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Association of CSF and Serum Neurofilament Light and Glial Fibrillary Acidic Protein, Injury Severity, and Outcome in Spinal Cord Injury

Sophie Stukas, PhD, Jennifer Cooper, BMLSc, Jasmine Gill, MSc, Nader Fallah, PhD, Michael A. Skinnider, BASc, Lise Belanger, RN, Leanna Ritchie, RN, Angela Tsang, RN, Kevin Dong, BSc, Femke Streijger, PhD, John Street, MD, PhD, FRCSI, Scott Paquette, MD, Med, FRSCS, Tamir Ailon, MD, MPH, FRCSC, Nicolas Dea, MD, MSc, FRCSC, Raphaele Charest-Morin, MD, FRCSC, Charles G. Fisher, MD, MPH, FRCSC, Christopher S. Bailey, MD, MSc, FRCSC, Sanjay Dhall, MD, Jean-Marc Mac-Thiong, MD, PhD, FRCSC, Jefferson R. Wilson, MD, PhD, FRCSC, Sean Christie, MD, FRCSC, Marcel F. Dvorak, MD, FRCSC, Cheryl L. Wellington, PhD, and Brian K. Kwon, MD, PhD, FRCSC

Correspondence
Dr. Kwon
brian.kwon@ubc.ca

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Abstract

Background and Objectives

Traumatic spinal cord injury (SCI) is highly heterogeneous, and tools to better delineate pathophysiology and recovery are needed. Our objective was to profile the response of 2 biomarkers, neurofilament light (NF-L) and glial fibrillary acidic protein (GFAP), in the serum and CSF of patients with acute SCI to evaluate their ability to objectively characterize injury severity and predict neurologic recovery.

Methods

Blood and CSF samples were obtained from prospectively enrolled patients with acute SCI through days 1–4 postinjury, and the concentration of NF-L and GFAP was quantified using Simoa technology. Neurologic assessments defined the ASIA Impairment Scale (AIS) grade and motor score (MS) at presentation and 6 months postinjury.

Results

One hundred eighteen patients with acute SCI (78 AIS A, 20 AIS B, and 20 AIS C) were enrolled, with 113 (96%) completing 6-month follow-up. NF-L and GFAP levels were strongly associated between paired serum and CSF specimens, were both increased with injury severity, and distinguished among baseline AIS grades. Serum NF-L and GFAP were significantly ($p = 0.02$ to <0.0001) higher in AIS A patients who did not improve at 6 months, predicting AIS grade conversion with a sensitivity and specificity (95% CI) of 76% (61, 87) and 77% (55, 92) using NF-L and 72% (57, 84) and 77% (55, 92) using GFAP at 72 hours, respectively. Independent of clinical baseline assessment, a serum NF-L threshold of 170 pg/mL at 72 hours predicted those patients who would be classified as motor complete (AIS A/B) compared with motor incomplete (AIS C/D) at 6 months with a sensitivity of 87% (76, 94) and specificity of 84% (69, 94); a serum GFAP threshold of 13,180 pg/mL at 72 hours yielded a sensitivity of 90% (80, 96) and specificity of 84% (69, 94).

Discussion

The potential for NF-L and GFAP to classify injury severity and predict outcome after acute SCI will be useful for patient stratification and prognostication in clinical trials and inform communication of prognosis.

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Class of Evidence
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From the Djavad Mowafaghian Centre for Brain Health (S.S., J.C., J.G., C.L.W.), Department of Pathology and Laboratory Medicine (S.S, J,C, J.G.,C.L.W.) Division of Neurology, Department of Medicine (N.F.), Division of Neurosurgery (S.P., T.A., N.D.), Michael Smith Laboratories (M.A.S.), and School of Biomedical Engineering (C.L.W.), University of British Columbia, Vancouver, British Columbia; Praxis Spinal Cord Institute (N.F.), and Vancouver Spine Research Program (L.B., L.R., A.T.), Vancouver General Hospital, Blusson Spinal Cord Center, Vancouver, British Columbia; International Collaboration on Repair Discoveries (ICORD) (K.D., F.S., J.S., M.F.D., C.L.W., B.K.K.) and Vancouver Spine Surgery Institute, Department of Orthopaedics (J.S., R.C.-M., C.G.F., M.F.D., B.K.K.), University of British Columbia, Blusson Spinal Cord Center, Vancouver, British Columbia; Division of Orthopaedics (C.S.B.), Schulich School of Medicine, University of Western Ontario, London, Canada; Department of Neurosurgery (S.D.), University of California San Francisco; Department of Surgery (J-M., M-T.), Hôpital du Sacré-Coeur de Montréal, Quebec; Department of Surgery (J.-M., M.-T.), Chu Sainte-Justine, University of Montreal, Quebec; Division of Neurosurgery (J.R.W.), University of Toronto, St. Michael's Hospital, Ontario; and Division of Neurosurgery (S.C.), Halifax Infirmary, Dalhousie University, Nova Scotia, Canada.

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Glossary

AIS = ASIA Impairment Scale; **ASIA** = American Spinal Injury Association; **AUROC** = area under the ROC; **BTI** = brain trauma indicator; **GFAP** = glial fibrillary acidic protein; **ISNCSCI** = International Standards for Neurological Classification of Spinal Cord Injury; **IQR** = interquartile range; **LDA** = linear discriminant analysis; **LEMS** = lower extremity MS; **MS** = motor score; **NF-L** = neurofilament light; **OR** = odds ratio; **ROC** = receiver operating characteristic; **SCI** = spinal cord injury; **TBI** = traumatic brain injury; **UEMS** = upper extremity MS; **ULOD** = upper limit of detection; **ULOQ** = upper limit of quantification; **ZPP** = zone of partial preservation.

Classification of Evidence

This study provides Class I evidence that higher serum NF-L and GFAP are associated with worse neurological outcome after acute SCI.

Trial Registration Information

Registered on ClinicalTrials.gov: NCT00135278 (March 2006) and NCT01279811 (January 2012).

Traumatic spinal cord injury (SCI) causes devastating paralysis for which few treatment options exist. Early assessment of neurologic impairment currently depends on clinical examination using the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI). Despite psychometric validation, this examination is subjective, time consuming, and often unreliable or impossible to perform in the acute setting due to concomitant injuries.[1] Even if the American Spinal Injury Association (ASIA) Impairment Scale (AIS) baseline injury severity grade can be established clinically, significant variability in spontaneous neurologic recovery poses difficulty in predicting long-term outcome[2] creating challenges for clinical decision-making and communicating prognosis. Furthermore, this variability requires a large enrollment target to achieve statistical power in clinical trials, slowing the pace of therapeutic development.[3]

Fluid biomarkers represent a low-cost, accessible, and objective tool for SCI research and care. Several protein biomarkers have been studied in SCI and have demonstrated some efficacy in stratifying patients by initial injury severity and predicting neurologic outcome.[4,5] To date, most SCI biomarker studies focus on CSF, enroll a small (i.e., 10–50) number of patients, typically have a single sampling time point, and lack data on neurologic recovery.[6] In this study, we focused on neurofilament light (NF-L) and glial fibrillary acidic protein (GFAP), both of which have demonstrated associations with injury severity and neurologic recovery in neurotrauma.[6-10] GFAP is an astroglial protein widely accepted as a diagnostic traumatic brain injury (TBI) biomarker.[11] NF-L is a marker of axonal injury, correlating with diffuse axonal injury observed on MRI and predicting poor outcome following severe TBI.[7,10,12,13] Our previous acute SCI work demonstrated that CSF GFAP levels differ between AIS grades and predict AIS grade conversion 6 months postinjury, but these data have not been replicated using serum.[14-16] A single study demonstrated that serum NF-L is higher in patients with SCI compared with healthy controls and associates with motor score (MS) after SCI.[17] Our objective was to profile serum and CSF GFAP and NF-L in the first 4 days post-SCI as a function of injury severity and determine whether these markers predict neurologic outcome with respect to AIS grade and MS improvement at 6 months postinjury.

Methods

Study Design and Patients

Patients who sustained an acute, traumatic SCI were enrolled into a series of prospective clinical trials between 2006 and 2019 using the following inclusion criteria: AIS grade A, B, or C on admission; neurologic level of injury between C1-L1; and the ability to collect a valid, reliable baseline neurologic examination within 24 hours of injury. Patients were excluded if they had concomitant TBI, major axial or appendicular trauma, or who were too sedated or intoxicated on admission to assess neurologically. All studies maintained the same inclusion and exclusion criteria and biospecimen protocols as detailed in eFigure 1, links.lww.com/WNL/C584.[18-20] Healthy adults undergoing routine lumbar surgery for spinal stenosis or disc herniations were recruited as a control group. Lumbar intrathecal catheters were inserted within 48 hours of injury, with the dual objectives of (1). monitoring CSF pressure for evaluation of spinal cord perfusion pressure[18-20] and (2) acquiring CSF and serum samples for biomarker studies.[14,15,21] This study is a secondary analysis of NF-L and GFAP and included all patients from whom sufficient volumes of CSF and serum remained. All patients with SCI underwent an ISNCSCI examination at admission before surgery and 6 months postinjury to establish baseline and follow-up AIS grade and MS. All sites received formal ISNCSCI training to ensure that the neurologic examinations were conducted by qualified individuals.

Standard Protocol Approvals, Registrations, and Patient Consents

Institutional ethics approvals were in place at each site, and all patients provided written informed consent. Trials are registered (ClinicalTrials.gov): NCT00135278 and NCT01279811.

Outcomes and Procedures

Before spinal surgery, a lumbar intrathecal catheter was inserted distal to L2 under strict aseptic technique and remained in place for 3–5 days postinjury to allow for serial CSF sampling every 24 hours. A serum specimen was collected at the same time as each CSF specimen. Biospecimen collection and processing was performed as described.[21]

The concentrations of NF-L and GFAP were quantified using the NF-L Advantage Assay (cat. 103186) and the GFAP Discovery Assay (cat. 102336) from Quanterix (Lexington, KY) following the manufacturer's protocol using adapted dilution strategies as described in the eMethods and eFigure 2, links.lww.com/WNL/C584. For complete assay specifications, see eTable 1.

For all analyses, we first assessed NF-L and GFAP individually at each time point and then evaluated them in combination to test for improved biomarker performance in classifying initial injury severity or predicting 6-month neurologic recovery. The first outcome was the accuracy with which NF-L and GFAP distinguished among baseline injury severities of AIS A, B, and C. The second outcome was the accuracy with which biomarkers predicted AIS grade conversion at 6 months in those initially assessed as AIS A at baseline. To expand on this analysis, we determined whether prediction of AIS grade conversion using biomarkers could be improving by the incorporation of motor (cervical) or sensory (cervical and thoracolumbar) zone of partial preservation (ZPP) lengths. The third outcome was the accuracy with which biomarkers, independent of baseline AIS grade, predicted motor-complete (AIS A and B) or motor-incomplete (AIS C and D) injury at 6 months postinjury. We further tested whether knowledge of baseline AIS grade, either A or B, improved the prognostic model for patients who started with motor-complete injuries. The fourth outcome was the accuracy with which biomarkers predicted ≤ or > 8-point change in total MS at 6 months in patients with cervical SCI. Here, 8 motor points was chosen as a cutoff as it was considered to be clinically meaningful, approximated the effect size of interventions such as surgical decompression, and resulted in comparable group sizes. This was assessed in all patients with cervical SCI and the subgroup with AIS A injuries.

Statistical Analysis

Descriptive statistics using mean and SD or median and interquartile range (IQR) and frequency were used to describe continuous and categorical variables. We binned the postinjury time of specimen draw (t) as follows: 24 hours ($0 < t < 36$ hours), 48 hours (36 hours $\leq t < 60$ hours), 72 hours (60 hours $\leq t < 84$ hours), and 96 hours (84 hours $\leq t < 108$ hours). In the case where 2 specimens were drawn in the 24-hour bin (serum: 6 AIS A, 4 AIS B, and 3 AIS C; CSF: 4 AIS A, 2 AIS B, and 2 AIS C patients), an average of the 2 measured concentrations was generated. We tested the association between NF-L and GFAP and demographic and injury characteristics using 2-sided Spearman rank correlation tests, Mann-Whitney *U* tests, Kruskal-Wallis tests for continuous variables, or Fisher exact tests for categorical variables. Data were log transformed, and receiver operating characteristic (ROC) curves were produced to assess biomarker performance on the 4 described outcomes. Linear discriminant analysis (LDA) was used to assess the combination of NF-L and GFAP for these outcomes. Goodness of fit for logistic regression models was assessed using the Hosmer-Lemeshow test. The robustness of the LDA results was assessed with a leave-one-out cross-validation where the model was trained on all data except one data point and then used to predict that point. After repeating this process for all samples, the average error was computed to evaluate model performance. To determine whether integration of clinical parameters improved on the biomarker-based prediction models, we first determined whether there was an independent association with outcome using contingency tables and the Fisher exact test and then compared the fit of logistic regression models using biomarker vs biomarker plus clinical data using the likelihood ratio test. To determine whether ZPP was predictive of 6-month AIS conversion in AIS A patients, ZPP scores were dichotomized based on segment length: motor ZPP 0–1 vs 2+ and sensory ZPP 0–2 vs 3+, based on previous literature.[22,23] For the prediction of 6-month motor complete vs incomplete using the baseline AIS grade, we dichotomized the group as AIS A or B (we excluded AIS C as this was a perfect predictor of motor incomplete at 6 months). All graphical data represent the median and IQR, with the median concentration in pg/mL per time pointed listed below in tabular format. A p value of <0.05 was considered to be significant, where $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

Data Availability

Deidentified data are available on request.

Results

NF-L and GFAP were quantified in serum and CSF specimens collected from 118 patients (68 cervical and 50 thoracolumbar) with acute traumatic SCI (Table, eTable 2, and eFigure 1, links.lww.com/WNL/C584). As minimal differences were observed for patient characteristics across the consecutive studies (eTable 3), for the purposes of this secondary analysis study, we treated this patient population as one unified cohort. Baseline AIS grades were AIS A in 78 (66%), AIS B in 20 (17%), and AIS C in 20 (17%) patients, respectively. Follow-up ISNCSCI assessments were conducted 6 months postinjury in 113/118 patients (96%). A minimum of 1 and up to 4 CSF and serum specimens were collected per patient between 4.3 and 107 hours post-SCI. A single paired CSF and serum specimen was also collected from 19 non-SCI control patients (n = 8 [42%] male, mean [SD] age 59.5 years [14.5]).

NF-L and GFAP were measured in 390 CSF and 421 serum specimens. Despite extensive dilution, 50 (12%) serum specimens and 98 (23%) CSF specimens were above the ULOD for GFAP (eTable 4, links.lww.com/WNL/C585). CSF concentrations of NF-L and GFAP were orders of

Table Demographics, Clinical Characteristics, and Biospecimen Distribution for Patients With SCI Categorized by Baseline Injury Severity (AIS Grade)

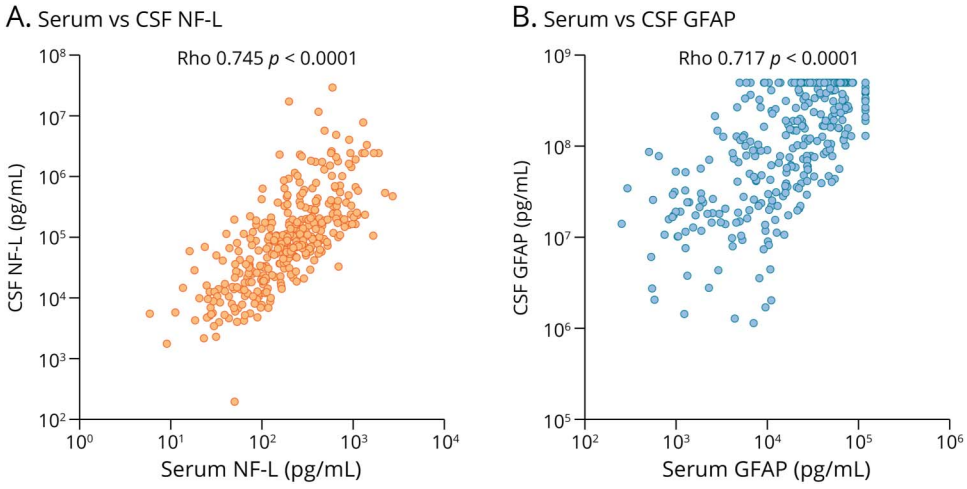
	All	AIS A	AIS B	AIS C
Patient, N (%)	118	78 (66)	20 (17)	20 (17)
Male, N (%)	94 (80)	61 (78)	18 (90)	15 (75)
Female, N (%)	24 (20)	17 (22)	2 (10)	5 (25)
Age, y, mean (SD)	42.9 (17.0)	39.6 (16.8)	46.9 (16.0)	52.2 (15.0)
Time from injury to baseline assessment, hours, mean (SD)	12.4 (11.3)	12.4 (12.0)	13.1 (10.9)	11.5 (8.8)
Time from injury to surgical decompression, hours, mean (SD)	22.6 (8.0)	23.3 (7.7)	21.8 (9.4)	20.5 (8.1)
Cervical injury level, N (%)	68 (58)	39 (50)	14 (70)	15 (75)
Thoracolumbar injury level, N (%)	50 (42)	39 (50)	6 (30)	5 (25)
Biospecimen (serum and CSF) measurement at 24, 48, 72, and 96 hours after injury				
Serum, 24 hours, N (%)	92 (78)	65 (83)	13 (65)	14 (70)
Serum, 48 hours, N (%)	99 (84)	70 (90)	14 (70)	15 (75)
Serum, 72 hours, N (%)	104 (88)	71 (91)	18 (90)	15 (75)
Serum, 96 hours, N (%)	94 (80)	67 (86)	15 (75)	12 (60)
CSF, 24 hours, N (%)	93 (79)	65 (83)	14 (70)	14 (70)
CSF, 48 hours, N (%)	90 (76)	65 (83)	12 (60)	13 (65)
CSF, 72 hours, N (%)	96 (81)	66 (85)	15 (75)	15 (75)
CSF, 96 hours, N (%)	84 (71)	59 (76)	14 (70)	11 (55)
6-month neurologic outcome				
Patient, N (%)	113 (96)	74 (95)	19 (95)	20 (100)
Time to follow-up, days, mean (SD)	187 (84)	179 (60)	232 (156)	174 (46)
AIS grade improvement, N (%)	57 (50)	25 (34)	13 (68)	19 (95)

Abbreviations: AIS = ASIA Impairment Scale; SCI = spinal cord injury.

magnitude greater than their corresponding serum levels, and were associated (NF-L: rho = 0.745, $p < 0.0001$; GFAP: rho = 0.717, $p < 0.0001$) between matrices, suggesting that serum levels reflect CSF levels (Figure 1). The pattern of NF-L and GFAP concentrations over time differed between matrices and baseline injury severities (AIS A, B, and C) (eFigure 3). There was no difference in serum or CSF biomarkers based on age, sex, neurologic level of injury (cervical vs thoracolumbar), timing of surgical decompression, or clinical trial of enrollment (eFigure 4).

NF-L and GFAP were elevated as a function of injury severity in both serum (Figure 2 and eFigure 5, links.lww.com/WNL/C584) and CSF (eFigure 6). Compared with controls, serum NF-L (median [IQR]: 16.9 pg/mL [12.4–31.8]) was significantly higher in AIS A and AIS B patients at all 4 time points, with the greatest difference observed at 96 hours (AIS A 376 pg/mL [238–678] $p < 0.0001$; AIS B 208 pg/mL [118–275] $p = 0.0024$). Likewise, compared with controls, serum GFAP (median [IQR]: 103 pg/mL [51.3–184]) was significantly higher in AIS A and AIS B patients at all 4 time points, with the maximum difference observed at 48 hours (AIS A 56,900 pg/mL [27,400–88,900] $p < 0.0001$; AIS B 19,600 pg/mL [10,800–34,800] $p = 0.0019$). Although there was no overlap in serum GFAP between controls and AIS C patients at 24 hours, this difference did not reach statistical significance ($p = 0.063$). The ability of NF-L or GFAP at 24, 48, 72, and 96 hours postinjury to discriminate between AIS grades at baseline (A vs B, A vs C, and B vs C) was determined using ROC curves (eFigure 5, eTable 5, and eTable 6). All ROC curves for serum GFAP and NF-L were statistically significant (except for serum GFAP's AIS A vs B at 24 hours), with an area under the ROC (AUROC) between 0.734 and 0.962, demonstrating a moderate-to-strong ability to distinguish among AIS grades. Using LDA, serum GFAP and NF-L correctly classified baseline AIS grades between AIS A and B patients 67% to 79%; AIS A and C patients 82%–90%; and AIS B and C patients 73%–86% of the time, with cross-validation models yielding similar accuracy, suggesting robustness of the models (eTable 7). Finally, we used multinominal logistic regression to determine

Figure 1 Association of NF-L and GFAP in Paired Serum and CSF Specimens Taken Following SCI

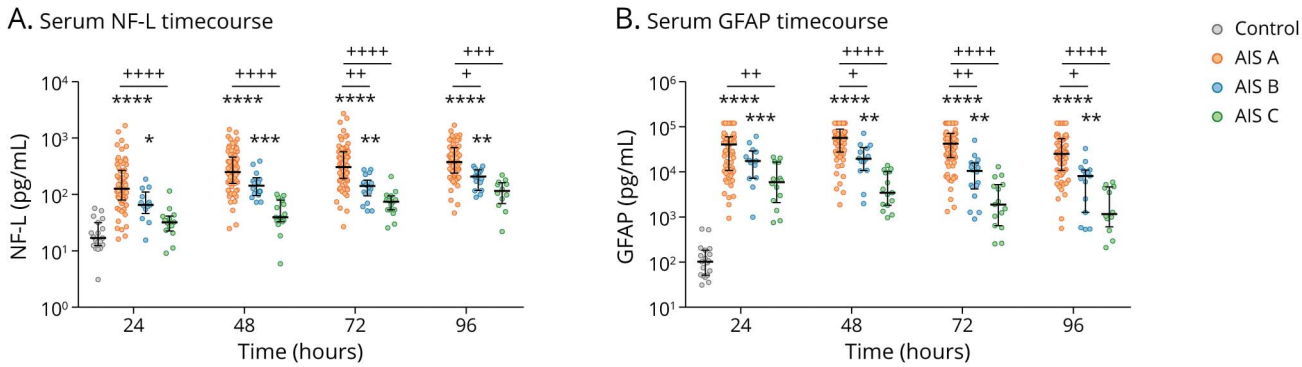


The concentration of (A) NF-L and (B) GFAP was quantified in 349 paired serum and CSF samples from 113 patients with SCI collected between 4 and 107 hours following injury. Data were analyzed using a Spearman correlation. GFAP = glial fibrillary acidic protein; NF-L = neurofilament light; SCI = spinal cord injury.

the ability of serum NF-L and GFAP to accurately classify AIS A, B, or C patients simultaneously (eTable 8). Although AIS A patients were classified correctly 92%–97% of the time, serum biomarkers were poor at identifying AIS B or C patients. Combining NF-L with GFAP did not improve the ability of these models to distinguish AIS grades.

Analyses to predict conversion were performed only in AIS A patients, recognizing that most AIS B/C injuries convert spontaneously.[2,3] Of the 74 AIS A patients with 6-month follow-up, 49 (66%) remained AIS A, whereas 25 (34%) improved and were classified as AIS B (n = 15), C (n = 8), or D (n = 2). Twenty-four-hour serum NF-L and GFAP were significantly higher in nonimproving AIS A patients compared with those who improved AIS grade (median [IQR]: NF-L 198 pg/mL [101, 352] vs 82 pg/mL [51.3, 124] $p < 0.0001$; GFAP 45,200 pg/mL [13,500, 65,200] vs 16,100 pg/mL [8,330, 49,200] $p = 0.017$), respectively, remaining 1.3–3.7-fold higher at 48, 72, and 96 hours postinjury (Figure 3). ROC curve analysis demonstrated a moderate-to-strong discrimination between groups (eTable 9, links.lww.com/WNL/C585); using a serum NF-L threshold of 264 pg/mL at 72 hours predicted those patients who would remain an AIS A with a sensitivity of 76% (95% CI 61, 87) and specificity of 77% (95% CI 55, 92), whereas a serum GFAP threshold of 35,200 pg/mL at 72 hours predicted nonconversion with a sensitivity of 72% (95% CI 57,

Figure 2 Evaluation of Serum NF-L and GFAP as Biomarkers of Baseline Injury Severity (AIS Grade)

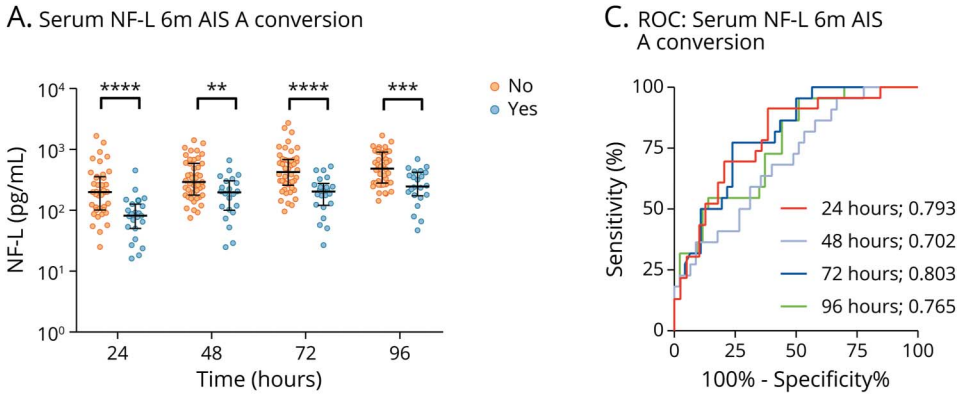


	24 hours	48 hours	72 hours	96 hours
AIS A	127	250	306	376
AIS B	65.3	143	142	208
AIS C	32.1	39.4	74.4	115
Control	16.9 pg/mL			

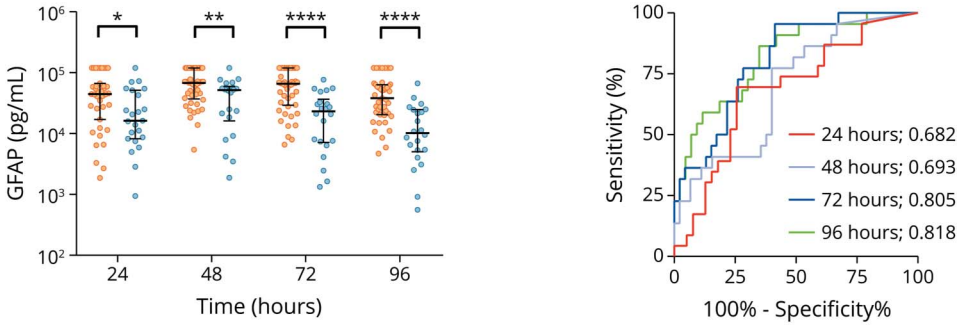
	24 hours	48 hours	72 hours	96 hours
AIS A	41,000	56,900	42,200	25,100
AIS B	17,600	19,600	10,500	8,120
AIS C	5,960	3,470	1,900	1,160
Control	103 pg/mL			

(A) NF-L and (B) GFAP were measured in serum samples from controls (n = 19, gray), AIS A (n = 78, orange), AIS B (n = 20, blue), and AIS C (n = 20, green) patients with SCI taken up to 4 days after injury. *Statistical results compared with control (shown once in 24-hour bin). +Statistical results compared within SCI groups. AIS = ASIA Impairment Scale; GFAP = glial fibrillary acidic protein; NF-L = neurofilament light; SCI = spinal cord injury.

Figure 3 Comparison of Serum NF-L and GFAP in AIS A Patients, Distinguished by Whether AIS Grade Conversion Occurred (Yes/No) at 6 Months Postinjury



	24 hours	48 hours	72 hours	96 hours
No	198	284	392	479
Yes	81.5	197	203	246



	24 hours	48 hours	72 hours	96 hours
No	45,200	67,600	65,300	38,200
Yes	16,100	51,800	23,200	10,200

Of the 74 AIS A patients with outcome assessed at 6 months, 49 (66%) remained an AIS A (no conversion, orange), whereas 25 (34%) improved in their AIS grade (yes conversion, blue). Serum (A) NF-L and (B) GFAP were graphed based on AIS A conversion status at 6 months, where *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001 NF-L and GFAP medians expressed as pg/ml below the graphs. ROC curves were generated comparing the concentration of (C) NF-L and (D) GFAP at each time point (24 hours orange, 48 hours blue, 72 hours gray, and 96 hours teal) based on conversion status. AUROC values and errors are listed in eTable 9, links.lww.com/WNL/C585. AIS = ASIA Impairment Scale; AUROC = area under the ROC; GFAP = glial fibrillary acidic protein; NF-L = neurofilament light; ROC = receiver operating characteristic; SCI = spinal cord injury.

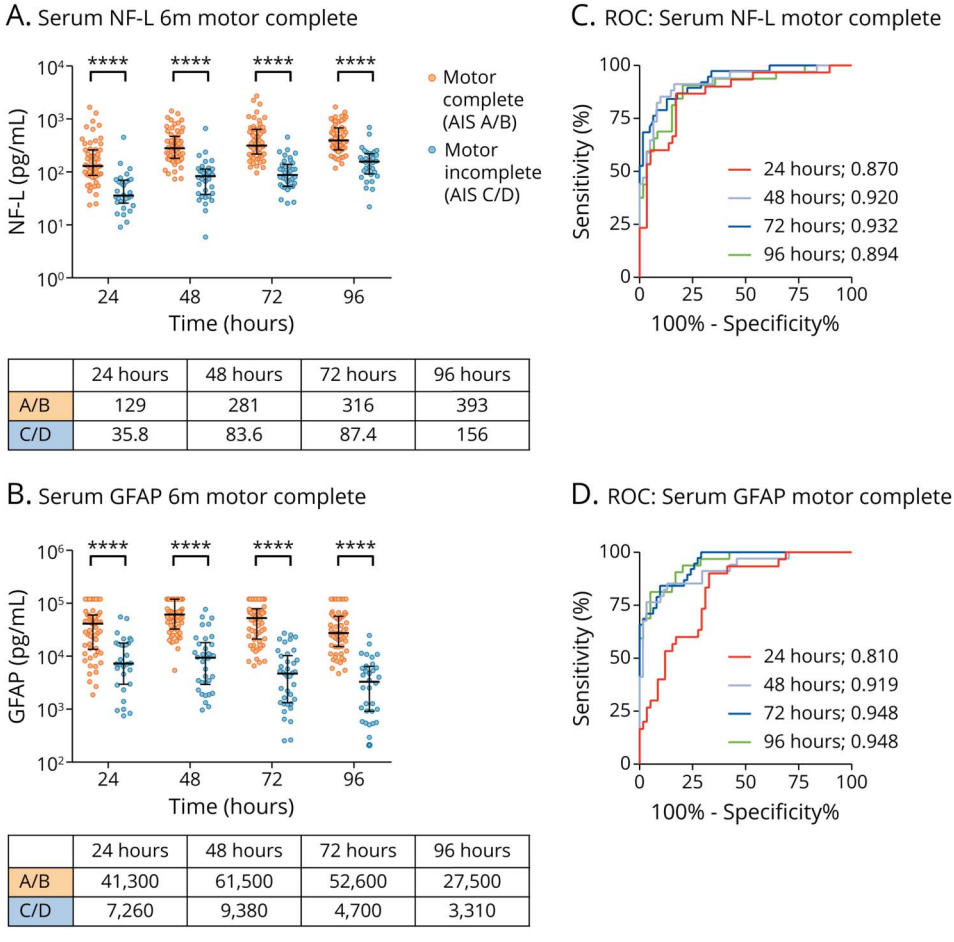
84) and specificity of 77% (95% CI 55, 92). Similar results were observed in the CSF; NF-L was 3.2–4.5-fold higher, and GFAP was 1.9 to 12-fold higher in AIS A patients who remained AIS A (eFigure 7), with AUROC between 0.678 and 0.899 (eTable 10). Using LDA, serum NF-L and GFAP correctly predicted 75%–81% of AIS A patients who remained AIS A (eTable 11). Leave-one-out cross-validation demonstrated the robustness of the results and showed less than 5% difference between model performance on training vs validation data. Next, we evaluated whether motor or sensory ZPP in the 34 cervical and 34 thoracolumbar AIS A patients with baseline ZPP (ZPP values missing in 6 patients) predicted 6-month AIS grade conversion. Consistent with previous reports,[22,23] while motor ZPP was not, sensory ZPP was associated with AIS A conversion in both cervical (odds ratio [OR] 8.67, 95% CI 1.65–34.7; p = 0.007) and thoracolumbar patients (OR 40.3, 95% CI 4.4–242; p = 0.0002). With the exception of 96 hours NF-L, serum NF-L and GFAP correctly predicted conversion with 67%–73% accuracy in cervical patients; addition of sZPP into the logistic regression model improved this prediction by 7%–20% (eTable 12). Serum NF-L and GFAP correctly predicted conversion in 69%–83% of thoracolumbar patients, and addition of sZPP into the model improved the accuracy by 15%–19% at 24 and 48 hours; this comparison could not be performed at 72 or 96 hours due to perfect separation of the data (eTable 13).

Given the challenges of conducting a detailed neurologic assessment and assigning a baseline AIS grade, we sought to determine whether NF-L or GFAP (independent of baseline AIS grade) could predict which patients would be motor complete (AIS A or B) or motor incomplete (AIS C or D) at 6 months postinjury. At 6 months, 70 (62%) patients were classified as motor complete, and 43 (38%) were classified as motor incomplete. As shown in Figure 4, median levels of serum NF-L and GFAP were significantly higher in motor

complete vs incomplete at 24 hours (median [IQR]: NF-L 129 pg/mL [86.3–261] vs 35.8 pg/mL [25.9–70.1] $p < 0.0001$; GFAP 41,300 pg/mL [13,600–59,900] vs 7,260 pg/mL [2,960–17,000] $p < 0.0001$), and these differences were maintained out to 96 hours (Figure 3). The ability to distinguish these groups improved slightly over time, such that there was excellent discrimination (AUROC >0.9) after 48 hours using either serum NF-L or GFAP (eTable 14, links.lww.com/WNL/C585). A serum NF-L threshold of 170 pg/mL at 72 hours predicted those patients who would be classified as motor complete with a sensitivity of 87% (95% CI 76, 94) and specificity of 84% (95% CI 69, 94), whereas a serum GFAP threshold of 13,180 pg/mL at 72 hours predicted 6-month motor complete designation with a sensitivity of 90% (95% CI 80, 96) and specificity of 84% (95% CI 69, 94). CSF NF-L and GFAP were 6 to 15-fold higher in motor complete vs motor incomplete patients at 6 months (eFigure 8), with AUROC between 0.883 and 0.935, where the 72-hour time point once again demonstrated the strongest prognostic ability (eTable 15). In both the original and cross-validation LDA models, serum biomarkers had an 80%–90% accuracy to predict motor-complete vs motor-incomplete AIS grades at 6 months postinjury (eTable 16). Similar accuracies for cross-validation compared with index models indicate model robustness and suggests that, even in the absence of baseline neurologic assessment, serum GFAP and NF-L measured within the first few days postinjury can predict distal motor function at 6 months. Within AIS A and B patients, baseline AIS grade was unsurprisingly associated with being motor complete at 6 months (OR 13.9, 95% CI 4.5–48, $p < 0.0001$). By themselves, serum NF-L and GFAP correctly predicted motor-complete status 78% to 87% of the time, and knowledge of baseline AIS grade (A vs B) improved the predictive modeling by only 1%–4%, except in the case of 96-hour GFAP where the model using the biomarker alone was a better fit than one that also incorporated AIS grade (eTable 17).

As change in MS is often considered a primary outcome measure in clinical trials of cervical SCI, we examined the relationship between serum and CSF biomarkers with changes in upper extremity MS (UEMS), lower extremity MS (LEMS), and total MS at 6 months in 65 patients with cervical SCI. There was an almost universal negative association between serum and CSF biomarkers and the change in UEMS, LEMS, and total MS, indicating that higher biomarker levels were associated with worse MS recovery at 6 months (eFigure 9 and

Figure 4 Comparison of Serum NF-L and GFAP Concentration Based on the Observed AIS Grade at 6 Months



	24 hours	48 hours	72 hours	96 hours
A/B	129	281	316	393
C/D	35.8	83.6	87.4	156

	24 hours	48 hours	72 hours	96 hours
A/B	41,300	61,500	52,600	27,500
C/D	7,260	9,380	4,700	3,310

Six-month outcome assessments were available in 113/118 (96%) patients with SCI. Seventy patients (62%) were classified as AIS A or AIS B (motor complete, orange), whereas 43 (38%) were classified as AIS C or AIS D (motor incomplete, blue) at 6 months. Graph of 24-, 48-, 72-, and 96-hour (A) NF-L and (B) GFAP in patients with SCI dichotomized based on 6-month AIS outcome, where ****$p < 0.0001$. NF-L and GFAP medians expressed as pg/ml below the graphs. ROC curves comparing (C) NF-L and (D) GFAP concentration at each time point based on observed AIS grade. AUROC values and errors are listed in eTable 14, links.lww.com/WNL/C585. AIS = ASIA Impairment Scale; GFAP = glial fibrillary acidic protein; NF-L = neurofilament light; ROC = receiver operating characteristic; SCI = spinal cord injury.

eTable 18, links.lww.com/WNL/C584). For analytical purposes, we focused on total MS and dichotomized motor recovery into those who regained ≤8 motor points (n = 28, 43%) vs >8 motor points (n = 37, 57%) at 6 months, as this approximates the change in MS achievable after early surgical decompression.[24] NF-L and GFAP at 24 hours were 3-4-fold higher (median [IQR]: NF-L 145 pg/mL [92.3–265] vs 43.4 pg/mL [27.4–77.1] $p < 0.0001$; GFAP 41,600 pg/mL [12,300–59,100] vs 8,680 pg/mL [3,000–21,200] $p = 0.0012$) in serum (Figure 5) and 10-fold higher ($p < 0.0001$) in CSF (eFigure 10) of patients with SCI who regained ≤8 motor points at 6 months. The most accurate distinctions occurred using the biomarker levels measured at the 48–72-hour time points (eTables 19 and 20), as observed for other outcomes. Using a serum NF-L threshold of 170 pg/mL at 72 hours predicted those patients who would regain ≤8 motor points with a sensitivity of 88% (95% CI 69–97) and specificity of 91% (95% CI 75–98), whereas a serum GFAP threshold of 21,150 pg/mL at 72 hours predicted those patients who would regain ≤8 motor points at 6 months with a sensitivity of 80% (95% CI 59–93) and specificity of 88% (95% CI 71–96). Even when restricting this analysis to the 36 patients with cervical SCI with AIS A injuries, with the exception of 24-hour GFAP, serum NF-L and GFAP levels were significantly higher in those who regained ≤8 motor points at 6 months (Figure 6) with AUROC between 0.680 to 0.864 (eTable 21). CSF NF-L and GFAP were 5-11-fold higher at all 4 time points in cervical AIS A patients that gained ≤8 points, with AUROC of 0.773–0.879 (eFigure 11 and eTable 22). These data suggest that NF-L and GFAP may be useful biomarkers to predict motor recovery in patients with cervical SCI, including those with AIS A injuries.

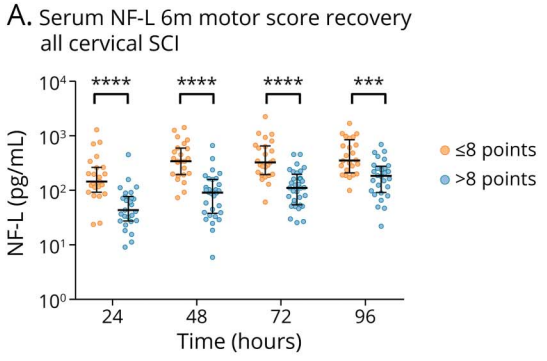
Classification of Evidence

This study provides Class I evidence that higher serum NF-L and GFAP are associated with worse neurological outcome after acute SCI.

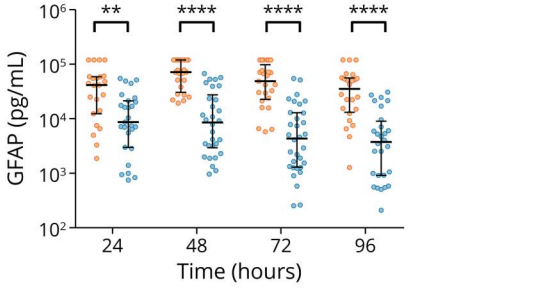
Discussion

In this comprehensive analysis of NF-L and GFAP in SCI, NF-L and GFAP concentrations were strongly associated between CSF and serum (Rho > 0.7), and similar, if not stronger, diagnostic

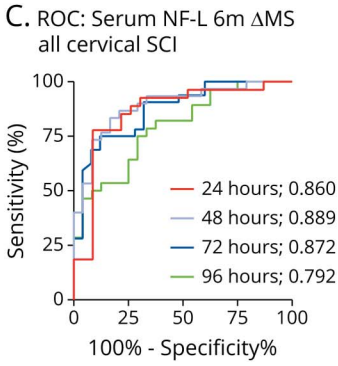
Figure 5 Association of Serum NF-L and GFAP and 6-Month Total Motor Score Recovery in All Patients With Cervical SCI

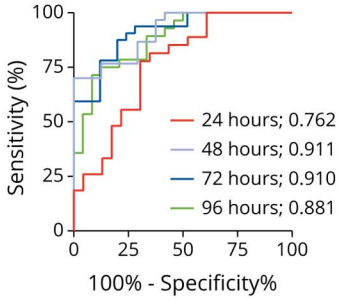


	24 hours	48 hours	72 hours	96 hours
≤8	145	342	325	353
>8	43.4	90.8	111	184



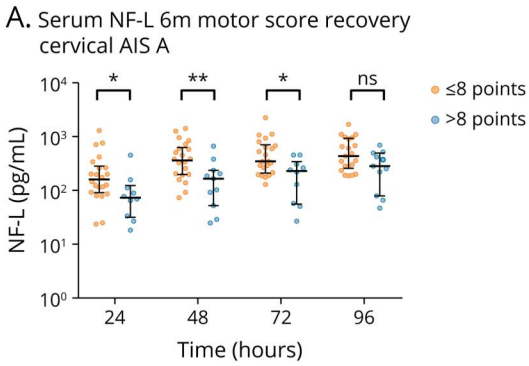
	24 hours	48 hours	72 hours	96 hours
≤8	41,600	71,300	48,800	35,400
>8	8,680	8,530	4,360	3,770



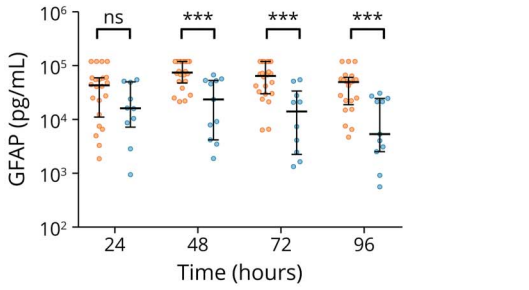


The change in total motor score (ΔMS) was dichotomized into patients who gained ≤8 points (orange, n = 28) vs those who gained >8 points (blue n = 37) at 6 months. Graphs of serum (A) NF-L and (B) GFAP based on motor score recovery, where $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$. NF-L and GFAP medians expressed as pg/ml below the graphs. ROC curves comparing (C) NF-L and (D) GFAP concentration at all time points based on dichotomized motor score recovery. AUROC values and error are listed in eTable 19, links.lww.com/WNL/C585. AUROC = area under the ROC; GFAP = glial fibrillary acidic protein; NF-L = neurofilament light; ROC = receiver operating characteristic; SCI = spinal cord injury.

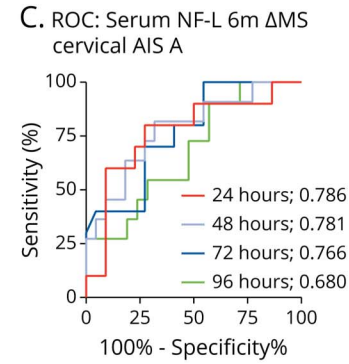
Figure 6 Association of Serum NF-L and GFAP and 6-Month Motor Score Recovery in Cervical AIS A Patients

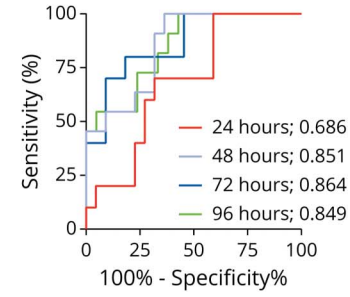


	24 hours	48 hours	72 hours	96 hours
≤8	160	361	348	433
>8	73.4	166	230	283



	24 hours	48 hours	72 hours	96 hours
≤8	43,000	74,600	64,500	49,600
>8	16,100	23,500	14,100	5,350





Serum (A) NF-L and (B) GFAP were compared in cervical AIS A patients who gained ≤8 points (orange, n = 25) vs those who gained >8 points (blue n = 11) of motor score at 6 months, where *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$, and ns =not significant. NF-L and GFAP medians expressed as pg/ml below the graphs. ROC curves comparing (C) NF-L and (D) GFAP concentration at all time points based on dichotomized motor score recovery. AUROC values and errors are listed in eTable 21, links.lww.com/WNL/C585. AIS = ASIA Impairment Scale; AUROC = area under the ROC; GFAP = glial fibrillary acidic protein; NF-L = neurofilament light; ROC = receiver operating characteristic; SCI = spinal cord injury.

and prognostic performance was observed in serum compared with CSF. Both serum NF-L and GFAP were strongly indicative of AIS grade improvement at 6 months in AIS A patients, with excellent distinction of being motor complete vs motor incomplete at 6 months, independent of baseline clinical assessment. Serum and CSF biomarkers were also associated with the degree of MS recovery in patients with cervical SCI. Intriguingly, NF-L and GFAP were equally strong biomarkers individually, and their linear combination did not improve predictive models.

Characterizing the dynamic response of NF-L and GFAP post-SCI revealed similarities to observations in TBI biomarker studies. For serum NF-L, 24-hour postinjury levels are similar between SCI and TBI, increasing 1.5–10× in TBI[7,12,25,26] and 1.9–7.5× in our SCI cohort relative to controls. Our results agree with those of Kuhle et al.,[17] who reported, in 27 patients with acute SCI, serum NF-L concentrations of 21–70 pg/mL 24 hours postinjury, with levels increasing up to 7 days postinjury, compared with 5 pg/mL in healthy controls. Serum GFAP has been reported to increase 4- to 200-fold in the first day following TBI compared with controls.[11] We observed median increases of 58-fold in AIS C patients to 399-fold in AIS A patients at 24 hours, with virtually no overlap observed between controls and patients with SCI for the first 48 hours. This response is interesting given that the volume of potential tissue damage is markedly less in SCI than TBI.

Fluid biomarkers may represent aspects of injury severity and biological response that cannot be captured fully by standard clinical examination or MRI. In the current study, serum and CSF biomarkers correctly predicted spontaneous AIS grade recovery in 75%–80% of AIS A patients. The possibility of accurately prognosticating spontaneous neurologic recovery could allow researchers to precisely stratify patients for clinical trial enrollment, reducing the number of patients required to demonstrate a clinically meaningful effect. The ability of serum NF-L and GFAP to distinguish between motor-complete vs motor-incomplete status at 6 months postinjury opens the possibility for clinicians to use a blood sample obtained within the first few days after injury to help communicate prognosis with patients and potentially inform management decisions in the acute and rehabilitation settings.

It is certainly conceivable that predicting outcome after acute SCI may be facilitated by combining fluid biomarkers

with clinical information and imaging biomarkers (e.g., MRI), a combinatorial approach that has shown promise in predicting outcome after TBI.[12,27-30] As we demonstrate, the serum biomarker predictions of conversion in AIS A patients or the AIS grade outcome (AIS A/B vs AIS C/D) at 6 months postinjury are improved with knowledge of baseline clinical parameters such as the sensory ZPP and baseline AIS grade. Although this shows that superior prognostication may be achieved by adding the neurologic examination, this clinical assessment is frequently impossible to perform or is quite unreliable in the acute setting. Of interest, for some models (e.g., the prediction of remaining motor complete vs being motor incomplete at 6 months postinjury), little was gained by adding clinical data to the biomarkers, indicating the strength with which the biology reflects neurologic outcome.

Given the similar performance of NF-L and GFAP as biomarkers of acute SCI and that combining them did not improve model performance, it is interesting that they are derived from distinct cells (neuronal vs astrocytic) and show different temporal profiles (slow vs fast decline over time) and magnitude of response to acute injury, with GFAP being 20–50 times higher than NF-L in serum. As SCI inevitably leads to damage of the neurons and glia, with disruption of the blood-brain and blood-CSF barrier, one could hypothesize that NF-L and GFAP are both indicators of overall injury severity on a granular scale and thus strong predictors of global neurologic outcome. GFAP is highly specific for the CNS, and given that point-of-care systems for GFAP quantification have been developed,[8,9] noteworthy for future clinical implementation. Although NF-L is CNS enriched, it is not CNS specific and thus may be elevated if there is concomitant injury to the peripheral nervous system. Albeit, a recent study of serum NF-L in a cohort of mild TBI, trauma controls, and healthy controls found no difference between the control groups,[31] suggesting that the peripheral contribution of NF-L may be negligible compared with what is elicited by SCI. As our cohort excluded individuals with multi-system trauma or major appendicular trauma, further studies in a real-world polytrauma setting will be needed to inform how this may influence serum biomarker levels. NF-L levels increase during normal aging[32,33] and are elevated in chronic neurodegenerative conditions such as Alzheimer disease.[34] This is important as the average age of patients with SCI is increasing and more elderly patients present with comorbidities. However, the magnitude of NF-L increase is vastly greater following acute neurotrauma compared with chronic neurodegeneration, with the difference becoming more apparent with serial samples taken in the days to weeks follow injury.

Another important consideration for future implementation is the temporal dynamics of biomarker expression and the timing of biospecimen sampling. Previous CSF biomarker studies focused largely on the 24-hour post-SCI time point,[14,35] but this current study demonstrates that serum NF-L and GFAP have optimal biomarker performance at 48–72 hours. Persistently high concentrations of GFAP, especially those that never fell below the ULOD, were associated with worse outcomes in AIS A patients. For both CSF and serum, all AIS A patients with samples over the ULOD for GFAP at 72 or 96 hours remained an AIS A at 6 months. Our observation of increasing NF-L over time is consistent with studies in TBI, which report increasing serum NF-L for at least the first 2 weeks following injury.[12,36] In this context, future SCI studies with extended sampling intervals at 1 or 2 weeks following injury may provide additional predictive information.

Strengths of our study include its cohort size, incorporation of precise, sensitive assays for an axonal (NF-L) and a glial (GFAP) marker in serum and CSF, characterization of dynamic responses over 4 days, 96% clinical follow-up at 6 months, and, ultimately, demonstration that these biomarkers may improve on currently available predictors of recovery. One limitation is the relatively low incidence of SCI resulting in enrollment that occurred over the course of 14 years in 3 clinical trial protocols. While these protocols to obtain CSF and serum evolved during this time period from a single center to a multicenter initiative, the inclusion/exclusion criteria, lumbar catheter insertion, and CSF/serum sampling were consistent. An analysis of biomarker levels within each of these clinical trial cohorts showed no significant differences between GFAP and NF-L levels, suggesting that pooling them was reasonable and that biospecimen degradation over time was not a confounding issue. Although all sites received formal ISNCSCI training, it was not possible to ensure that the same individual who performed the baseline assessment was the same individual who conducted the 6-month follow-up assessment, reflecting an inherent difficulty in conducting acute SCI clinical trials. These limitations notwithstanding, serum NF-L and GFAP appear to have great potential to help clinicians communicate prognosis and decision-making with patients with acute SCI and assist researchers in the conduct of clinical trials to assess novel interventions and potentially direct treatment.

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Disclosure

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Appendix Authors

Name	Location	Contribution
Sophie Stukas, PhD	Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, British Columbia, Canada; Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data
Jennifer Cooper, BMLSc	Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, British Columbia, Canada; Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada	Major role in the acquisition of data and analysis or interpretation of data
Jasmine Gill, MSc	Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, British Columbia, Canada; Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada	Major role in the acquisition of data
Nader Fallah, PhD	Praxis Spinal Cord Institute, Vancouver General Hospital, Blusson Spinal Cord Center, Vancouver, British Columbia, Canada; Division of Neurology, Department of Medicine, University of British Columbia, Vancouver, British Columbia, Canada	Analysis or interpretation of data
Michael A. Skinnider, BASc	Michael Smith Laboratories, University of British Columbia, Vancouver, British Columbia, Canada	Analysis or interpretation of data
Lise Belanger, RN	Vancouver Spine Research Program, Vancouver General Hospital, Blusson Spinal Cord Center, Vancouver, British Columbia, Canada	Major role in the acquisition of data
Leanna Ritchie, RN	Vancouver Spine Research Program, Vancouver General Hospital, Blusson Spinal Cord Center, Vancouver, British Columbia, Canada	Major role in the acquisition of data
Angela Tsang, RN	Vancouver Spine Research Program, Vancouver General Hospital, Blusson Spinal Cord Center, Vancouver, British Columbia, Canada	Major role in the acquisition of data
Kevin Dong, BSc	International Collaboration on Repair Discoveries (ICORD), University of British Columbia, Blusson Spinal Cord Center, Vancouver, British Columbia Canada	Analysis or interpretation of data
Femke Streijger, PhD	International Collaboration on Repair Discoveries (ICORD), University of British Columbia, Blusson Spinal Cord Center, Vancouver, British Columbia Canada	Analysis or interpretation of data
John Street, MD, PhD, FRCSI	International Collaboration on Repair Discoveries (ICORD), University of British Columbia, Blusson Spinal Cord Center, Vancouver, British Columbia Canada; Vancouver Spine Surgery Institute, Department of Orthopaedics, University of British Columbia, Blusson Spinal Cord Center, Vancouver, British Columbia, Canada	Major role in the acquisition of data and study concept or design
Scott Paquette, MD, Med, FRSCS	Division of Neurosurgery, University of British Columbia, Vancouver, British Columbia, Canada	Major role in the acquisition of data and study concept or design
Tamir Ailon, MD, MPH, FRCSC	Division of Neurosurgery, University of British Columbia, Vancouver, British Columbia, Canada	Major role in the acquisition of data and study concept or design
Nicolas Dea, MD, MSc, FRCSC	Division of Neurosurgery, University of British Columbia, Vancouver, British Columbia, Canada	Major role in the acquisition of data and study concept or design
Raphaele Charest-Morin, MD, FRCSC	Vancouver Spine Surgery Institute, Department of Orthopaedics, University of British Columbia, Blusson Spinal Cord Center, Vancouver, British Columbia, Canada	Major role in the acquisition of data and study concept or design
Charles G. Fisher, MD, MPH, FRCSC	Vancouver Spine Surgery Institute, Department of Orthopaedics, University of British Columbia, Blusson Spinal Cord Center, Vancouver, British Columbia, Canada	Major role in the acquisition of data and study concept or design
Christopher S. Bailey, MD, MSc, FRCSC	Division of Orthopaedics, Schulich School of Medicine, University of Western Ontario, London, Ontario, Canada;	Major role in the acquisition of data and study concept or design
Sanjay Dhall, MD	Department of Neurosurgery, University of California San Francisco, San Francisco, CA, USA	Major role in the acquisition of data and study concept or design

Continued

Appendix *(continued)*

Name	Location	Contribution
Jean-Marc Mac-Thiong, MD, PhD, FRCSC	Department of Surgery, Hôpital du Sacré-Coeur de Montréal, Montreal, Quebec, Canada; Department of Surgery, Chu Sainte-Justine, University of Montreal, Montreal, Quebec, Canada	Major role in the acquisition of data and study concept or design
Jefferson R. Wilson, MD, PhD, FRCSC	Division of Neurosurgery, University of Toronto, St. Michael's Hospital, Toronto, Ontario, Canada	Major role in the acquisition of data and study concept or design
Sean Christie, MD, FRCSC	Division of Neurosurgery, Halifax Infirmary, Dalhousie University, Halifax, Nova Scotia, Canada	Major role in the acquisition of data and study concept or design
Marcel F. Dvorak, MD, FRCSC	International Collaboration on Repair Discoveries (ICORD), University of British Columbia, Blusson Spinal Cord Center, Vancouver, British Columbia Canada; Vancouver Spine Surgery Institute, Department of Orthopaedics, University of British Columbia, Blusson Spinal Cord Center, Vancouver, British Columbia, Canada	Major role in the acquisition of data and study concept or design
Cheryl L. Wellington, PhD	Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, British Columbia, Canada; Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada;	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; and analysis or interpretation of data
Brian K. Kwon, MD, PhD, FRCSC	International Collaboration on Repair Discoveries (ICORD), University of British Columbia, Blusson Spinal Cord Center, Vancouver, British Columbia Canada; Vancouver Spine Surgery Institute, Department of Orthopaedics, University of British Columbia, Blusson Spinal Cord Center, Vancouver, British Columbia, Canada; School of Biomedical Engineering, University of British Columbia, Vancouver, British Columbia, Canada	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data

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Sophie Stukas, Jennifer Cooper, Jasmine Gill, et al.

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