Supplementary materials

eMethods and eResults – Biomarker Assays

Simoa Assays: For SCI patients we used the following dilutions: serum NF-L (10x), CSF NF-L (1,000x), serum GFAP (40x), CSF GFAP (100,000x). The 100,000-fold dilution was obtained by performing two serial 1:100 dilutions (5ul sample + 495ul dilution buffer) followed by a final 1:10 dilution (50ul sample + 450ul of dilution buffer). The same dilution strategy was used for controls, except that CSF was diluted 1,000x for GFAP. A theoretical upper limit of detection (ULOD) was generated based on the highest measurable concentration from samples above the upper limit of quantification (ULOQ) but that had not saturated the detector. Based on observed data for GFAP and NF-L (**eFig. 2**), we estimated the theoretical ULOD to be at least 3x (serum) or 5x (CSF) the ULOQ. As such, we used these inferred values to give a conservative estimate of the analyte concentration, as previously described,[21, 22] as further dilution of the samples may lead to challenges with dilution linearity or inability to quantify analytes with lower concentrations.

To validate imputed ULOD GFAP concentrations used for analysis, a sub-set of 5 serum and 9 CSF samples previously measured over the ULOD were selected for re-analysis in 2021 using an independent lot of the GFAP Discovery Assay (lot 503138). Samples over the ULOD were randomized and then selected such that all four timepoints were represented. A serial dilution strategy was used. Serum samples were serially diluted at 80, 160 and 320-fold; CSF samples were serially diluted at 200,000-, 400,000- and 800,000-fold using the same dilution strategy as described in the main text methods section with the exception being the third step was a 1:20 dilution as opposed to a 1:10 dilution. For each sample, a grand average GFAP concentration was calculated by combining the data generated at all three dilutions tested (or as many as produced data points). To allow for cross lot comparison, 10 serum and 20 CSF samples spanning the detectable GFAP range were analyzed using either a 40-fold (serum) or 100,000-fold (CSF) dilution. Data were analyzed using a Spearman Rho correlation and Bland Altman plot to determine cross lot bias. Linear regression was used to generate a cross lot correction factor that was used for CSF.

Overall, there was a very strong positive correlation between the GFAP data originally produced in 2019 compared to the analysis performed in 2021 among samples that were quantifiable in both sets of analysis. The Spearman Rho for serum was 0.98 ($n=10$, $p<0.0001$) and for CSF was 0.95

(n=20, p<0.0001). Bland Altman analysis revealed that the mean bias between lots was -2.4% for serum but 143% for CSF, implying that in CSF specifically, concentrations calculated in 2021 are significantly lower compared to 2019, when the primary analysis was done. A cross lot correction factor (y=8.065X + 57) was generated and applied to CSF concentrations calculated in 2021, prior to correction for dilution, to allow for the more accurate comparison between 2019 and 2021. The median concentration of GFAP in serum samples previously >ULOD was $2.11x10^5$ pg/ml and 4 out of 5 samples tested were above the theoretical ULOD that was set at 1.2×10^5 pg/ml (**eFigure 1C**). In CSF, the median GFAP was 2.11×10^9 pg/ml, following cross lot correction, with all 9 samples falling above the theoretical ULOD of 5×10^8 pg/ml (**eFigure 1D**).

Supplementary Tables

eTable 1. Discovery Human GFAP and NF-L Advantage Simoa assay specifications provided by Quanterix.

	NF-L	GFAP
Calibrator range, pg/ml	0 – 500	0 – 1000
Dynamic range, pg/ml, serum[a]	0 – 5000	0 – 40,000
Dynamic range, pg/ml, CSF[b]	0 – 50, 000	0 – $1x10^8$
LLOD[c], pg/ml	0.038	0.211
LLOQ[d], pg/ml	0.174	0.686
Spike-recovery (serum/plasma), mean recovery	90.7% [e]	92.8% [f]
Spike-recovery (CSF), mean recovery	118.5% [g]	N/A
Linearity, mean recovery	97.0% [h]	N/A
Dilution linearity [i] (serum), mean recovery	100.7%	106.2%
Dilution linearity[i] (CSF), mean recovery	100.4%	90.1%
Within run CV, mean[j]	7.8%	8.5%
Between run CV, mean[j]	4.1%	10.8%
Inter-lot CV, mean[k]	1.74%	N/A
Inter-instrument CV, mean[l]	2.07%	N/A

[a] Dynamic range is corrected for dilution factor. Serum NF-L 10-fold, GFAP 40-fold
[b] Dynamic range is corrected for dilution factor. CSF NF-L 1000-fold, GFAP 100,000-fold
[c] LLOD is defined as 2.5 SD from calibrator A (blank). NF-L: 3 replicates x 12 runs across 3 instruments, 2 reagent lots, 2 calibrator lots. GFAP: 3 reps x 5 runs, one instrument, one reagent lot
[d] LLOQ is defined as <20%, 80-120% recovery. NF-L: 12 runs across3 instruments, 2 reagent lots. GFAP: 5 runs, one instrument, one reagent lot
[e] NF-L spiked into 4 serum and 2 plasma samples at 10, 100, and 1000 pg/ml.
[f] GFAP spiked 2 serum and 2 plasma samples at two concentrations
[g] NF-L spiked into 6 CSF samples at 100, 1000, and 10000 pg/ml
[h] high NF-L plasma sample fractionally admixed with low NF-L plasma sample, mean of 10 levels
[i] spiked serum or CSF diluted 2x serially from minimum recommended dilution to 128-fold with sample diluent
[j] Reproducibility was determined with guidance from CLSI Protocol EP5-A. Three serum/plasma panels were assayed in replicates of three for: NF-L; two runs on each of three instruments and two reagent lots. GFAP; 5 runs on one instrument and one reagent lot
[k] Pool of CVs from 5 samples tested with 2 reagent lots across 2 runs x 3 instruments
[l] Pool of CVs from 5 samples tested with 3 instruments across 2 runs x 2 reagent lots

eTable 2. SCI patient enrollment by neurological lesion level and AIS grade at baseline.

Lesion level	AIS A	AIS B	AIS C	Total
C1	1			1
C2			1	1
C3	2		2	4
C4	15			15
C5	11	4	11	26
C6	8	7	1	16
C7	2	3		5
C8				0
T1		1	1	2
T2	2			2
T3	3		1	4
T4	2	1		3
T5	4		1	5
T6	5	1		6
T7	2			2
T8	1	1		2
T9	4			4
T10	2			2
T11	4		1	5
T12	4			4
L1	6	2	1	9
Total	*78*	*20*	*20*	**118**

eTable 3. SCI patient demographics and injury characterization by enrolling clinical trial designation.

	CSF Drainage	CSF Pressure	CAMPER	P-value
Patient N, %	28 (24)	21 (18)	69 (58)	
Male, N (%)	19 (68)	20 (95)	55 (80)	0.03/0.3/0.2[a]
Female, N (%)	9 (32)	1 (5)	14 (20)	
Age, y, mean (SD)	40.9 (12.3)	45.1 (18.0)	43.1 (18.4)	0.7[b]
Cervical injury level, N (%)	22 (79)	10 (48)	36 (52)	0.04/0.02/0.8
Thoracolumbar injury level, N (%)	6 (21)	11 (52)	33 (48)	
AIS A, n (%)	16 (57)	15 (62)	49 (71)	0.6/0.2/0.6
AIS B, n (%)	6 (21)	5 (24)	9 (13)	>0.9/0.4/0.3
AIS C, n (%)	6 (21)	3 (14)	11 (16)	0.5/0.6/>0.9

[a] Categorical data was analyzed using a Fisher's exact test comparing the following order of pairings: Drainage vs Pressure/ Drainage vs CAMPER / Pressure vs CAMPER

[b] Continuous group wise comparisons were done using a one-way ANOVA

eTable 4. Frequency and distribution for serum and CSF samples from SCI and control patients greater than the ULOQ or ULOD for GFAP and NF-L by baseline AIS grade and timepoint measured.

AIS grade	24h			48h			72h			96h			Grand total		
	Sample, n	> ULOQ[a]	> ULOD[a]	Sample, n	> ULOQ[a]	> ULOD[a]	Sample, n	> ULOQ[a]	> ULOD[a]	Sample, n	> ULOQ[a]	> ULOD[a]	Sample, n	> ULOQ[a]	> ULOD[a]
Serum NF-L															
AIS A	71	0 (0)	0 (0)	69	0 (0)	0 (0)	70	0 (0)	0 (0)	67	0 (0)	0 (0)	279	0 (0)	0 (0)
AIS B	17	0 (0)	0 (0)	15	0 (0)	0 (0)	19	0 (0)	0 (0)	15	0 (0)	0 (0)	64	0 (0)	0 (0)
AIS C	17	0 (0)	0 (0)	15	0 (0)	0 (0)	15	0 (0)	0 (0)	12	0 (0)	0 (0)	59	0 (0)	0 (0)
Cont.													19	0 (0)	0 (0)
Serum GFAP															
AIS A	71	25 (35)	12 (17)	69	31 (45)	17 (25)	70	24 (34)	14 (20)	67	15 (22)	7 (10)	279	95 (34)	50 (18)
AIS B	17	4 (24)	0 (0)	15	2 (13)	0 (0)	19	1 (5)	0 (0)	15	0 (0)	0 (0)	64	7 (11)	0 (0)
AIS C	17	0 (0)	0 (0)	15	0 (0)	0 (0)	15	0 (0)	0 (0)	12	0 (0)	0 (0)	59	0 (0)	0 (0)
Cont.													19	0 (0)	0 (0)
CSF NF-L															
AIS A	69	12 (17)	0 (0)	65	13 (20)	1 (2)	66	10 (15)	3 (5)	59	11 (19)	2 (3)	259	46 (18)	6 (2)
AIS B	16	0 (0)	0 (0)	12	0 (0)	0 (0)	15	0 (0)	0 (0)	14	0 (0)	0 (0)	57	0 (0)	0 (0)
AIS C	16	0 (0)	0 (0)	13	0 (0)	0 (0)	15	0 (0)	0 (0)	11	0 (0)	0 (0)	55	0 (0)	0 (0)
Cont.													19	0 (0)	0 (0)
CSF GFAP															
AIS A	69	26 (38)	33 (48)	65	26 (40)	27 (42)	66	22 (33)	22 (33)	59	19 (32)	15 (25)	259	93 (36)	97 (37)
AIS B	16	6 (38)	2 (13)	12	5 (42)	0 (0)	15	3 (20)	0 (0)	14	1 (7)	0 (0)	57	15 (26)	2 (4)
AIS C	16	1 (6)	0 (0)	13	2 (15)	0 (0)	15	0 (0)	0 (0)	11	0 (0)	0 (0)	55	3 (5)	0 (0)
Cont.													19	1 (5)	0 (0)

[a]Data is listed as n (%)

eTable 5. Serum NF-L and GFAP classification of baseline AIS grade using logistic regression. Serum ROC curve characterization for discrimination of baseline AIS grade [AIS A vs AIS B; AIS A vs AIS C; AIS B vs AIS C] of SCI patients using serum NF-L and GFAP either alone or in combination at 24h, 48h, 72h, or 96h. All analyses were performed on log transformed concentrations.

	24h	48h	72h	96h
Serum NF-L alone				
AIS A vs AIS B	0.751 (0.063) 0.627, 0.876 P=0.0044	0.734 (0.066) 0.651, 0.863 P=0.0060	0.825 (0.045) 0.736, 0.913 P<0.0001	0.793 (0.051) 0.693, 0.893 P=0.0004
AIS A vs AIS C	0.903 (0.039) 0.827, 0.980 P<0.0001	0.957 (0.021) 0.916, 0.998 P<0.0001	0.941 (0.026) 0.889, 0.995 P<0.0001	0.932 (0.029) 0.875, 0.988 P<0.0001
AIS B vs AIS C	0.830 (0.084) 0.664, 0.995 P=0.0036	0.943 (0.039) 0.866, 1.000 P<0.0001	0.800 (0.080) 0.642, 0.958 P=0.0034	0.811 (0.082) 0.651, 0.971 P=0.0063
Serum GFAP alone				
AIS A vs AIS B	0.666 (0.070) 0.529, 0.803 P=0.0596	0.806 (0.055) 0.698, 0.914 P=0.0003	0.855 (0.042) 0.772, 0.938 P<0.0001	0.816 (0.052) 0.713, 0.919 P=0.0001
AIS A vs AIS C	0.853 (0.047) 0.761, 0.944 P<0.0001	0.960 (0.019) 0.922, 0.998 P<0.0001	0.954 (0.021) 0.913, 0.995 P<0.0001	0.951 (0.023) 0.907, 0.996 P<0.0001
AIS B vs AIS C	0.775 (0.091) 0.596, 0.954 P=0.0153	0.881 (0.067) 0.751, 1.000 P=0.0005	0.796 (0.077) 0.646, 0.947 P=0.038	0.789 (0.090) 0.612, 0.966 P=0.0112
Serum NF-L and GFAP combined				
AIS A vs AIS B	0.753 (0.063) 0.629, 0.876 P=0.004	0.809 (0.054) 0.703, 0.915 P<0.0001	0.861 (0.041) 0.780, 0.941 P<0.0001	0.829 (0.052) 0.734, 0.924 P<0.0001
AIS A vs AIS C	0.907 (0.035) 0.838, 0.975 P<0.0001	0.962 (0.019) 0.925, 0.999 P<0.0001	0.956 (0.021) 0.915, 0.996 P<0.0001	0.955 (0.022) 0.912, 0.998 P<0.0001
AIS B vs AIS C	0.835 (0.082) 0.675, 0.996 P=0.003	0.952 (0.035) 0.884, 1.000 P<0.0001	0.830 (0.074) 0.685, 0.974 P=0.001	0.822 (0.080) 0.665, 0.980 P=0.005

ROC curve data: area under the receiver operating characteristic (AUROC) (SD); 95% confidence interval; P-value

eTable 6. CSF NF-L and GFAP classification of baseline AIS grade using logistic regression. CSF ROC curve characterization for discrimination of baseline AIS grade [AIS A vs AIS B; AIS A vs AIS C; AIS B vs AIS C] of SCI patients using CSF NF-L and GFAP either alone or in combination at 24h, 48h, 72h, or 96h. All analysis were performed on log transformed concentrations.

	24h	48h	72h	96h
CSF NF-L alone				
AIS A vs AIS B	0.804 (0.055) 0.696, 0.913 P=0.0004	0.814 (0.063) 0.691, 0.937 P=0.0006	0.862 (0.045) 0.773, 0.950 P<0.0001	0.848 (0.052) 0.746, 0.949 P<0.0001
AIS A vs AIS C	0.939 (0.030) 0.880, 0.997 P<0.0001	0.968 (0.024) 0.922, 1.014 P<0.0001	0.936 (0.032) 0.873, 1.000 P<0.0001	0.831 (0.066) 0.701, 0.960 P=0.0005
AIS B vs AIS C	0.878 (0.071) 0.738, 1.017 P=0.0007	0.904 (0.063) 0.781, 1.027 P=0.0006	0.756 (0.092) 0.576, 0.936 P=0.0171	0.597 (0.126) 0.351, 0.844 P=0.4115
CSF GFAP alone				
AIS A vs AIS B	0.734 (0.072) 0.593, 0.875 P=0.0062	0.853 (0.049) 0.757, 0.948 P=0.0001	0.832 (0.047) 0.741, 0.924 P<0.0001	0.866 (0.049) 0.769, 0.962 P<0.0001
AIS A vs AIS C	0.901 (0.034) 0.835, 0.968 P<0.0001	0.922 (0.031) 0.860, 0.983 P<0.0001	0.910 (0.034) 0.844, 0.976 P<0.0001	0.841 (0.052) 0.740, 0.943 P=0.0004
AIS B vs AIS C	0.827 (0.080) 0.671, 0.982 P=0.0033	0.712 (0.108) 0.499, 0.924 P=0.0727	0.667 (0.101) 0.468, 0.865 P=0.1198	0.584 (0.118) 0.352, 0.816 P=0.4767
CSF NF-L and GFAP combined				
AIS A vs AIS B	0.805 (0.056) 0.697, 0.914 P<0.0001	0.851 (0.049) 0.755, 0.947 P<0.0001	0.880 (0.041) 0.799, 0.960 P<0.0001	0.878 (0.050) 0.780, 0.976 P<0.0001
AIS A vs AIS C	0.941 (0.029) 0.883, 0.998 P<0.0001	0.976 (0.015) 0.947, 1.000 P<0.0001	0.946 (0.027) 0.894, 0.998 P<0.0001	0.871 (0.057) 0.760, 0.982 P<0.0001
AIS B vs AIS C	0.857 (0.072) 0.716, 0.999 P=0.001	0.904 (0.063) 0.781, 1.000 P=0.001	0.791 (0.084) 0.627, 0.955 P=0.007	0.571 (0.123) 0.330, 0.813 P=0.123

ROC curve data: area under the receiver operating characteristic (AUROC) (SD); 95% confidence interval; P-value

eTable 7. Serum NF-L and GFAP combined with linear discriminant analysis to classify baseline AIS grade. Percent accuracy of combining serum NF-L and GFAP with linear discriminant analysis for classifying baseline AIS grade [AIS A vs AIS B; AIS A vs AIS C; AIS B vs AIS C]. Subsequent leave-out-one cross validation was performed to assess the robustness of prediction models. Analyses were performed on log transformed serum NF-L and GFAP 24h, 48h, 72h, and 96h.

% correct classification	**24h**		**48h**		**72h**		**96h**	
	Original model	Cross validation	Original model	Cross validation	Original model	Cross validation	Original model	Cross validation
AIS A vs AIS B	66.7	64.1	72.6	72.6	78.7	77.5	74.4	74.4
AIS A vs AIS C	82.3	81.0	89.4	89.4	90.7	88.4	89.9	89.9
AIS B vs AIS C	81.5	70.4	86.2	79.3	72.7	69.7	77.8	66.7

eTable 8. Serum NF-L and GFAP classification of baseline AIS grade using multinomial logistic regression. Percent correct classification of baseline AIS A, B, and C grade using multinomial logistic regression with serum NF-L and GFAP either alone or in combination at 24h, 48h, 72h, and 96h. All analysis were performed on log transformed concentrations.

% correct classification	**24h**	**48h**	**72h**	**96h**
Serum NF-L alone				
AIS A	92.3	94.3	94.4	95.5
AIS B	0.0	0.0	0.0	0.0
AIS C	50.0	66.7	73.3	50.0
overall	72.8	76.8	75.0	74.5
Serum GFAP alone				
AIS A	93.8	93.8	94.4	97.0
AIS B	9.0	9.0	5.6	0.0
AIS C	35.7	35.7	60.0	58.3
overall	71.1	71.1	74	76.6
Serum NF-L and GFAP combined				
AIS A	90.8	95.7	95.8	95.5
AIS B	0.0	14.3	16.7	13.3
AIS C	50.0	73.3	60.0	58.3
overall	71.7	80.8	76.9	77.7
Comparison %	72.8/71.1/71.7	76.8/71.1/80.8	75/74/76.9	74.5/76.6/77.7

eTable 9. Serum NF-L and GFAP prediction of whether AIS grade conversion occurred in AIS A patients at 6 months using logistic regression. Serum ROC curve characterization for AIS A patients categorized by the occurrence (yes) or absence (no) of AIS grade conversion at 6-months using serum NF-L and GFAP either alone or in combination at 24h, 48h, 72h, or 96h. Analyses were conducted on log transformed concentrations.

Yes vs No	**24h**	**48h**	**72h**	**96h**
Serum NF-L alone				
AUROC (SD)	0.793 (0.058)	0.702 (0.066)	0.803 (0.053)	0.765 (0.060)
95% CI	0.678, 0.907	0.573, 0.831	0.699, 0.907	0.648, 0.883
P-value	P=0.0001	P=0.0076	P<0.0001	P=0.0005
Serum GFAP alone				
AUROC (SD)	0.682 (0.070)	0.693 (0.067)	0.805 (0.053)	0.818 (0.054)
95% CI	0.544, 0.820	0.562, 0.825	0.702, 0.909	0.712, 0.925
P-value	P=0.0175	P=0.0106	P<0.0001	P<0.0001
Serum NF-L and GFAP combined				
AUROC (SD)	0.795 (0.058)	0.720 (0.065)	0.829 (0.050)	0.829 (0.051)
95% CI	0.681, 0.909	0.593, 0.847	0.732, 0.926	0.729, 0.929
P-value	P<0.0001	P=0.004	P<0.0001	P<0.0001

eTable 10. CSF NF-L and GFAP prediction of whether AIS grade conversion occurred in AIS A patients at 6 months using logistic regression. CSF ROC curve characterization for AIS A patients categorized by the occurrence (yes) or absence (no) of AIS grade conversion at 6-months using CSF NF-L and GFAP either alone or in combination at 24h, 48h, 72h, or 96h. Analyses were conducted on log transformed concentrations.

Yes vs No	24h	48h	72h	96h
CSF NF-L alone				
AUROC (SD)	0.806 (0.055)	0.808 (0.056)	0.837 (0.049)	0.878 (0.044)
95% CI	0.698, 0.914	0.699, 0.918	0.740, 0.934	0.791, 0.965
P-value	P<0.0001	P<0.0001	P<0.0001	P<0.0001
CSF GFAP alone				
AUROC (SD)	0.678 (0.074)	0.733 (0.074)	0.899 (0.040)	0.794 (0.059)
95% CI	0.533, 0.824	0.589, 0.878	0.820, 0.977	0.679, 0.910
P-value	P=0.0197	P=0.0032	P<0.0001	P=0.0003
CSF NF-L and GFAP combined				
AUROC (SD)	0.813 (0.054)	0.812 (0.055)	0.898 (0.040)	0.879 (0.044)
95% CI	0.706, 0.919	0.704, 0.920	0.820, 0.975	0.793, 0.965
P-value	P<0.0001	P<0.0001	P<0.0001	P<0.0001

eTable 11. Serum NF-L and GFAP combined with linear discriminant analysis to predict AIS grade conversion at 6 months in AIS A patients. Percent accuracy of combining serum NF-L and GFAP with linear discriminant analysis for predicting the occurrence (yes) or absence (no) of AIS grade conversion in AIS A patients 6-months post injury. Subsequent leave-out-one cross validation was performed to assess the robustness of prediction models. Analyses were performed on log transformed serum NF-L and GFAP 24h, 48h, 72h, and 96h.

% correct classification	24h		48h		72h		96h	
	Original model	Cross validation	Original model	Cross validation	Original model	Cross validation	Original model	Cross validation
Yes vs No	75.0	73.9	75.8	73.7	81.0	81.0	80.2	79.1

eTable 12. Serum NF-L or GFAP combined with sensory ZPP to predict AIS grade conversion in cervical AIS A patients at 6 months. Fifteen out of the 34 cervical AIS A patients with 6-month outcome and ZPP scoring experienced improvement in AIS grade. Logistic regression models were generated to predict AIS grade improvement using serum NF-L or GFAP 24h, 48h, 72h, or 96h either alone or in combination with sensory ZPP (length dichotomized 0-2 vs 3+). Model fit was compared using the Likelihood ratio (LR) test. All analysis were performed on log transformed concentrations.

% correct classification yes vs no		24h	48h	72h	96h
Serum NF-L -/+ sZPP					
% Correct classification	NF-L	73.3	71.0	70.0	56.7
	NF-L + sZPP	80.0	77.4	86.7	86.7
Likelihood ratio test	LR	12.2	6.36	6.44	7.33
	p-value	0.0005	0.012	0.011	0.008
Serum GFAP -/+ sZPP					
% Correct classification	GFAP	66.7	67.7	70.0	70.0
	GFAP + sZPP	76.7	77.4	83.3	90.0
Likelihood ratio test	LR	13.9	4.64	4.92	6.82
	p-value	0.0002	0.031	0.027	0.009

eTable 13. Serum NF-L or GFAP combined with sensory ZPP to predict AIS grade conversion in thoracolumbar AIS A patients at 6 months. Nine out of the 34 thoracolumbar AIS A patients with 6-month outcome and ZPP scoring experienced improvement in AIS grade. Logistic regression models were generated to predict AIS grade improvement using serum NF-L or GFAP 24h, 48h, 72h, or 96h either alone or in combination with sensory ZPP (length dichotomized 0-2 vs 3+). Model fit was compared using the Likelihood ratio (LR) test. All analysis were performed on log transformed concentrations.

% correct classification		24h	48h	72h	96h
Serum NF-L -/+ sZPP					
% Correct classification	NF-L	73.1	76.7	81.3	83.3
	NF-L + sZPP	88.5	93.3	Cannot computer logistic regression model due to perfect separation	
Likelihood ratio test	LR	11.5	19.7		
	p-value	0.0007	<0.0001		
Serum GFAP -/+ sZPP					
% Correct classification	GFAP	69.2	76.7	81.3	80.0
	GFAP + sZPP	88.5	93.3	Cannot computer logistic regression model due to perfect separation	
Likelihood ratio test	LR	13.3	18.5		
	p-value	0.0003	<0.0001		

eTable 14. Serum NF-L and GFAP prediction of 6-month AIS grade using logistic regression. Serum ROC curve characterization for prediction of 6-month AIS grade for patients categorized as having motor complete (AIS A and AIS B) versus motor incomplete (AIS C and AIS D) injuries using serum NF-Land GFAP either alone or in combination at 24h, 48h, 72h, or 96h. Analyses were performed on log transformed concentrations.

Motor complete vs incomplete	24h	48h	72h	96h
Serum NF-L alone				
AUROC (SD)	0.870 (0.042)	0.920 (0.032)	0.932 (0.025)	0.894 (0.037)
95% CI	0.787, 0.952	0.859, 0.982	0.884, 0.981	0.822, 0.966
P-value	P<0.0001	P<0.0001	P<0.0001	P<0.0001
Serum GFAP alone				
AUROC (SD)	0.810 (0.046)	0.919 (0.031)	0.948 (0.019)	0.948 (0.021)
95% CI	0.720, 0.900	0.858, 0.979	0.910, 0.986	0.906, 0.990
P-value	P<0.0001	P<0.0001	P<0.0001	P<0.0001
Serum NF-L and GFAP combined				
AUROC (SD)	0.875 (0.041)	0.939 (0.026)	0.962 (0.016)	0.957 (0.019)
95% CI	0.796, 0.955	0.889, 0.990	0.930, 0.993	0.920, 0.994
P-value	P<0.0001	P<0.0001	P<0.0001	P<0.0001

eTable 15. CSF NF-L and GFAP prediction of 6-month AIS grade using logistic regression. CSF ROC curve characterization for prediction of 6-month AIS grade for patients categorized as having motor complete (AIS A and AIS B) versus motor incomplete (AIS C and AIS D) injuries using CSF NF-Land GFAP either alone or in combination at 24h, 48h, 72h, or 96h. Analyses were performed on log transformed concentrations.

Motor complete vs incomplete	24h	48h	72h	96h
CSF NF-L alone				
AUROC (SD)	0.908 (0.032)	0.932 (0.030)	0.930 (0.026)	0.907 (0.034)
95% CI	0.845, 0.971	0.873, 0.991	0.879, 0.980	0.840, 0.974
P-value	P<0.0001	P<0.0001	P<0.0001	P<0.0001
CSF GFAP alone				
AUROC (SD)	0.883 (0.041)	0.917 (0.033)	0.935 (0.023)	0.901 (0.033)
95% CI	0.802, 0.965	0.856, 0.982	0.889, 0.980	0.836, 0.966
P-value	P<0.0001	P<0.0001	P<0.0001	P<0.0001
CSF NF-L and GFAP combined				
AUROC (SD)	0.921 (0.029)	0.943 (0.028)	0.954 (0.021)	0.933 (0.026)
95% CI	0.864, 0.978	0.889, 0.998	0.913, 0.995	0.882, 0.985
P-value	P<0.0001	P<0.0001	P<0.0001	P<0.0001

eTable 16. Serum NF-L and GFAP combined with linear discriminant analysis to predict AIS grade observed at 6 months. Percent accuracy of combining serum NF-L and GFAP with linear discriminant analysis for classifying SCI patients as having motor complete (AIS A and AIS B) versus motor incomplete (AIS C and AIS D) SCI injuries at 6 months. Subsequent leave-out-one cross validation was performed to assess the robustness of prediction models. Analyses were performed on log transformed serum NF-L and GFAP 24h, 48h, 72h, and 96h.

% correct classification	**24h**		**48h**		**72h**		**96h**	
	Original model	Cross validation	Original model	Cross validation	Original model	Cross validation	Original model	Cross validation
Yes vs No	81.8	79.5	89.5	88.4	88.0	87.0	86.8	86.8

eTable 17. Serum NF-L or GFAP combined with baseline AIS grade to predict 6-month motor complete (AIS A/B) versus motor incomplete (AIS C/D) in AIS A and B patients. Of the 74 AIS A patients with outcome, 64 were considered motor complete (86%) and 10 (14%) were motor incomplete; for AIS B patients, 6 out of 19 (32%) were motor complete and 13 (68%) were scored as motor incomplete at 6 months. Logistic regression models were generated to predict outcome using serum NF-L or GFAP at 24h, 48h, 72h, or 96h alone or combined with baseline AIS grade (A or B). Model fit was compared using the Likelihood ratio (LR) test. All analysis were performed on log transformed concentrations.

% correct classification		**24h**	**48h**	**72h**	**96h**
Serum NF-L -/+ baseline AIS					
% Correct classification	NF-L	83.8	82.5	85.9	82.3
	NF-L + AIS	82.4	86.3	88.2	86.1
Likelihood ratio test	LR	4.50	8.54	7.00	6.67
	p-value	0.034	0.004	0.008	0.0097
Serum GFAP -/+ baseline AIS					
% Correct classification	GFAP	78.4	85.0	84.7	87.3
	GFAP +AIS	82.4	86.3	87.1	88.6
Likelihood ratio test	LR	6.85	5.19	4.51	ns
	p-value	0.0089	0.023	0.034	

eTable 18. Correlation of 24h, 48h, 72h, and 96h serum and CSF NF-L and GFAP and the change (6 month minus baseline) in UEMS, LEMS, and TMS in all cervical SCI patients.

		24h	48h	72h	96h
Serum NF-L					
ΔUEMS[a]	Rho[d]	-0.596	-0.591	-0.515	-0.410
	95% CI	-0.754 to -0.374	-0.745 to -0.376	0.688 to -0.287	-0.619 to -0.146
	P-value	P<0.0001	P<0.0001	P<0.0001	P=0.0026
ΔLEMS[b]	Rho	-0.565	-0.672	-0.650	-0.596
	95% CI	-0.733 to -0.333	-0.800 to -0.487	-0.782 to -0.462	-0.751 to -0.379
	P-value	P<0.0001	P<0.0001	P<0.0001	P<0.0001
ΔTMS[c]	Rho	-0.599	-0.686	-0.601	-0.516
	95% CI	-0.756 to -0.378	-0.809 to -0.506	-0.748 to -0.397	-0.696 to -0.275
	P-value	P<0.0001	P<0.0001	P<0.0001	P<0.0001
Serum GFAP					
ΔUEMS	Rho	-0.411	-0.625	-0.631	-0.525
	95% CI	-0.624 to -0.142	-0.768 to -0.423	-0.769 to -0.437	-0.702 to -0.286
	P-value	P=0.003	P<0.0001	P<0.0001	P<0.0001
ΔLEMS	Rho	-0.501	-0.740	-0.742	-0.760
	95% CI	-0.688 to -0.251	-0.843 to -0.583	-0.842 to -0.591	-0.857 to -0.609
	P-value	P=0.0002	P<0.0001	P<0.0001	P<0.0001
ΔTMS	Rho	-0.448	-0.731	-0.707	-0.678
	95% CI	-0.651 to -0.186	-0.838 to -0.570	-0.820 to -0.542	-0.806 to -0.491
	P-value	P=0.0011	P<0.0001	P<0.0001	P<0.0001
CSF NF-L					
ΔUEMS	Rho	-0.601	-0.572	-0.526	-0.294
	95% CI	-0.756 to -0.383	-0.742 to -0.333	-0.702 to -0.291	-0.545 to 0.00465
	P-value	P<0.0001	P<0.0001	P<0.0001	P=0.0472
ΔLEMS	Rho	-0.604	-0.718	-0.708	-0.607
	95% CI	-0.758 to -0.387	-0.836 to -0.537	-0.824 to -0.536	-0.767 to -0.377
	P-value	P<0.0001	P<0.0001	P<0.0001	P<0.0001
ΔTMS	Rho	-0.655	-0.661	-0.636	-0.486
	95% CI	-0.792 to -0.457	-0.800 to -0.454	-0.777 to -0.435	-0.685 to -0.220
	P-value	<0.0001	<0.0001	<0.0001	0.0006
CSF GFAP					
ΔUEMS	Rho	-0.678	-0.515	-0.668	-0.248
	95% CI	-0.807 to -0.489	-0.703 to -0.259	-0.797 to -0.478	-0.509 to 0.0541
	P-value	P<0.0001	P=0.0002	P<0.0001	P=0.0961
ΔLEMS	Rho	-0.684	-0.678	-0.662	-0.668
	95% CI	-0.810 to -0.496	-0.811 to -0.478	-0.794 to -0.471	-0.806 to -0.462
	P-value	P<0.0001	P<0.0001	P<0.0001	P<0.0001
ΔTMS	Rho	-0.719	-0.630	-0.702	-0.518
	95% CI	-0.833 to -0.547	-0.780 to -0.412	-0.820 to -0.526	-0.707 to -0.260
	P-value	P<0.0001	P<0.0001	P<0.0001	P=0.0002

[a] UEMS, upper extremity motor score
[b] LEMS, lower extremity motor score
[c] TMS, total motor score
[d] data was analyzed using a Spearman Rho correlation

eTable 19. Serum NF-L and GFAP prediction of change in total motor score in all cervical SCI patients at 6 months using logistic regression. Serum ROC curve characterization for prediction of ≤ 8 point or > 8 point change in 6-month total motor score in all cervical SCI patients using serum NF-L and GFAP either alone or in combination at 24h, 48h, 72h, or 96h. Analyses were performed on log transformed concentrations.

≤ 8pt versus > 8pt ΔMS	24h	48h	72h	96h
Serum NF-L alone				
AUROC (SD)	0.860 (0.058)	0.889 (0.045)	0.872 (0.046)	0.792 (0.061)
95% CI	0.747, 0.973	0.801, 0.977	0.781, 0.963	0.672, 0.912
P-value	P<0.0001	P<0.0001	P<0.0001	P=0.0003
Serum GFAP alone				
AUROC (SD)	0.762 (0.069)	0.911 (0.037)	0.910 (0.037)	0.881 (0.046)
95% CI	0.627, 0.897	0.839, 0.983	0.838, 0.982	0.791, 0.971
P-value	P=0.0016	P<0.0001	P<0.0001	P<0.0001
Serum NF-L and GFAP combined				
AUROC (SD)	0.860 (0.058)	0.917 (0.040)	0.910 (0.037)	0.884 (0.045)
95% CI	0.747, 0.973	0.839, 0.995	0.837, 0.983	0.796, 0.972
P-value	P<0.0001	P<0.0001	P<0.0001	P<0.0001

eTable 20. CSF NF-L and GFAP prediction of change in total motor score in all cervical SCI patients at 6 months using logistic regression. CSF ROC curve characterization for prediction of ≤ 8 point or > 8 point change in 6-month total motor score in all cervical SCI patients using CSF NF-L and GFAP either alone or in combination at 24h, 48h, 72h, or 96h. Analyses were performed on log transformed concentrations.

≤ 8pt versus > 8pt ΔMS	24h	48h	72h	96h
CSF NF-L alone				
AUROC (SD)	0.897 (0.049)	0.882 (0.051)	0.869 (0.049)	0.813 (0.064)
95% CI	0.801, 0.992	0.783, 0.981	0.773, 0.965	0.688, 0.938
P-value	P<0.0001	P<0.0001	P<0.0001	P=0.0003
CSF GFAP alone				
AUROC (SD)	0.880 (0.046)	0.858 (0.057)	0.893 (0.042)	0.851 (0.056)
95% CI	0.789, 0.970	0.747, 0.969	0.811, 0.975	0.742, 0.960
P-value	P<0.0001	P<0.0001	P<0.0001	P<0.0001
CSF NF-L and GFAP combined				
AUROC (SD)	0.917 (0.040)	0.882 (0.051)	0.911 (0.038)	0.854 (0.055)
95% CI	0.839, 0.995	0.782, 0.982	0.838, 0.985	0.747, 0.962
P-value	P<0.0001	P<0.0001	P<0.0001	P<0.0001

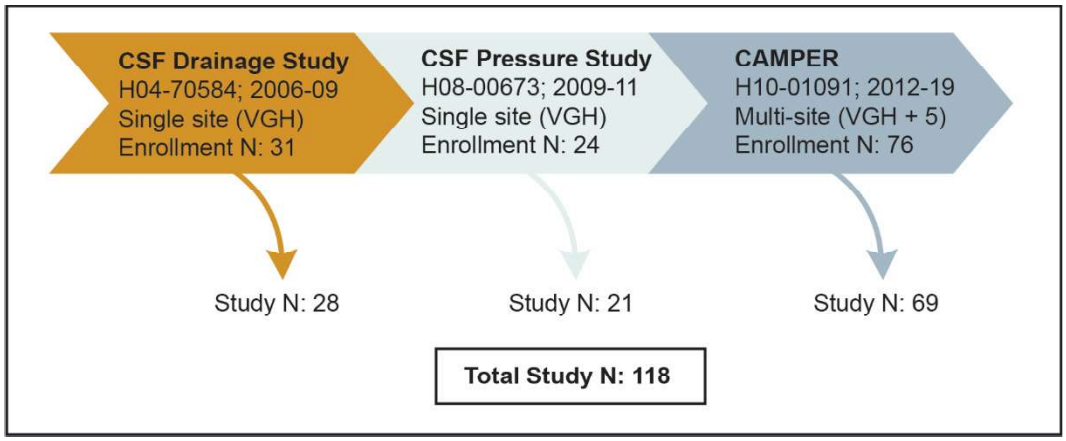
eTable 21. Serum NF-L and GFAP prediction of change in total motor score in cervical AIS A patients at 6 months using logistic regression. Serum ROC curve characterization for prediction of ≤ 8 point or > 8 point change in 6-month total motor score in cervical AIS A patients using serum NF-L and GFAP either alone or in combination at 24h, 48h, 72h, or 96h. Analyses were performed on log transformed concentrations.

≤ 8pt versus > 8pt ΔMS	24h	48h	72h	96h
Serum NF-L alone				
AUROC (SD)	0.768 (0.096)	0.781 (0.087)	0.766 (0.087)	0.680 (0.098)
95% CI	0.580, 0.956	0.611, 0.951	0.595, 0.937	0.488, 0.872
P-value	P=0.0165	P=0.0094	P=0.0174	P=0.0996
Serum GFAP alone				
AUROC (SD)	0.686 (0.096)	0.851 (0.066)	0.864 (0.068)	0.849 (0.068)
95% CI	0.498, 0.874	0.721, 0.981	0.7301, 0.997	0.715, 0.982
P-value	P=0.0955	P=0.0012	P=0.0011	P=0.0014
Serum NF-L and GFAP combined				
AUROC (SD)	0.768 (0.096)	0.860 (0.064)	0.850 (0.069)	0.835 (0.073)
95% CI	0.581, 0.956	0.734, 0.985	0.714, 0.986	0.693, 0.978
P-value	P=0.016	P=0.001	P=0.002	P=0.002

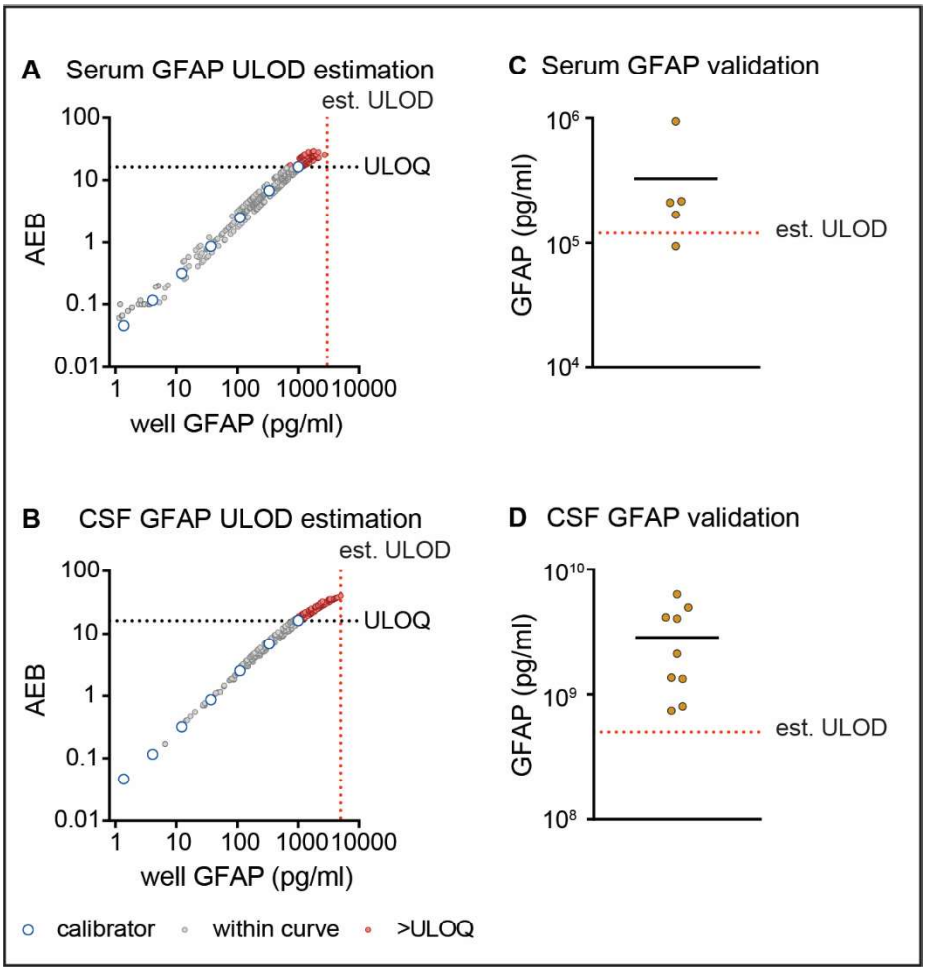
eTable 22. CSF NF-L and GFAP prediction of change in total motor score in cervical AIS A patients at 6 months using logistic regression. CSF ROC curve characterization for prediction of ≤ 8 point or > 8 point change in 6-month total motor score in cervical AIS A patients using CSF NF-L and GFAP either alone or in combination at 24h, 48h, 72h, or 96h. Analyses were performed on log transformed concentrations.

≤ 8pt versus > 8pt ΔMS	**24h**	**48h**	**72h**	**96h**
CSF NF-L alone				
AUROC (SD)	0.848 (0.068)	0.773 (0.095)	0.813 (0.078)	0.850 (0.078)
95% CI	0.715, 0.981	0.587, 0.958	0.661, 0.965	0.697, 1.003
P-value	P=0.0017	P=0.0198	P=0.0070	P=0.0030
CSF GFAP alone				
AUROC (SD)	0.822 (0.073)	0.818 (0.093)	0.879 (0.068)	0.833 (0.078)
95% CI	0.673, 0.965	0.636, 0.999	0.746, 1.012	0.680, 0.987
P-value	P=0.0037	P=0.0066	P=0.0011	P=0.0047
CSF NF-L and GFAP combined				
AUROC (SD)	0.865 (0.063)	0.810 (0.091)	0.874 (0.069)	0.878 (0.067)
95% CI	0.742, 0.989	0.632, 0.987	0.739, 1.000	0.746, 1.000
P-value	P=0.001	P=0.008	P=0.001	P=0.001

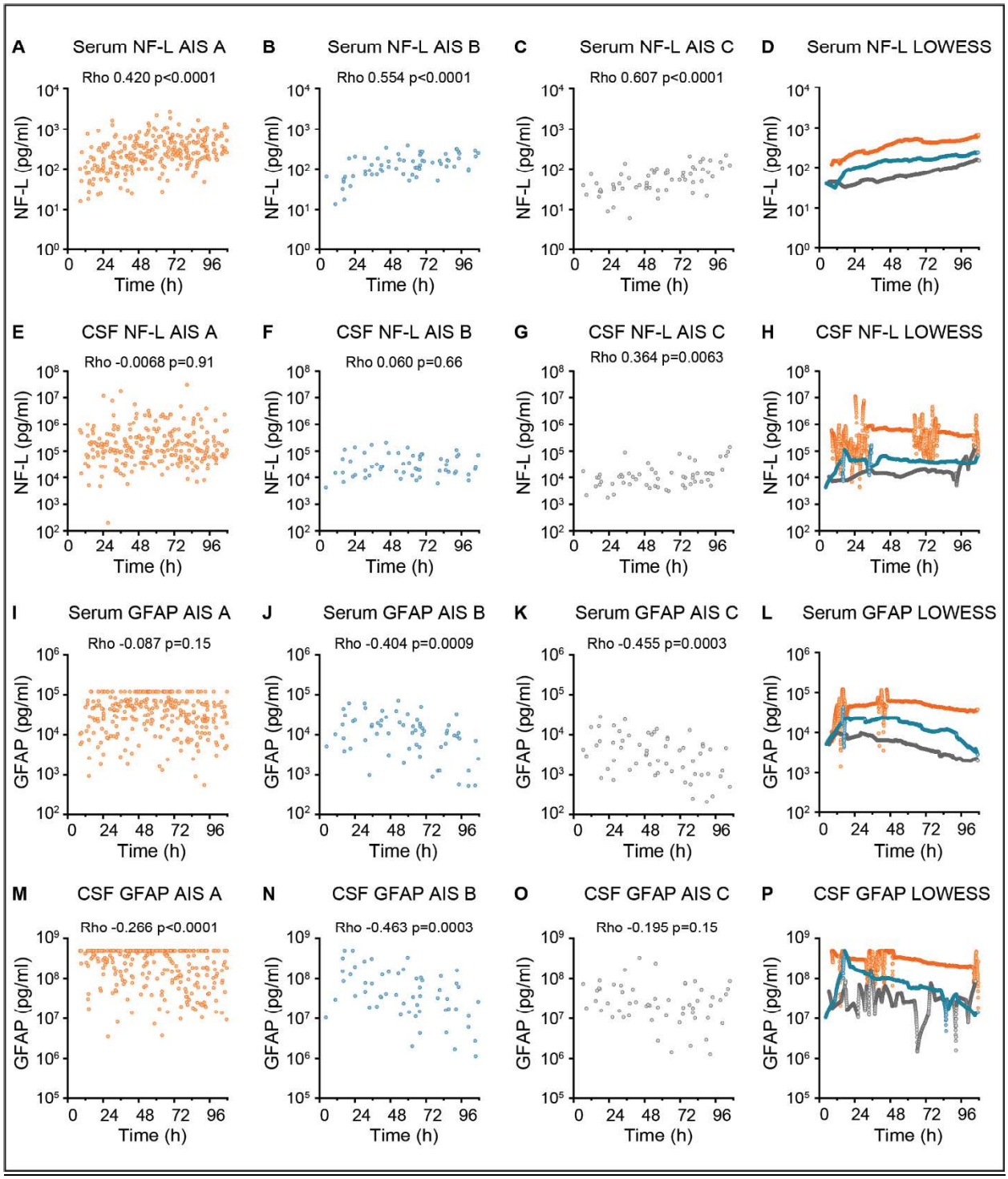
Supplementary figures



eFigure 1. SCI patient enrollment and sample selection for biomarker analysis. SCI patients, using the same inclusion/exclusion criteria, enrolled into one of three consecutive studies between 2006 and 2019. For each study, the name, UBC clinical research ethics board study number, study duration, site involvement, and total study N enrolled are listed. Note for CAMPER a total of 86 patients were enrolled as of trial completion on Sept 30, 2020; a total of 76 has been enrolled at the time of biomarker analysis for this manuscript in 2019. Patients were selected for analysis based on sample availability, where we were seeking to have as many patients with paired CSF and serum samples at all four time points where possible. The original study (CSF Drainage Study) was a prospective study of CSF drainage in which we instituted a drainage protocol that was very conservative, resulting in negligible CSF drainage (less than 2 ml/hour) and no lowering of intrathecal pressure. This was the impetus for abandoning the CSF drainage component of the study for "CSF Pressure" (which was a single-center prospective initiative at Vancouver General Hospital to just monitor CSF pressures over the first 5 days post-injury) and "CAMPER" (which was the expansion of this single-center initiative to 5 additional North American sites). The protocol for intrathecal catheter insertion and CSF sampling and processing was the same throughout all studies. For the specific demographics and injury characteristics per enrollment sub-cohort see **eTable 3**; for acute serum and CSF NF-L and GFAP see **eFigure4**.

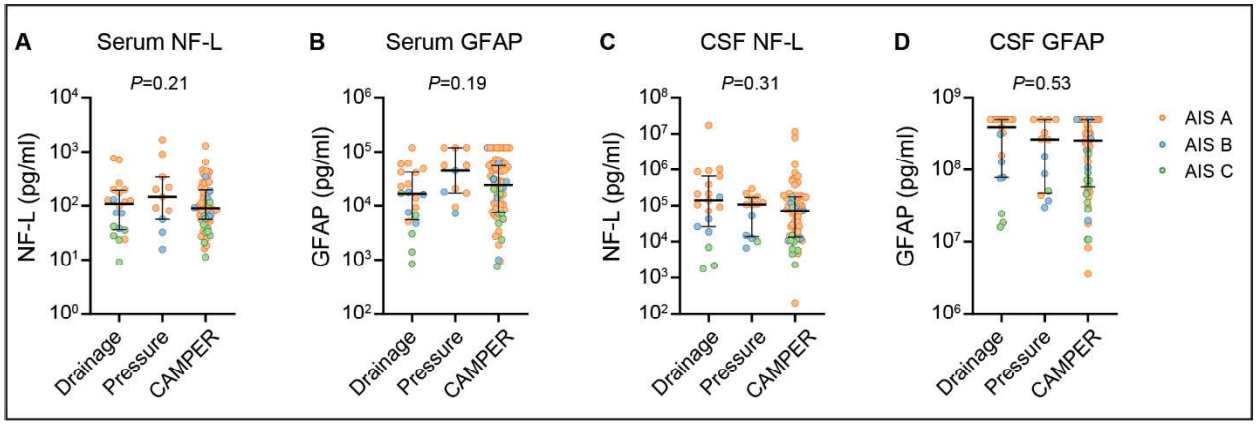


eFigure 2. Estimation and validation of serum and CSF GFAP concentration for samples over the upper limit of detection. A theoretical upper limit of detection (ULOD) was determined for **(A)** serum GFAP and **(B)** CSF GFAP by creating a graph of the average enzyme bound (AEB) versus the calculated concentration of GFAP in the well (prior to correction for dilution) for: the calibrator curve (blue open circles; calibrator B-H displayed as calibrator A=0. Grand average across all runs), and all detectable samples measured for GFAP in the cohort. Samples above the upper limit of quantification (ULOQ, determined by calibrator H) are displayed in red. The estimated theoretical ULOD (red dashed line) was inferred based off of the highest detectable samples prior to detector saturation (typically between AEB of 25-35). In 2021, a subset of **(C)** serum (n=5) and **(D)** CSF (n=9) samples that were previously determined to be over the ULOD were re-run using an extended dilution. Graph shows data following correction for the dilution factor with the black line denoting the group median. The previously estimated ULOD (corrected for dilution), red dashed line, is shown for reference.

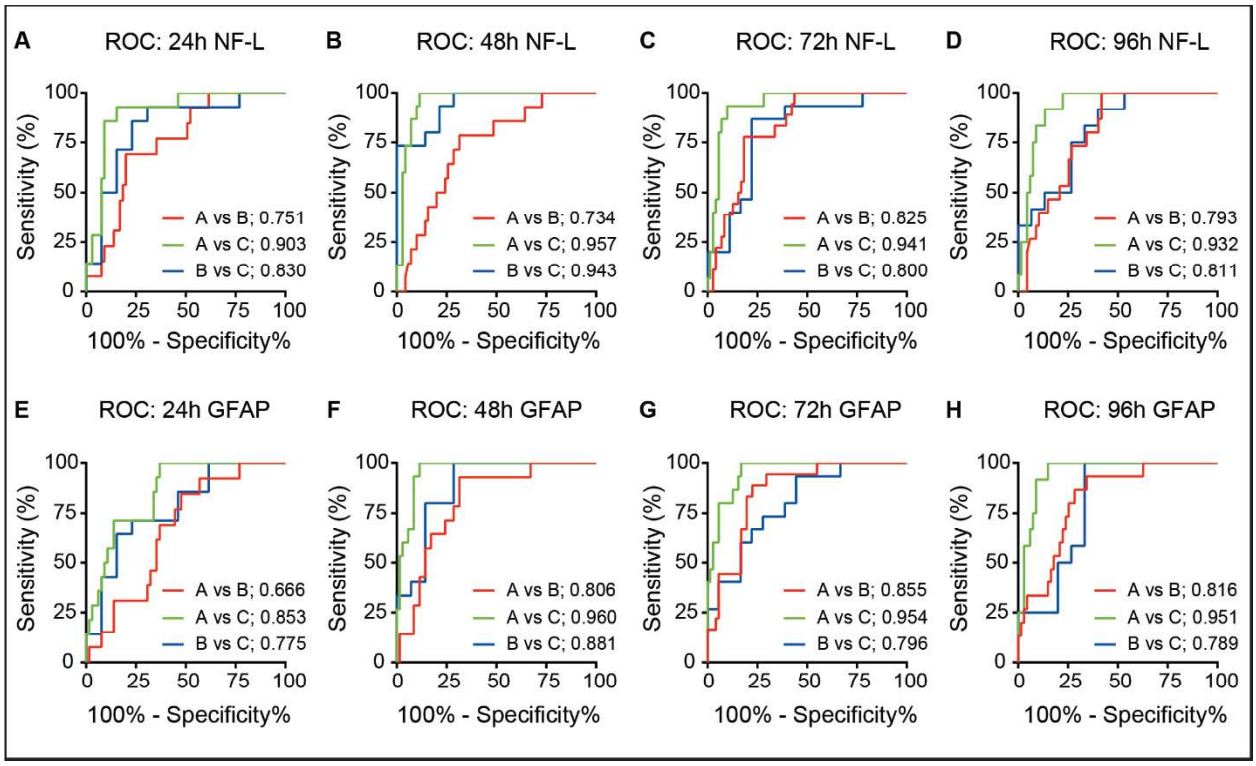


eFigure 3. The association of serum and CSF NF-L and GFAP with time post-injury and severity of neurologic impairment (AIS grade). The concentration of **(A-H)** NF-L and **(I-P)** GFAP was quantified in **(A-D, I-L)** 402 serum samples and **(E-H, M-P)** 371 CSF samples collected from 78 AIS A patients (orange), 20 AIS B patients (blue) and 20 AIS C patients (grey). The concentration of NF-L or GFAP was plotted against the exact time of sample draw (4.3-107h). Data was analyzed using a Spearman correlation. Locally weighted scatterplot

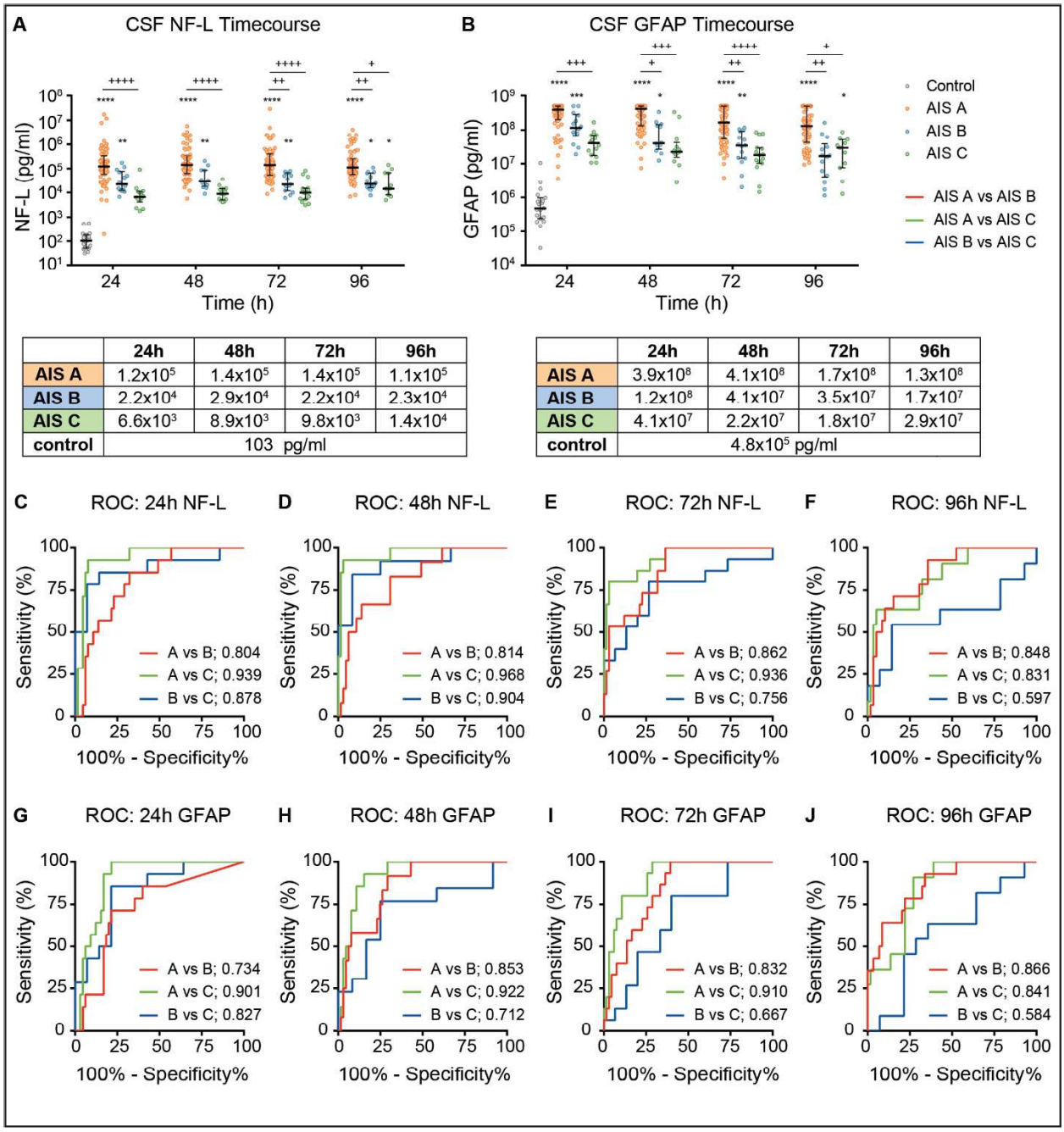
smoothing (LOWESS) was used to generate trajectories of **(D)** serum NF-L, **(H)** CSF NF-L, **(L)** serum GFAP and **(P)** CSF GFAP versus time for each AIS grade (A- orange; B-blue; C-grey).



eFigure 4. Comparison of 24h serum and CSF biomarker concentrations based on initial enrollment clinical trial. SCI patients were drawn from one of three consecutive prospective studies; CSF Drainage Study (n=28), CSF Pressure Study (n=21), and CAMPER (n=69). The concentration of 24h **(A)** serum NF-L, **(B)** serum GFAP, **(C)** CSF NF-L and **(D)** CSF GFAP was compared between the sub-cohorts. All severities of SCI were grouped and compared together, but are coloured for visual purposes as: AIS A=orange, AIS B=blue, and AIS C=green. Graphs display median and IQR; data was analyzed using a Kruskal Wallis test. Demographic and injury information is displayed in **eTable 3**.



eFigure 5. Diagnostic accuracy of serum NF-L and GFAP in acute SCI. The ability of serum **(A-D)** NF-L and **(E-H)** GFAP to differentiate between SCI patients based on AIS grade was assessed using ROC curves. At each time-point, 3 comparisons were made: AIS A vs AIS B (orange), AIS A vs AIS C (grey) and AIS B vs AIS C (blue). The AUROC for each comparison (all statistically significant) are noted in the graphs; for complete AUROC values and errors, see **eTable 5**.

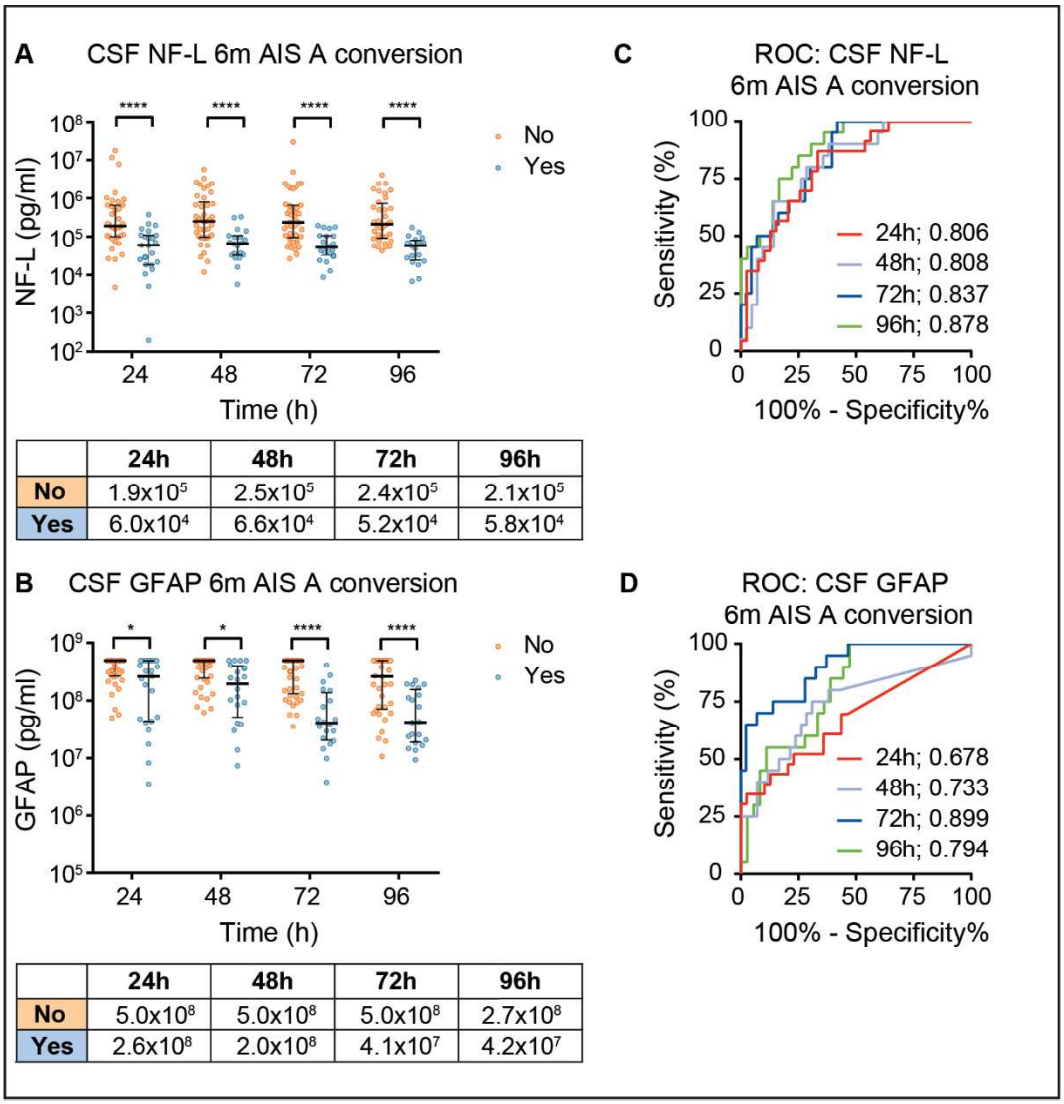


	24h	48h	72h	96h
AIS A	$1.2x10^5$	$1.4x10^5$	$1.4x10^5$	$1.1x10^5$
AIS B	$2.2x10^4$	$2.9x10^4$	$2.2x10^4$	$2.3x10^4$
AIS C	$6.6x10^3$	$8.9x10^3$	$9.8x10^3$	$1.4x10^4$
control	103 pg/ml			

	24h	48h	72h	96h
AIS A	$3.9x10^8$	$4.1x10^8$	$1.7x10^8$	$1.3x10^8$
AIS B	$1.2x10^8$	$4.1x10^7$	$3.5x10^7$	$1.7x10^7$
AIS C	$4.1x10^7$	$2.2x10^7$	$1.8x10^7$	$2.9x10^7$
control	$4.8x10^5$ pg/ml			

eFigure 6. Evaluation of CSF NF-L and GFAP as biomarkers of baseline injury severity (AIS grade). (A) NF-L and **(B)** GFAP were measured in CSF samples from controls (N=19, grey), AIS A (N=78, orange), AIS B (N=20, blue), and AIS C (N=20, green) SCI patients taken up to 4d after injury. Bars denotes the median and IQR of **(A)** NF-L and **(B)** GFAP, with each patient represented as a point. The median concentration in pg/ml for each AIS grade per time point is listed in tabular format below the corresponding graph. Within each time bin, data was compared within SCI severities and control using a Kruskal Wallis test with Dunn's multiple comparison test, where * $p<0.05$, ** $p<0.01$, and *** $p<0.0001$ compared to control (shown once in 24h bin) and + $p<0.05$, ++ $p<0.01$, and +++ $p<0.001$ compared within SCI groups. The ability of CSF **(C-F)** NF-L and **(G-J)** GFAP to differentiate between SCI patients based on AIS

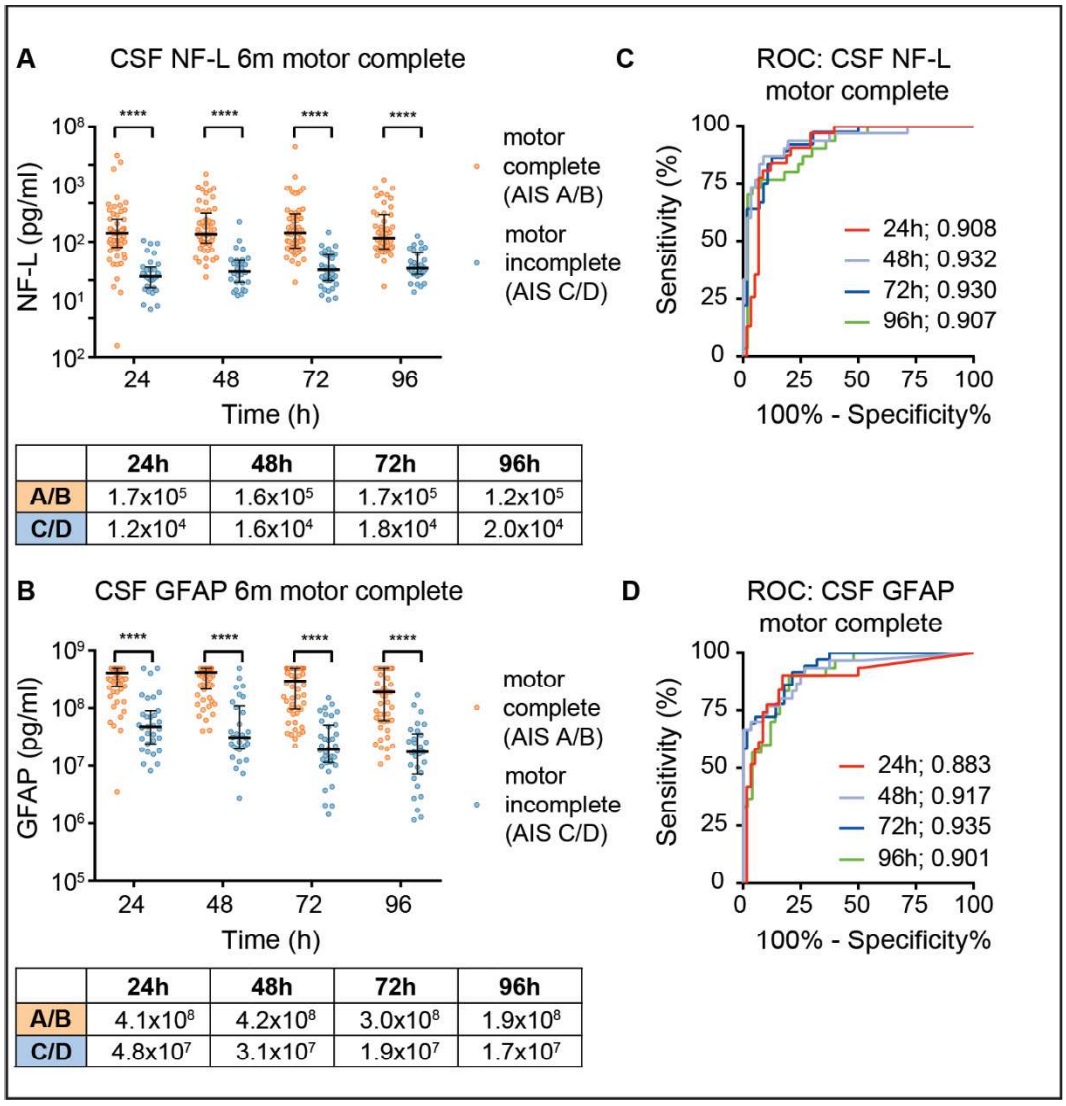
grade was assessed using ROC curves. At each time-point, 3 comparisons were made: AIS A vs AIS B (orange), AIS A vs AIS C (green) and AIS B vs AIS C (blue). The AUROC for each comparison (all statistically significant) are noted in the graphs; for complete AUROC values and error see **eTable 6**.



	24h	48h	72h	96h
No	$1.9x10^5$	$2.5x10^5$	$2.4x10^5$	$2.1x10^5$
Yes	$6.0x10^4$	$6.6x10^4$	$5.2x10^4$	$5.8x10^4$

	24h	48h	72h	96h
No	$5.0x10^8$	$5.0x10^8$	$5.0x10^8$	$2.7x10^8$
Yes	$2.6x10^8$	$2.0x10^8$	$4.1x10^7$	$4.2x10^7$

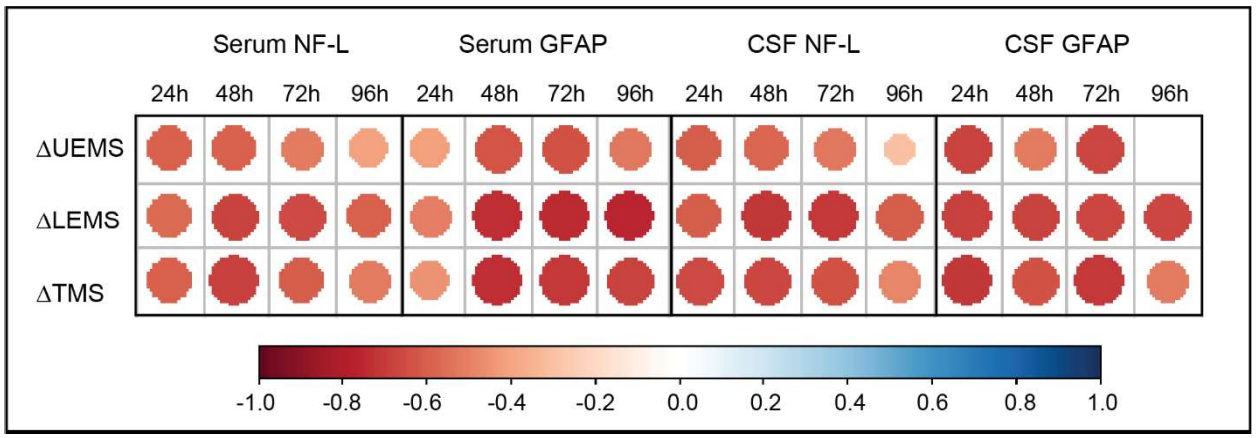
eFigure 7. Comparison of CSF NF-L and GFAP in AIS A patients, distinguished by whether AIS grade conversion occurred (Yes/No) at 6 months post-injury. Of the 74 AIS A patients, 49 (66%) remained an AIS A at 6 months (no conversion, orange), while 25 (34%) improved in their AIS grade (yes conversion, blue). CSF **(A)** NF-L and **(B)** GFAP were graphed based on AIS A conversion status at 6 months. Graphs represent median and IQR. The median concentration in pg/ml based on AIS A conversation status, per time point, is listed in tabular format below the corresponding graph. Data was analyzed using a Mann-Whitney U test at each time-point, where ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. ROC curves were generated comparing the concentration of **(C)** NF-L and **(D)** GFAP at each time-point (24h orange, 48h grey, 72h blue, 96h green) based on conversion status. The AUROC is listed beside the legend in each graph; for complete AUROC values and errors see **eTable 10**.



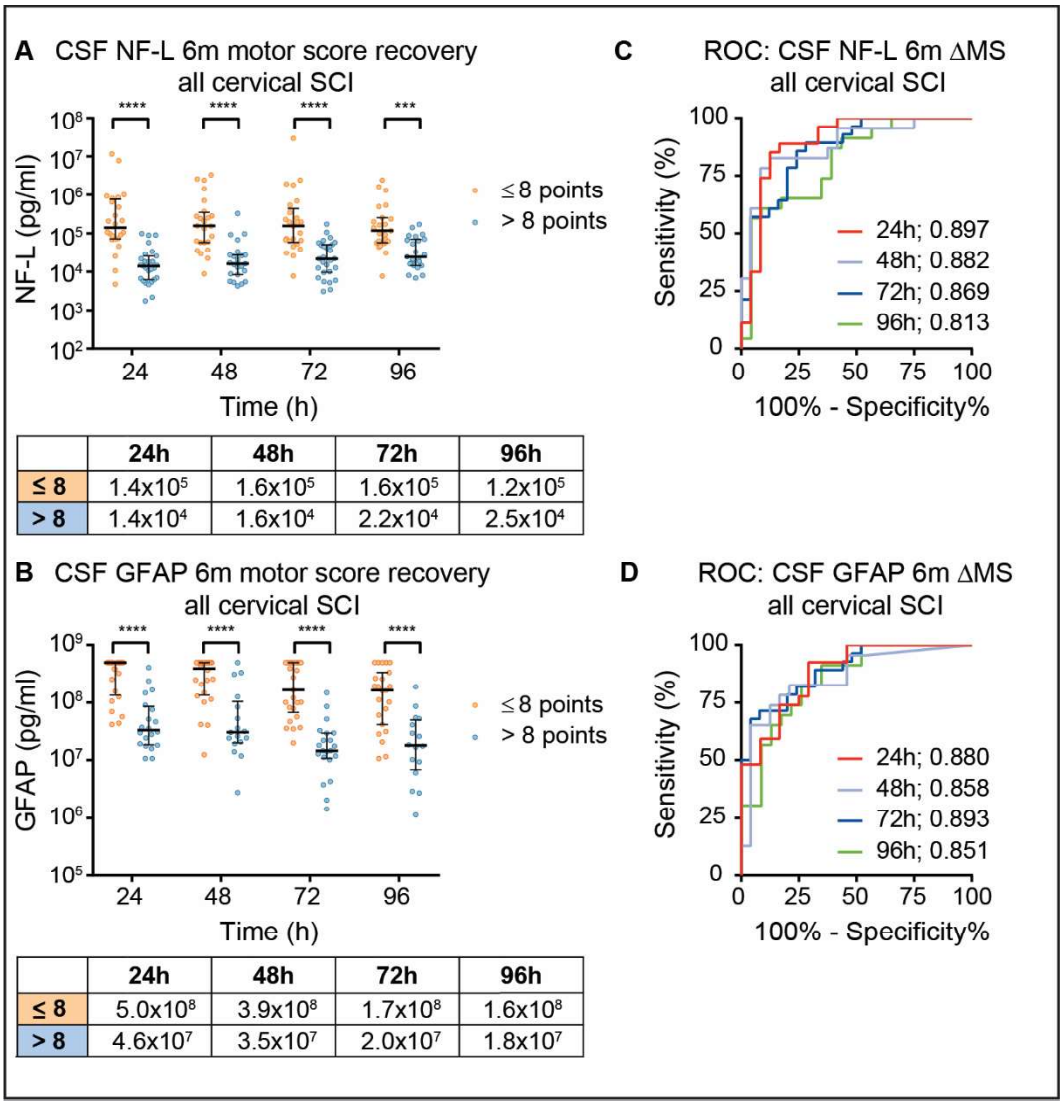
	24h	48h	72h	96h
A/B	$1.7x10^5$	$1.6x10^5$	$1.7x10^5$	$1.2x10^5$
C/D	$1.2x10^4$	$1.6x10^4$	$1.8x10^4$	$2.0x10^4$

	24h	48h	72h	96h
A/B	$4.1x10^8$	$4.2x10^8$	$3.0x10^8$	$1.9x10^8$
C/D	$4.8x10^7$	$3.1x10^7$	$1.9x10^7$	$1.7x10^7$

eFigure 8. Comparison of CSF NF-L and GFAP concentration based on the observed AIS grade at 6 months. 6-month outcome assessments were available in 113/118 (96%) SCI participants. Seventy patients (62%) were classified as AIS A or AIS B (motor complete, orange) while 43 (38%) were classified as AIS C or AIS D (motor incomplete, blue) at 6 months. Graph of 24h, 48h, 72, and 96h CSF **(A)** NF-L and **(B)** GFAP in SCI patients dichotomized based on 6-month AIS outcome. Graph represent median and IQR. The median concentration in pg/ml for AIS A&B versus AIS C&D per time point is listed in tabular format below the corresponding graph. Data pairs at each timepoint were analyzed using a Mann Whitney U test, where ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. ROC curves comparing **(C)** NF-L and **(D)** GFAP concentration at 24h (orange), 48h (grey), 72h (blue) or 96h (green) between motor complete (AIS A/B) and motor incomplete (AIS C/D). The AUROC is listed beside the legend in each graph; for complete AUROC values and errors see **eTable 15**.



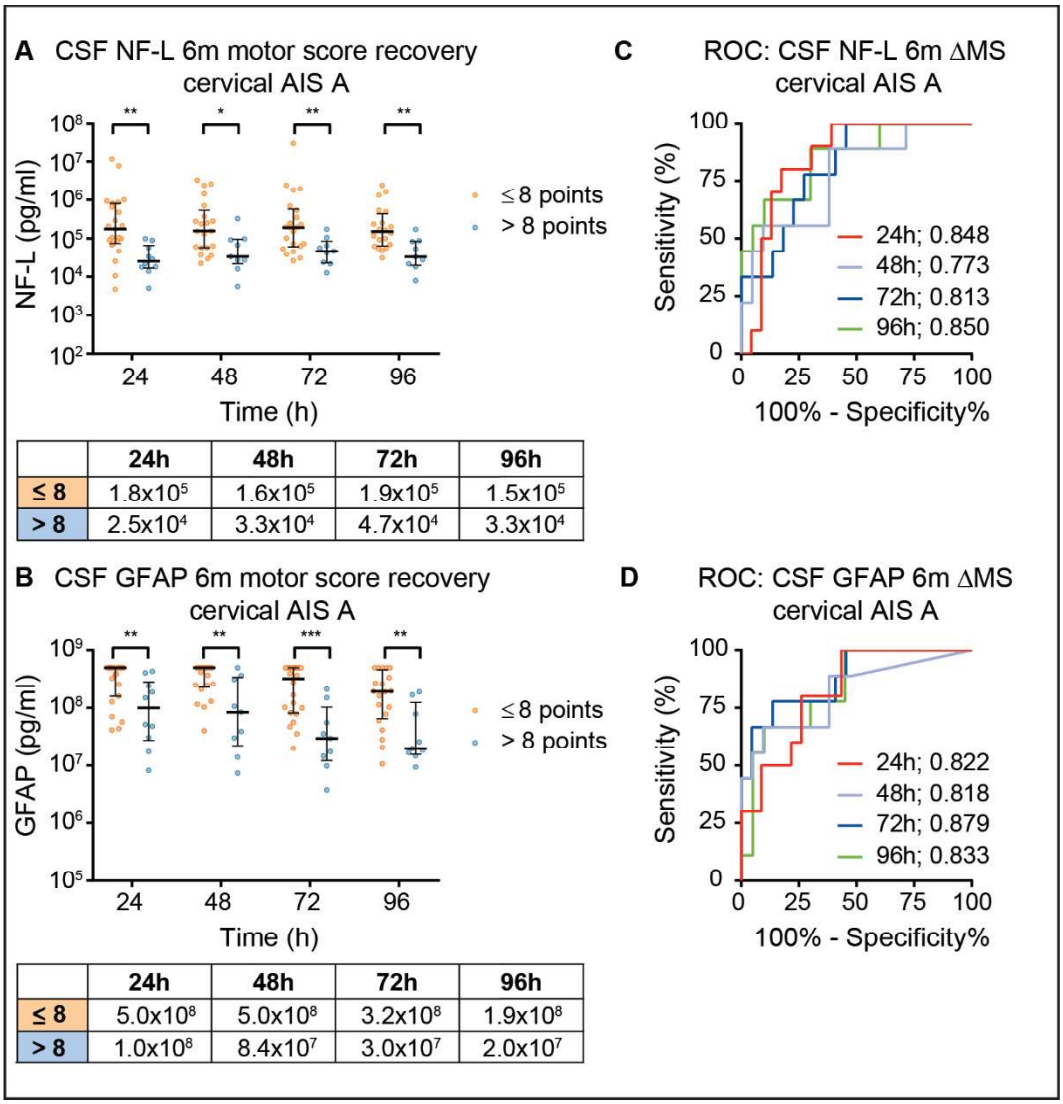
eFigure 9. Correlation of 24h, 48h, 72h, and 96h serum and CSF NF-L and GFAP and the change (6 month minus baseline) in UEMS, LEMS, and TMS in all cervical SCI patients. Correlation matrix depicts the strength and significance of the correlation between each biomarker and the change (Δ) in upper extremity (UE), lower extremity (LE) and total (T) motor score (MS). Only statistically significant relationships are shown; for Spearman Rho coefficient, 95% CI, and P-value see **eTable 18**.



	24h	48h	72h	96h
≤ 8	$1.4x10^5$	$1.6x10^5$	$1.6x10^5$	$1.2x10^5$
> 8	$1.4x10^4$	$1.6x10^4$	$2.2x10^4$	$2.5x10^4$

	24h	48h	72h	96h
≤ 8	$5.0x10^8$	$3.9x10^8$	$1.7x10^8$	$1.6x10^8$
> 8	$4.6x10^7$	$3.5x10^7$	$2.0x10^7$	$1.8x10^7$

eFigure 10. Association of CSF NF-L and GFAP and 6-month motor score recovery in all cervical SCI patients. A total of 65 patients who were classified as having a cervical level of injury at baseline were followed up at 6 months. The change in total motor score (ΔMS) was calculated by subtracting the baseline from the 6-month MS and data was dichotomized into patients that gained ≤8 points (orange, n=28) vs those who gained >8 pts (blue, n=37). Graphs represent the median and IQR of CSF **(A)** NF-L and **(B)** GFAP based on motor score recovery. The median concentration in pg/ml for patients based on MS gain, per time point, is listed in tabular format below the corresponding graph. Data pairs at each timepoint were analyzed using a Mann Whitney U test, where * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001. ROC curves comparing **(C)** NF-L and **(D)** GFAP concentration at 24h (orange), 48h (grey), 72h (blue) or 96h (green) between patients who gained ≤8 pts vs >8pts in total motor score at 6 months. The AUROC is listed beside the legend in each graph; for complete AUROC values and error see **eTable 20.**



	24h	48h	72h	96h
≤ 8	$1.8x10^5$	$1.6x10^5$	$1.9x10^5$	$1.5x10^5$
> 8	$2.5x10^4$	$3.3x10^4$	$4.7x10^4$	$3.3x10^4$

	24h	48h	72h	96h
≤ 8	$5.0x10^8$	$5.0x10^8$	$3.2x10^8$	$1.9x10^8$
> 8	$1.0x10^8$	$8.4x10^7$	$3.0x10^7$	$2.0x10^7$

eFigure 11. Association of CSF NF-L and GFAP and 6-month motor score recovery in cervical AIS A patients. A total of 36 patients who were assessed as AIS A and cervical level of injury at baseline were followed up at 6 months. The change in total motor score (ΔMS) was calculated by subtracting the baseline from the 6-month MS and data was dichotomized into patients that gained ≤8 points (orange or teal, n=25) vs those who gained >8 pts (blue or grey n=11). Graphs represent the median and IQR of CSF **(A)** NF-L and **(B)** GFAP based on motor score recovery. The median concentration in pg/ml for patients based on MS gain, per time point, is listed in tabular format below the corresponding graph. Data pairs at each timepoint were analyzed using a Mann Whitney U test, where * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. ROC curves comparing **(C)** NF-L and **(D)** GFAP concentration at 24h (orange), 48h (blue), 72h (grey) or 96h (teal) between patients who gained ≤8 pts vs >8pts in total motor score at 6 months. The AUROC is listed beside the legend in each graph; for complete AUROC values and error see **eTable 22**.