ORIGINAL ARTICLE - SPINE TRAUMA

Glial fibrillary acidic protein is a robust biomarker in cerebrospinal fluid and peripheral blood after traumatic spinal cord injury: a prospective pilot study

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Abstract

Purpose Biochemical biomarkers to determine the injury severity and the potential for functional recovery of traumatic spinal cord injury (TSCI) are highly warranted; however, it remains to be clarified whether cerebrospinal fluid (CSF) or peripheral blood (PB) is the ideal sample media. This study aims to measure and compare biomarker concentrations in CSF and PB and to explore associations between biomarker concentrations and injury severity, i.e., American Spinal Injury Association (ASIA) Impairment Scale (AIS) grade, and biomarker concentrations and clinical outcome, i.e., AIS grade improvement and Spinal Cord Independent Measure version III (SCIM-III) score.

Methods From 2018 to 2020, we conducted a single-center prospective pilot study of TSCI patients (*n*=15) and healthy controls (*n*=15). Sample collection and clinical outcome assessment were performed at median 13 h [IQR: 19], 9 days [IQR: 2], and 148 days [IQR: 49] after TSCI. Concentrations of neuron-specific enolase (NSE); glial fibrillary acid protein (GFAP); neurofilament light chain (NfL); interferon-γ (IFN-γ); interleukin (IL)-1ß, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, and IL-13; and tumor necrosis factor α (TNF-α) were measured and associated to clinical outcomes.

Results The biomarker concentrations were higher in CSF than PB. CSF concentrations of GFAP, NSE, IFN-y, TNF-a, IL-2, IL-12p70, IL-4, IL-10, and IL-13 and PB concentrations of GFAP and IFN-y were significantly associated with AIS grade, but not with AIS grade improvement or SCIM-III score.

Conclusions Our results support GFAP as a potential diagnostic biomarker that may be measured in CSF as well as PB.

Keywords Traumatic spinal cord injury · Inflammation · Biomarkers · Blood · Cerebrospinal fluid

Introduction

Traumatic spinal cord injury (TSCI) is a heterogenic condition with limited options for determining correct diagnosis and prognosis [24, 34]. The American Spinal Injury Association (ASIA) Impairment Scale (AIS) grade is the current standard for diagnostic assessment of

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Previous presentations.

Some of the data has been published elsewhere, but the data is presented in new analyses in the current manuscript.

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injury severity. It is also used for assessment of functional recovery, although not intended for this purpose [35]. Several limitations are inherent to the AIS grade [5, 16, 20], and supplements or substitutes for diagnostic assessment of injury severity and assessment of functional recovery are warranted. Extensive research in the field of biochemical biomarkers has therefore been conducted striving to refine and improve diagnostic and prognostic assessment [3, 38]. Biochemical biomarkers that reflect aspects of the underlying pathophysiological processes can provide valuable diagnostic and prognostic information. Biomarkers of the structural spinal cord injury and the inflammatory response are released into the cerebrospinal fluid (CSF) and the peripheral blood (PB); though the pathways of biomarker release into PB are poorly understood. It is therefore questionable if biomarker concentrations in PB mirror the degree of spinal cord damage, and thereby the

injury severity or the potential for functional recovery [45], to the same extent as biomarker concentrations in CSF. Still, biomarkers measured in PB have considerable clinical advantage over CSF [14, 25]. Among the most studied biochemical biomarkers are cytokines, glial fibrillary acidic protein (GFAP), neurofilament (Nf), and neuron-specific enolase (NSE) [2, 8, 9, 11–13, 15, 17, 18, 23, 25, 26, 28, 30, 32, 33, 37]. Yet, results are conflicting, and very few studies have investigated selected proteins in both CSF and PB [25, 37]. The purpose of this study was to (1) measure biomarker concentrations in CSF and PB to assess the utility of PB for biomarker measurement and to (2) explore any correlation between protein concentrations and clinical measures to assess the diagnostic and prognostic utility of CSF and PB biomarkers.

Methods

Study population

TSCI patients were recruited between 2018 and 2020. Patients above 18 years of age with acute blunt TSCI were considered eligible if they met the following inclusion criteria: (1) TSCI at level of C1/L1, (2) Glasgow Coma Scale 15, and (3) AIS grades A–D upon admission. Patients were excluded if they had prior surgery at the level of TSCI, major co-injuries including traumatic brain injury, major co-morbidities including immunological and neurological diseases, immunosuppressive treatment, penetrating TSCI, >72 h from TSCI to sample collection, substance abuse, or mental diseases preventing compliance with the study. A reference group of healthy controls without neurological diseases scheduled for elective hip surgery in spinal anesthesia were recruited in 2020.

CSF and PB collection

Samples of CSF and PB were collected from TSCI patients at three follow-ups and healthy controls at one follow-up. We used the methods for sample collection, handling, and storage as described in previous studies [41, 42].

Clinical outcome

Clinical examination was performed by the AIS grade at the three follow-ups parallel to sample collection [35]. Additional clinical examination by the SCIM-III score was performed at the third follow-up [20]. Clinical examinations were conducted by trained physicians or physiotherapists.

Biochemical analyses

Roche Cobas 8000 Modular Analyzer (Roche Diagnostics International AG, Rotkreuz, Switzerland) was used to measure CSF and serum concentrations of NSE (µl/L). The clinical laboratory has a reference interval for NSE <17.3 µl/L. As serum concentrations of NSE for healthy controls were influenced by pre-analytical hemolysis, only CSF concentrations of NSE were considered for further analyses. The Neurology 2-plex B assay (Quanterix, Billerica, USA) read on Simoa HD-1 platform was used according to the manufacturer's instructions to measure CSF and plasma concentrations of GFAP and NfL (pg/mL). The limit of detection for this assay is 0.065 pg/mL for NfL and 0.457 pg/mL for GFAP. The upper limit of the standard curves was 473 pg/ml for NfL and 8418 pg/mL for GFAP. Controls at two levels (NfL at 5.7 pg/mL and 1880 pg/mL, GFAP at 94 pg/mL and 12434 pg/mL) were used, and the intermediate precisions at these levels were determined for NfL (9.4% and 9.9%, respectively) and GFAP (8.1% and 8.4%, respectively). Plasma was analyzed at four times dilution, and those exceeding the standard curve were reanalyzed in a 100-fold dilution. CSF was analyzed at 100-fold dilution and reanalyzed at 1000 and 10.000 dilution if exceeding the standard curve. Samples exceeding the standard curve at 10.000-fold dilution were assigned a value of 10.000 times the upper standard. V-PLEX Proinflammatory Panel 1 Human Kit (Meso Scale Discovery, Inc.) read on MESO QuickPlex SQ 120 was used according to the manufacturer's instructions to measure CSF and plasma concentrations (pg/mL) of interferon-y (IFN-y); interleukin (IL)-1ß, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, and IL-13; and tumor necrosis factor α (TNF-α). Concentrations below detection limit were assigned with half the value of the lower limit of detection (LLOD) as provided by the manufacturer. Values for IL-8 were above the higher standard of the standard curve in 4 TSCI patients. These are reported as measured. All samples were batch analyzed as single measurements by a certified laboratory technician.

Statistical analyses

Concentrations of NSE, GFAP, and NfL were compared between TSCI patients and healthy controls by a mixed regression model. Kenward-Roger method was used to calculate the degrees of freedom [22]. The model assumptions were examined by inspection of QQ plots for the residuals and scatter plots of the residuals versus fitted values. Few proteins were log-transformed to fulfill the model assumptions. Marginal medians with 95% CI and comparisons between groups were tested using post

hoc analyses following the model. Regression analyses were used to test the association between biomarker concentrations and AIS grades at the first follow-up, the association between biomarker concentrations and AIS grade improvement at the third follow-up, and the association between biomarker concentrations and SCIM-III scores at the third follow-up. *p*-values ≤0.05 were considered statistically significant. All statistical analyses were conducted in Stata/IC 16.0 (StataCorp LP, College Station, TX) by the Biostatistical Advisory Service, and graphical presentations were performed using GraphPad Prism version 9.3.1 for MacOS (GraphPad Software, San Diego, CA, USA).

Results

Study population

A total of 30 patients (15 TSCI patients and 15 healthy controls) were included. Follow-up was performed at median 13 h [IQR: 19], 9 days [IQR: 2], and 148 days [IQR: 49] following TSCI. Four TSCI patients withdrew their informed consent after the first follow-up. Details on patient characteristics are provided in Table 1. All TSCI patients underwent primary decompressive and stabilizing spine surgery. AIS grades were A (*n*=6), B (*n*=2), C (*n*=4), and D (*n*=3) at the first follow-up, and AIS grade improvement was observed in 4 TSCI patients at the third follow-up. No AIS grade deterioration was observed. Mean total SCIM-III score was 59 ±42 at the third follow-up.

Structural biomarker dynamics

Concentrations of GFAP, NSE, and NfL in CSF were significantly increased at the first and second follow-up compared to healthy controls. Only CSF concentrations of NfL remained significantly increased throughout the study period. The CSF concentrations of GFAP and NSE were highest at the first follow-up, while the CSF concentrations of NfL were highest at the second follow-up (Fig. 1). Similar findings were found for NfL and GFAP in PB (Fig. 2). The greatest increase in CSF and PB was found for GFAP.

Inflammatory biomarker dynamics

Concentrations of all cytokines have been reported and graphical presented previously [42]. Concentrations of cytokines in CSF were significantly increased at the first and second follow-up compared to healthy controls. Only CSF concentrations of IL-4, IL-8, and TNF-α remained significantly increased throughout the study period. Concentrations of IL-6, IL-8, and IL-10 were significantly

increased, and concentrations of IFN-γ were significantly decreased at the first follow-up. Only concentrations of IL-4 were significantly different at the third follow-up.

Biomarker concentrations and AIS grade

Biomarker concentrations measured at the first follow-up were compared to simultaneous measured AIS grades (Table 2 and Table 3). Results showed that CSF concentrations of GFAP (*p*=0.024), IFN-y (*p*=0.009), TNF-α (*p*=0.032), IL-2 (*p*=0.034), IL-12p70 (*p*=0.028), IL-4 (*p*=0.027), IL-10 (*p*=0.018), and IL-13 (*p*=0.032) are associated with AIS grade. There was no statistically significant association between AIS grades and NfL (*p*=0.116), IL-6 (*p*=0.085), and IL-8 (*p*=0.062). Only PB concentrations of GFAP (*p*=0.030) and IFN-y (*p*=0.026) were statistically significant associated with AIS grade. There was no statistically significant association between AIS grades and the remaining biomarkers.

Biomarker concentrations and AIS grade conversion

Biomarker concentrations at the first follow-up were compared to AIS grade improvement at the third follow-up (Table 2 and Table 3). Neither the CSF concentrations nor the PB concentrations of NSE, GFAP, NfL, IL-1ß, IL-6, IL-8, IFN-y, TNF-α, IL-2, IL-12p70, IL-4, IL-10, and IL-13 were significantly different between those who remained at the same AIS grade and those who improved. IL-1ß was omitted from analysis in PB as many concentrations were below LLOD.

Biomarker concentrations and SCIM-III score

Biomarker concentrations at the first follow-up were compared to SCIM-III scores at the third follow-up (Table 2 and Table 3). None of the biomarker concentrations in CSF were significantly associated with SCIM-III scores, i.e., NSE, GFAP, NfL, IL-1ß, IL-6, IL-8, IFN-y, TNF-α, IL-2, IL-12p70, IL-4, IL-10, and IL-13. Similarly, none of the biomarker concentrations in PB were significantly associated with SCIM-III scores, i.e., NSE, GFAP, NfL, IL-1ß, IL-6, IL-8, IFN-y, TNF-α, IL-2, IL-12p70, IL-4, IL-10, and IL-13. Again, IL-1ß was omitted from analysis as many concentrations were below LLOD.

Discussion

Our study uncovers the trajectory of structural and inflammatory biomarkers in CSF and PB after TSCI. We observed that several inflammatory biomarkers in CSF are associated with injury severity, whereas only PB IFN-y

Table 1 Characteristics of the study population

Sex	Age	Group	Mechanism of injury	Level of injury	AIS grade at first follow-up	AIS grade at second follow-up	AIS grade at third follow-up	SCIM-III score at third follow-up	Hours from injury to first follow-up	Days from injury to second follow-up	Days from injury to third follow-up
Male	70	TSCI	Fall	Cervical	AIS B	AIS C	AIS C	18	6.5 h	7 days	199 days
Male	24	TSCI	Fall	Thoracic	AIS A	AIS A	AIS A	63	6.5 h	9 days	184 days
Male	62	TSCI	Traffic accident	Thoracic	AIS A				6.5 h		
Male	48	TSCI	Traffic accident	Cervical	AIS C	AIS C	AIS D	68	8.5 h	8 days	160 days
Male	32	TSCI	Traffic accident	Cervical	AIS C	AIS C	AIS D	98	40 h	9 days	122 days
Female	70	TSCI	Fall	Cervical	AIS B	AIS B	AIS B	18	35 h	9 days	196 days
Female	29	TSCI	Traffic accident	Thoracic	AIS C				19 h		
Female	24	TSCI	Fall	Cervical	AIS A				67 h		
Male	52	TSCI	Traffic accident	Cervical	AIS A	AIS A	AIS A	0	11.5 h	7 days	137 days
Male	59	TSCI	Traffic accident	Cervical	AIS D	AIS D	AIS D	100	39 h	10 days	148 days
Female	56	TSCI	Traffic accident	Cervical	AIS C	AIS C	AIS D	83	6 h	12 days	125 days
Male	21	TSCI	Fall	Cervical	AIS A				16.5 h		
Male	59	TSCI	Sports	Cervical	AIS D	AIS D	AIS D	100	16 h	11 days	119 days
Male	75	TSCI	Fall	Cervical	AIS D	AIS D	AIS D	97	11.5 h	8 days	149 days
Male	72	TSCI	Fall	Thoracic	AIS A	AIS A	AIS A	30	13 h	10 days	97 days
Male	52	Healthy									
Female	38	Healthy									
Female	69	Healthy									
Female	34	Healthy									
Male	64	Healthy									
Female	27	Healthy									
Male	18	Healthy									
Female	78	Healthy									
Female	80	Healthy									
Male	51	Healthy									
Male	66	Healthy									
Male	82	Healthy									
Female	39	Healthy									
Male	62	Healthy									
Male	40	Healthy									

AIS American Spinal Injury Association Impairment Scale, *SCIM-III* Spinal Cord Independence Measure version III, *TSCI* traumatic spinal cord injury
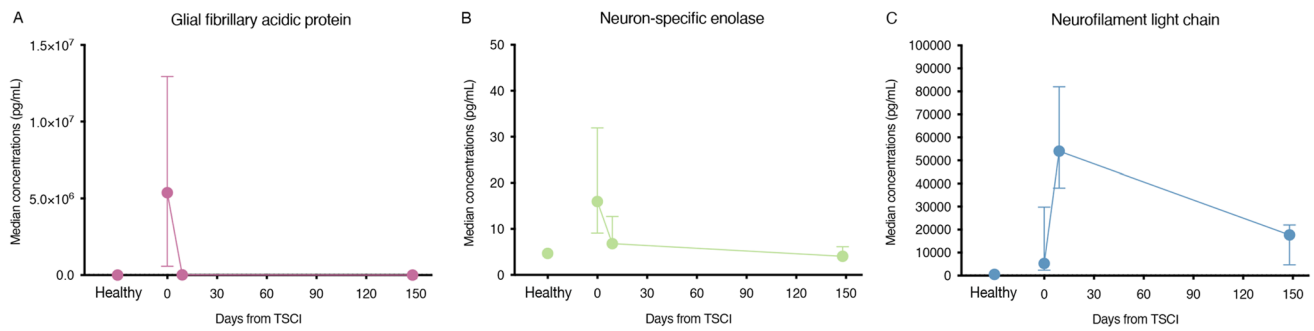
Fig. 1 Graphical presentation of **A** glial fibrillary acidic protein (GFAP), **B** neuron-specific enolase (NSE), and **C** neurofilament light chain (NfL) concentrations in cerebrospinal fluid from non-traumatic spinal cord injury (TSCI) patients and TSCI patients. All concentrations are presented as medians with interquartile range. As the concentrations reached different values, the protein concentrations are presented on different scales. Compared to healthy controls, the concentrations of GFAP and NSE were highest at first follow-up, notably the concentrations of GFAP. At second and third follow-up, the concentrations of GFAP decreased; however, NSE remained slightly increased. The concentrations of NfL from TSCI patients did not reveal the same expression pattern as observed of GFAP and NSE, and the concentrations of NfL were increased through the study period with highest concentrations at second follow-up
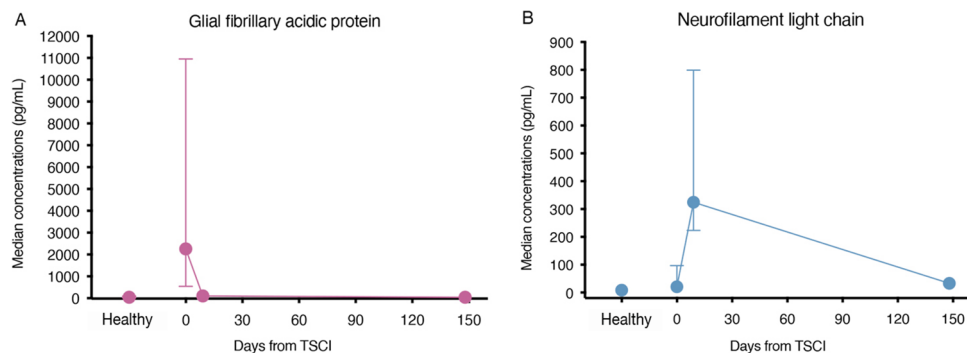

Fig. 2 Graphical presentation of **A** glial fibrillary acidic protein (GFAP) and **B** neurofilament light chain (NfL) concentrations in peripheral blood from non-traumatic spinal cord injury (TSCI) patients and TSCI patients. All concentrations are presented as medians with interquartile range. As the concentrations reached different values, the proteins concentrations are presented on different scales. Compared to healthy controls, the concentrations of GFAP were highest at first follow-up with concentrations in some patients reaching high values. At second and third follow-up, the concentrations of GFAP decreased to concentrations resembling healthy controls. The concentrations of NfL from TSCI patients did not reveal the same expression pattern as observed of GFAP, and the concentrations of NfL were increased through the study period with highest concentrations at second follow-up

demonstrated this association. Among the structural biomarkers, only GFAP were associated with injury severity in CSF as well as PB. None of the investigated biomarkers were associated with clinical outcome 148 days after injury.

Biomarker concentrations decrease with time

It is critical to understand the dynamics of biomarkers, i.e., the time to reach peak concentrations and the half-life to evaluate the clinical potential of biomarkers. Our results showed that protein concentrations in CSF and PB decline over time after TSCI with only concentrations of NfL remaining significantly high throughout the study period. Contrary to the other proteins, concentrations of NfL were highest at the second follow-up, which may challenge the diagnostic utility of NfL. Yet, the prognostic utility of NfL

might be improved by waiting day or weeks to measure the concentration of all the NfL that has been released. Only few studies measure protein concentrations beyond the earliest time after injury [6, 8, 9, 11, 15, 25, 26, 31, 36], and when they do, they use either PB or CSF samples [12, 15, 18, 19, 31, 36]. This hampers the cross-comparison between studies.

Few biomarkers are associated to clinical outcome

Although being the most studied proteins in TSCI studies, none of the measured proteins are specific for TSCI. GFAP is found in the cytoskeleton of glial cells [2]; NfL is found in the cytoskeleton of axons [44]; NSE is found in the cytoplasm of neuronal cells, neuroendocrine cells, platelets, and erythrocytes [44]; and inflammatory proteins are found throughout the body. Our results showed that GFAP was

Table 2 Association between biomarkers in cerebrospinal fluid and clinical measures

	AIS grade at day 0			AIS grade conversion at day 148			SCIM-III score at day 148		
	Coef	[95% CI]	p	OR	[95% CI]	p	Coef	[95% CI]	p
NSE	−0.015	[−0.030; −0.0012]	0.035*	1.22	[0.92; 1.61]	0.153	−0.506	[−2.05; 1.03]	0.478
GFAP	−2.43e-08	[−4.49e-08; −3.68e−09]	0.024*	1.00	[0.99; 1.00]	0.272	−7.80e−07	[−3.19e−06; 1.63e−06]	0.483
NfL	−3.12e-07	[−7.11e-07; 8.79e−08]	0.116	1.00	[0.99; 1.00]	0.301	−0.001	[−0.003; 0.001]	0.219
IL-6	−0.0003	[−0.0006; 0.0001]	0.085	1.00	[0.99; 1.01]	0.437	−0.0163	[−0.103; 0.070]	0.681
IL-8	−0.0001	[−0.0002; 8.56e−06]	0.062	1.00	[0.99; 1.00]	0.568	−0.0015	[−0.008; 0.005]	0.615
IFN-y	−0.1019	[−0.174; −0.029]	0.009*	1.06	[0.88; 1.29]	0.502	−0.9979	[−4.60; 2.60]	0.546
TNF-a	−0.087	[−0.166; −0.008]	0.032*	1.07	[0.88; 1.30]	0.481	−1.01	[−4.53; 2.50]	0.532
IL-2	−0.086	[−0.165; −0.007]	0.034*	1.07	[0.87; 1.31]	0.482	−0.731	[−4.32; 2.86]	0.656
IL-12p70	−0.091	[−0.172; −0.011]	0.028*	1.20	[0.74; 1.93]	0.445	−2.11	[−10.7; 6.53]	0.593
IL-4	−0.340	[−0.637; −0.044]	0.027*	2.10	[0.27; 16.3]	0.474	−11.5	[−49.0; 25.9]	0.503
IL-10	−0.182	[−0.327; −0.036]	0.018*	1.27	[0.76; 2.10]	0.355	−2.02	[−8.86; 4.82]	0.521
IL-13	−0.040	[−0.076; −0.004]	0.032*	1.02	[0.944; 1.11]	0.523	−0.410	[−2.07; 1.25]	0.591

*Statistical significance is designated at $p \leq 0.05$

AIS American Spinal Injury Association Impairment Scale, *SCIM-III* Spinal Cord Independence Measure version III, *Coef* coefficient, *95% CI* 95% confidence interval, *OR* odds ratio

Table 3 Association between biomarkers in peripheral blood and clinical measures

	AIS grade at day 0			AIS grade conversion at day 148			SCIM-III score at day 148		
	Coef	[95% CI]	p	OR	[95% CI]	p	Coef	[95% CI]	p
GFAP	0.00004	[−0.0001; −4.66e-06]	0.030*	1.00	[0.99; 1.00]	0.644	−0.0008	[−0.002; 0.001]	0.365
NfL	−0.003	[−0.01; 0.0006]	0.054	1.08	[0.90; 1.30]	0.395	−0.05	[−0.19; 0.09]	0.409
IL-6	−0.03	[−0.09; 0.25]	0.248	1.08	[0.92; 1.26]	0.314	−1.14	[−3.62; 1.35]	0.328
IL-8	−0.07	[−0.15; 0.01]	0.075	0.99	[0.85; 1.15]	0.938	−2.11	[−5.04; 0.81]	0.136
IFN-y	0.37	[0.05; 0.68]	0.026*	1.09	[0.56; 2.11]	0.806	10.3	[−2.37; 22.9]	0.09
TNF-a	−0.28	[−1.52; 0.96]	0.636	0.87	[0.11; 6.94]	0.903	−16.4	[−61.7; 28.3]	0.428
IL-2	−0.01	[−1.56; 1.54]	0.994	0.18	[0.01; 3.06]	0.233	10.2	[−45.3; 65.7]	0.687
IL-12p70	0.29	[−1.19; 1.78]	0.676	0.69	[0.07; 6.40]	0.752	9.07	[−40.8; 58.9]	0.690
IL-4	−0.14	[−7.48; 7.18]	0.966	9.77	[0.00; 1387356]	0.706	60.5	[−184.0; 305.1]	0.589
IL-10	−0.04	[−0.14; 0.07]	0.422	0.99	[0.84; 1.17]	0.961	−0.73	[−4.5; 2.97]	0.665
IL-13	0.03	[−0.69; 0.76]	0.920	0.67	[0.21; 2.20]	0.516	11.5	[−12.8; 35.8]	0.312

*Statistical significance is designated at $p \leq 0.05$

AIS American Spinal Injury Association Impairment Scale, *SCIM-III* Spinal Cord Independence Measure version III, *Coef* coefficient, *95% CI* 95% confidence interval, *OR* odds ratio

significantly associated with AIS grades in CSF and PB, but not AIS grade improvement or SCIM-III score. This is in line with previous small-scale [2, 25–27, 33] and large-scale studies who also found GFAP to be significantly associated with AIS grades and AIS grade improvement in CSF [37]; however, they did not find the same association in PB [37]. These findings suggest GFAP as a potential biomarker to determine injury severity. Yet, the pre-analytical stability and variability of GFAP along with the preferred analytical method remains to be fully characterized [1, 4]. The roles of NfL and NSE are more controversial with pre-clinical and clinical studies reporting conflicting results [7, 13, 23, 29, 33, 38]. Our results showed that only NSE was significantly associated with AIS grades. None of the proteins were significantly associated with AIS grade improvement or SCIM-III score. Consistent with our observations, studies report that hemolysis and concomitant injuries external to the central nervous system can cause false positive NSE concentrations in CSF and PB [10, 39]. The lack of association for NfL might relate to the presence of highest concentrations at the second follow-up, thereby questioning the diagnostic utility of NfL.

As inflammatory proteins are found throughout the body, the utility of these proteins as biomarkers are also controversial, although they are thought to reflect the structural spinal cord damage. Our results showed that CSF concentrations of IFN-y, TNF-α, IL-2, IL-12p70, IL-4, IL-10, and IL-13 were significantly associated with AIS grades and that PB concentrations of IFN-y were significantly associated with AIS grades. This indicates an association between the degree of local inflammation and the injury severity. Studies have demonstrated that concentrations of IL-6 and IL-8 in CSF are significantly different between AIS grades A–C [25, 26], while another study found 27 proteins that were significantly different among AIS grades A–C [38]. Our results further showed that none of the proteins were significantly associated with AIS grade improvement; however, previous studies report conflicting results [25, 26, 38]. Finally, our results showed no significant association between biomarker concentrations and SCIM-III scores. These findings are consistent with those of another study showing no association between SCIM-III scores and CSF concentrations of NSE, IL-13, IL-16, IL-8, and TNF [8]. Although rarely used in TSCI studies, the SCIM-III score is more sensitive to detect changes in the degree of functional recovery than AIS grade conversion. It is surprising that none of the biomarkers are significantly associated to the SCIM-III score. The lack of association may be a type II error and may also point towards the heterogeneity in TSCI that is found at the molecular level and in clinical presentation.

The utility of PB remains questionable

PB has practical advantages over CSF [14]; however, the utility of PB is questionable due to the lack of knowledge about the pathways of biomarker release into PB and the extraspinal sources of some biomarkers. The lack of knowledge might result in misinterpretation of the findings in PB. Although some biomarkers were present in undetectable concentrations, our results demonstrated higher concentrations in PB in TSCI patients compared to healthy controls; however, only GFAP was persistently elevated and correlated to injury severity in both PB and CSF. These findings are similar to the results of a large-scale study [37]. Although it is generally believed that biomarkers are released into PB through a mechanical disrupted blood-spinal cord barrier, more research is warranted to clarify whether the release occurs through a disrupted blood-spinal cord barrier or an impaired glymphatic system [1]. It has been proposed that trauma may impair the glymphatic system and thereby influence the amount of biomarkers released into PB, suggesting that the biomarker concentration might reflect inactivity of the glymphatic system rather than tissue injury [21]. It may furthermore reflect a systemic response [37]. Several studies have collected CSF by lumbar puncture despite the practical advantages of PB [8, 9, 15, 25, 26, 33, 36–38, 40, 43]; however, as TSCI most frequently involve the cervical spinal cord [34], collection of CSF from the lumbar spine might also result in misinterpretation of the findings in CSF. A prior study explored the feasibility of collecting CSF by lumbar puncture in one TSCI patient [42]. Not surprisingly, the highest protein concentrations were found in CSF from the injury site, although protein concentrations were higher in the TSCI patient compared to the healthy controls. These findings indicate that CSF sampled from the lumbar spine is feasible for protein studies; however, the protein concentrations depend on injury level; thus, the measured protein concentrations are not precise. Our data suggest that future research would benefit from a focus on CNS-specific biomarkers such as GFAP in comparison to more general inflammatory-related markers. More research is warranted to avoid misinterpretation of the findings in CSF and PB.

Strengths and limitations

Our prospective pilot study presents simultaneously collected PB and CSF biochemical biomarkers. The results of our study must be interpreted in the context of the study design. The limited sample size does not provide sufficient statistical power to draw definitive conclusions about biomarkers; however, our results may provide guidance for further research. We included patients with AIS grades A–D injuries to achieve a sufficient sample size and to reflect the whole spectrum of injury severities, although it generates a heterogenic population. Importantly, we excluded patients with significant concomitant injuries, e.g., traumatic brain injuries, and we included a reference group of healthy controls without neurological diseases that might alter the protein composition in CSF. As healthy controls were not exposed to similar therapeutic interventions as TSCI patients, we cannot adjust for the effect of these interventions. We cannot reject that the analysis between biomarkers and clinical outcome measures can be confounded by variability in type, duration, and intensity of rehabilitation and the heterogeneity of the study population. Furthermore, we cannot reject that the large spread in timing of sample collection has affected the levels of some biomarkers with short half-lives. Finally, our panel of biomarkers might favor the proteins present in the central nervous system as these can be found in very small concentrations in blood using the current assay platforms, thereby introducing bias.

Conclusions

The results of our prospective pilot study support GFAP as a potential diagnostic biomarker in CSF and PB. Though our results cannot definitively point out CSF or PB as the ideal sample source for biomarker research, we suggest CSF

for cytokine measurements at early time points after TSCI but emphasize that CSF and PB should be applied in future biomarker research to clarify the utility of PB as it depends on the biomarker of interest.

Declarations

Ethical approval This research was approved by the Central Denmark Region Committees on Health Research Ethics (1-10-72-382-17) and the Danish Data Protection Agency (1-16-02-754-17) and registered on ClinicalTrials.gov (NCT03505463). Written informed consent was obtained from all patients.

Conflict of interests The authors declare no competing interests.

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