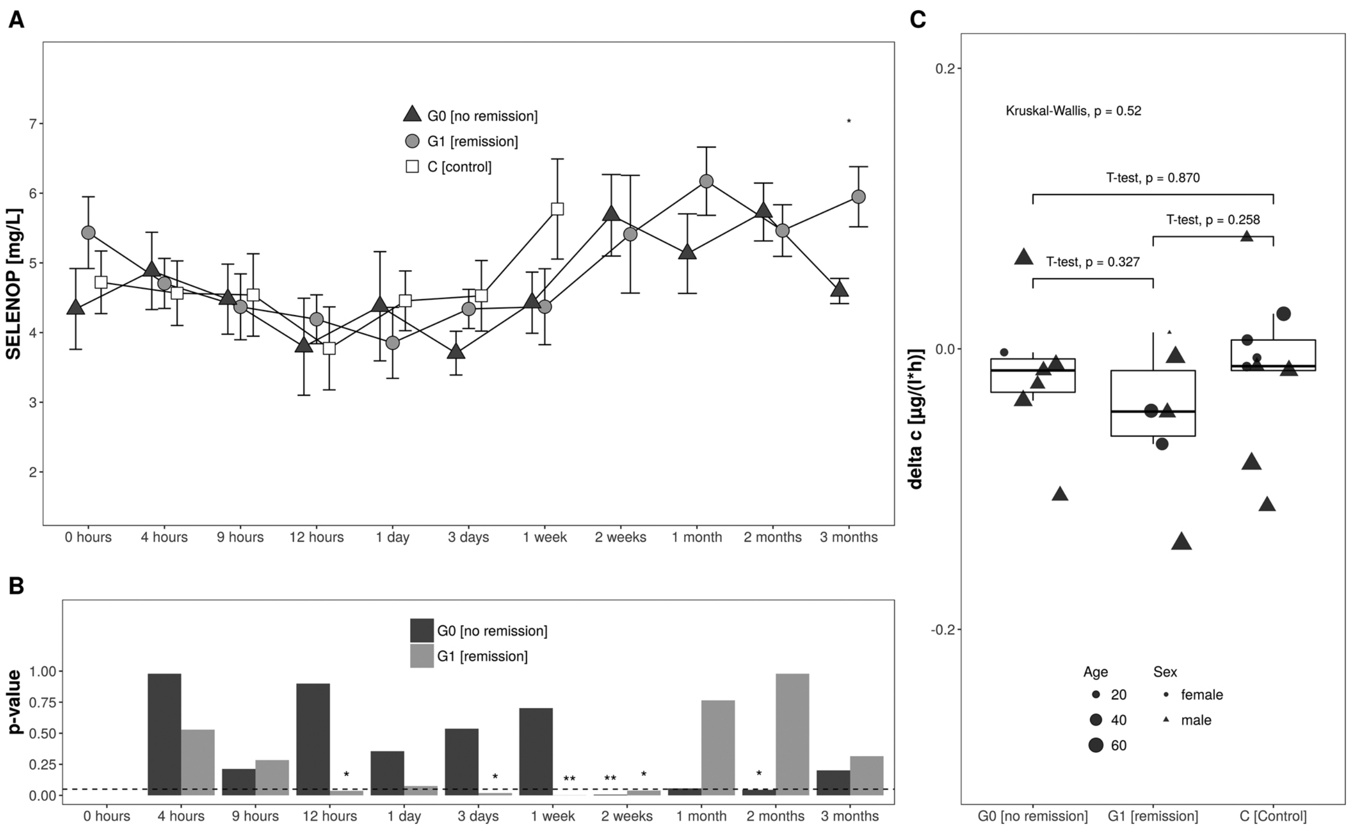

Fig. 4. Dynamic changes in serum SELENOP concentrations. Average SELENOP concentrations (mean ± SE) in the groups of patients with no remission (G0), remission (G1) and control show a dynamic pattern (A). Significant differences to the initial value do particularly develop in the remission group G1 from 3 d to 2 weeks after admission (B). The SELENOP concentrations at 24 h after admission do not differ significantly (C). Test and p-values are indicated as follows: ⁎, $p < 0.05$; ⁎⁎, $p < 0.01$; and ⁎⁎⁎, $p < 0.001$. Normal distributions were assessed by Shapiro-Wilks Test followed by *t*-test analyses; (+) indicates Wilcoxon signed rank test analysis.

3.2. Serum analysis and comparison of the remission (G1) and no-remission (G0) group

Dynamic serum patterns of serum Zn, Se and SELENOP concentrations were determined (Figs. 2–4). Part A indicates the serum concentrations in mean ± SE. Part B shows the group differences of each timepoint compared to the initial value estimated via the p-value (Wilcoxon signed rank test indicated with "+", otherwise via *t*-test if normal distribution was given). Part C analyses the difference between the initial value and the corresponding timepoint within the first 24 h where the biggest difference is detected.

3.3. Zinc

Fig. 2 indicates the dynamic pattern of serum Zn concentrations in the three groups of patients. Zinc decreased initially in all groups followed by a constant increase from 9 h to 1 month. Thereafter mean Zn levels showed a discordant development in G1 versus G0, and a significant difference was detected at 3 months after admission.

3.4. Selenium and Selenoprotein-P

Fig. 3 shows Se and Fig. 4 presents the SELENOP concentrations. During the observational period of 3 months, mean serum levels of Se and SELENOP initially declined reaching their minimum 24 h after admission in G1. In G0 both parameters showed little variation in the first seven days after admission. After 1 week, Se and SELENOP concentrations increased in both G0 and G1 and remained elevated as compared to admission.

3.5. Dynamics within the first 24 h

The highest delta during the first 24 h is calculated by subtracting the lowest value of each patient from the corresponding value at admission. The results are shown in part C of Figs. 2–4, indicating that changes in the Se concentration differ significantly 24 h after admission (Se 24 h: $p = 0.044$). SELENOP and Zn concentrations showed no significantly different dynamics during the first 24 h in G0 and G1 (results at maximal deviations from admission: SELENOP 24 h: $p = 0.327$; Zn 9 h: $p = 0.236$).

3.6. Binary logistic model

A binary logistic regression model was set up to evaluate the diagnostic value of the dynamic changes in Se, SELENOP and Zn concentrations within the first 24 h as a tool for prediction of neurological remission. Fig. 5 presents the sample size (n), Akaike Information Criterion (AIC) [40], Bayesian Information Criterion (BIC) [41], and Area under the Curve (AUC) for the relation of concentration changes to outcome within the first 24 h in an univariate model (Model 1, Model 2 and Model 3) and combined in a multivariate analysis (Model 4 and Model 5). With respect to the AIC the AUC indicated that the combined Model 5 provided the overall best predictive performance. The ROC curve for the favoured logistic regression model is shown in Fig. 6 and presented a value of 90.0% (CI: 67.4%–100.0%), while the model specifics are given in Table 3. The ROC analysis provides additional deeper insight into the predictive capacity of the variables as the adjusted odds ratios (Table 3) are strongly differing from 1 (Se: 0; SELENOP: 0.05).

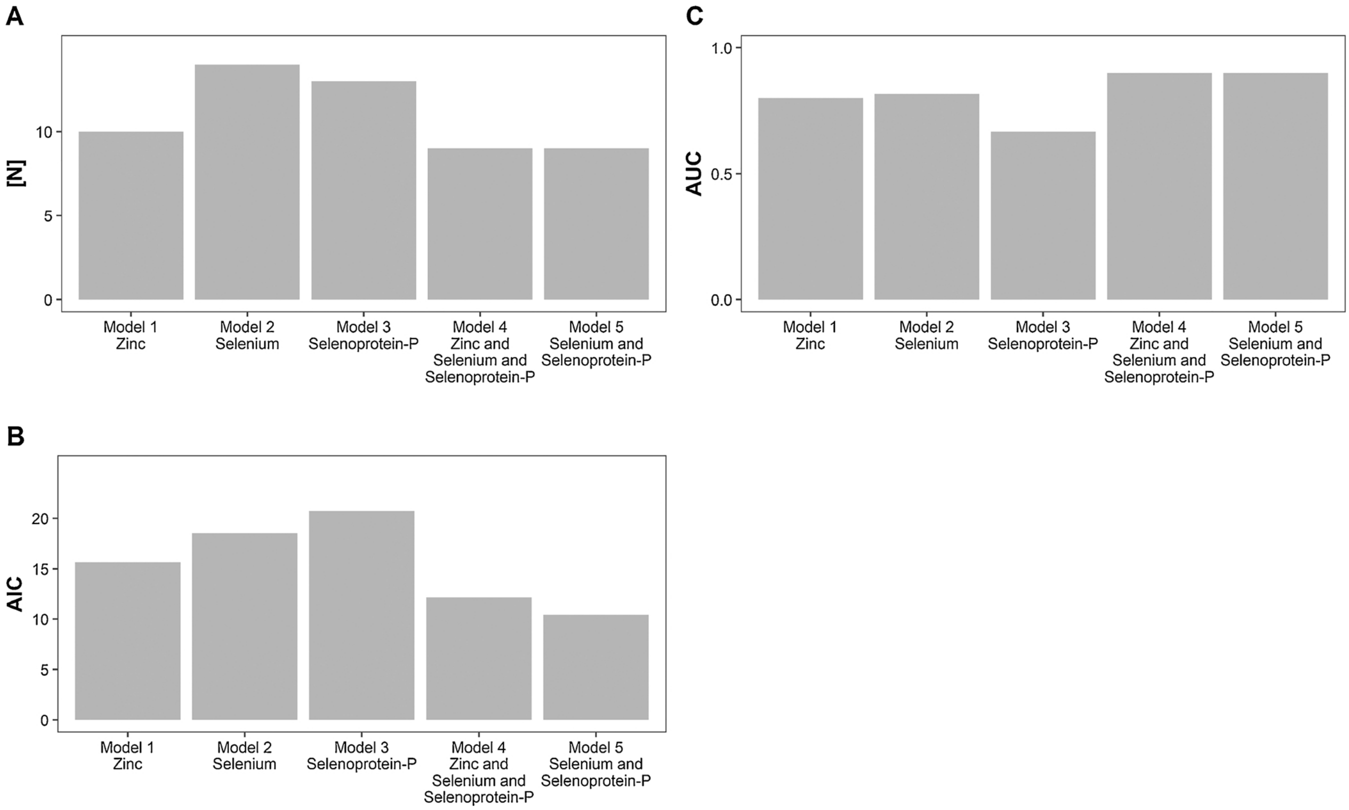

Fig. 5. Model characteristics for univariate (Model 1, Model 2 and Model 3) and multivariate binary logistic regression analysis (Model 4 and Model 5). The Figure displays the included number of complete cases (A), the resulting models Akaike Information Criterion (AIC) (B), the Bayesian Information Criterion (BIC) (C) and the corresponding Area under the Curve (AUC) of the Receiver Operator Characteristics analysis (ROC) with respect to remission (D).

4. Discussion

The current study analysed the dynamic concentration patterns of Se, SELENOP and Zn in relation to the clinical outcome after TSCI to evaluate their potential as prognostic biomarkers for neuroregeneration. Serum samples from patients were obtained during the acute, subacute and intermediate phases subsequent to TSCI. The first 24 h are of particular interest and relevance for clinicians [42], therefore blood samples were initially obtained at a higher rate. Our database covers 144 patients enrolled from 2011 to 2016, and enabled us to perform a matched-pair analysis.

Concentration patterns of Zn provided no significant information regarding the presence or absence of neurological remission after 3 months. These findings were in contrast to our expectations and question the need for supplemental Zn as an adjuvant treatment option for SCI [43,44]. In contrast, the analysis of the Se biomarkers total serum Se and SELENOP concentrations may offer the perspective of utilizing their changes for predicting regeneration success in the context of TSCI. The Se and SELENOP data are in agreement with results from previous studies investigating the relevance of Se in the context of traumatic brain injury (TBI) [45,46], ALS [47,48] and SCI [17,49,50] confirming the positive association of Se status with a favourable clinical outcome after central nervous tissue injury. Previous animal experiments also attempted to pharmacologically reduce lipid peroxidation during the anterograde degeneration process via pre-treatment of cats with a combination of the antioxidants α-tocopherol (200 IU) and Se (50 μg) once daily for 5 days (p.o.). The results indicated the potential to significantly diminish the anterograde degeneration process by supplemental antioxidants or trace elements like Se that are supporting the biosynthesis of endogenous enzymatic antioxidative defense systems [17]. Confirming results have been obtained by Mitchell et al. in 1986 suggesting that free radicals are of prime importance in motor neuron disease [50]. Similarly, Chen et al. reported that Se-enriched supplements provided some protection in SCI through up-regulating the expression of ciliary neurotrophic factor (CNTF) and its receptor-α (CNTF-Rα) [49]. As Se-related effects are exerted mainly through changes in the biosynthesis of selenoproteins, it can be assumed that a sufficiently high Se status at the time of injury contributes to a better expression of antioxidative enzymes, to an improved and timely adapted immune response and a more beneficial clinical outcome.

This assumption is supported by the results of our study due to higher Se concentrations in G1 as compared to G0 at admission. The observed significant decline of serum Se concentrations in patients with a beneficial neurological remission may be explained by an increased demand of actively regenerating tissue for selenoprotein biosynthesis and might thus present a surrogate marker of the repair process. And inversely, the absence of changes in Se status after TSCI may indicate an insufficient tissue repair activity, likely associated with a failing regeneration process. If these interpretations of the study data proved reproducible in larger follow-up studies, active Se supplementation after injury may constitute a promising adjuvant treatment option for supporting selenoprotein biosynthesis at the site of injury. In addition, such an intervention would increase hepatic SELENOP production as a readily available circulating Se transport form directly available to uptake by the injured tissue for increasing intracellular selenoprotein expression and supporting the regenerative process. Similar strategies using supplemental Se have been tested in patients with sepsis [51] or undergoing cardiac surgery [52], providing a useful blueprint for the underlying kinetics and suitable dosages of Se supplementation.

Further studies are needed to better evaluate the predictive potential of Se and SELENOP as biomarkers of regeneration odds, and to test the clinical potential of adjuvant Se supplementation to injured patients with proven low Se status to support their repair processes and increase

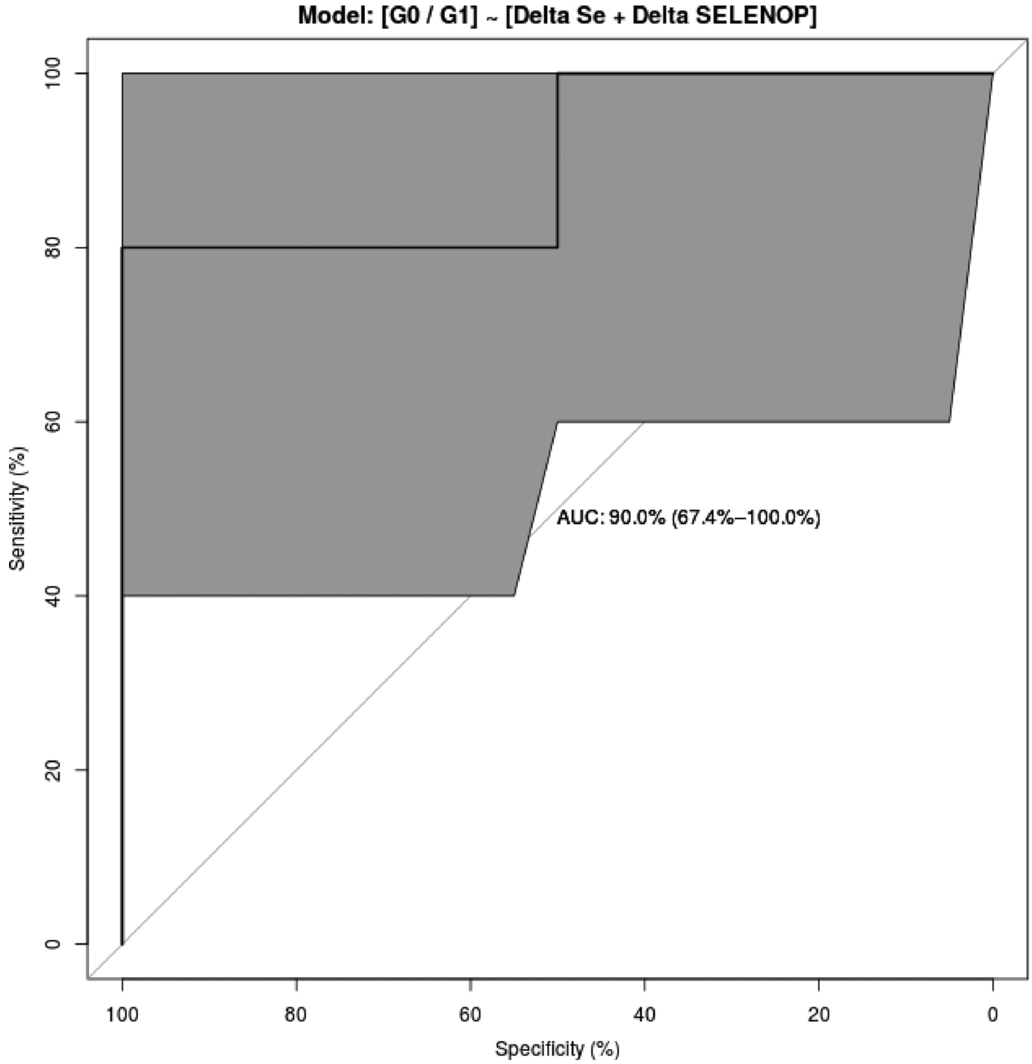

Fig. 6. ROC (Receiver Operator Characteristic) curve analysis of the favoured multivariate Model 5 indicating its AUC (Area under the Curve) and the corresponding confidence interval (CI) for predicting remission as a function of changes in serum Se and SELENOP concentrations during the first 24 h after admission.

Table 3
Specifics of the predictive model.

Variable	crude OR (95%CI)	adjusted OR (95%CI)	P (Wald's test)	P (LR-test)
Delta Se	0.05 (0,2.4)	0 (0,392.3)	0.343	0.01
Delta SELENOP	0.45 (0.1,2.1)	0.05 (0,124.5)	0.448	0.245

the likelihood of regeneration.

4.1. Limitations

Our study is limited by the relatively small patient size due to the infrequency of this debilitating injury in a single study unit. In this explorative study we were only able to enrolling patients with slightly differing initial AIS into both the remission and non-remission group. Differences between the patients in these groups may thus contribute to possible misinterpretation. Therefore, the current data and conclusions need to be interpreted with care and require a verification in larger studies.

4.2. Strengths

This study provides a detailed overview regarding the dynamics of changes in serum Se, SELENOP und Zn concentrations during the post-traumatic period. Two important biomarkers of Se status were evaluated in parallel at a remote lab from the clinics, thereby reducing experimental bias of our analyses. Moreover, despite the rarity of this disease, the data and biobank that were successfully established allowed an effective matching of the groups. Moreover, the frequent blood drawings and standardized asservation of samples allowed a detailed time-resolved analysis of several diagnostic parameters.

5. Conclusion

High normal Se levels may contribute to a positive outcome after traumatic spinal cord injury (TSCI). The dynamic changes of serum Se and SELENOP concentrations within the first 24 h of admission were translated into a promising tool for predicting neurological remission with an AUC of 90.0% in the ROC analysis. These results support the importance of trace elements in severe diseases and support strategies considering an adjuvant Se supplementation during therapy. Larger studies and intervention trials are now needed to evaluate whether supplemental Se contributes to recovery and neurological remission after severe trauma.

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Conflict of interest

RAH, JS, TB, PH, PAG, AM and BB have nothing to declare in relation to this study. LS holds shares in selenOmed GmbH, a company involved in Se status assessment and supplementation.

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