SUPPLEMENTARY MATERIALS and METHODS

New Astroglial Injury Defined Biomarkers for Neurotrauma Assessment

Julia Halford[1], Sean Shen[2], Kyohei Itamura[1], Jaclynn Levine[1], Albert C. Chong[1], Gregg Czerwieniec[2], Thomas C. Glenn[4], David A. Hovda[4], Paul Vespa[5], Ross Bullock[6], Dalton W. Dietrich[7], Stefania Mondello[8], Joseph A. Loo[2, 3] and Ina-Beate Wanner[1]

[1]Semel Institute for Neuroscience and Human Behavior, [2]Department of Chemistry and Biochemistry, [3]Department of Biological Chemistry, [3]UCLA Molecular Biology Institute, and UCLA/DOE Institute for Genomics and Proteomics, [4]Department of Neurosurgery, Brain Injury Research Center, Department of Molecular and Medical Pharmacology, [5]Department of Neurology, UCLA-David Geffen School of Medicine, Los Angeles; [6]Department of Neurological Surgery, Jackson Memorial Hospital; Miami; [7]The Miami Project to Cure Paralysis, University of Miami-Miller School of Medicine; [8]Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Messina, Italy

Corresponding Author: Ina B. Wanner, PhD

Semel Institute for Neuroscience and Human Behavior

635 Charles E Young Drive South, NRB 260, UCLA, Los Angeles, CA 90095

E-mail: iwanner@mednet.ucla.edu

Phone: 310 825 8847

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Additional Information on donors, patients and samples

CSF and blood samples were aliquoted and stored at −80°C until use. Control subject and TBI patients' information is listed in Supplementary Table 1. Severe TBI patients requiring a ventricular catheter for intracranial pressure (ICP) monitoring were included. Treatment of patients, according to international guidelines, was targeted at maintaining normal ICP and cerebral perfusion pressure.

CSF Proteomics

Equal CSF volumes from TBI patients and healthy subjects were dried by vacuum centrifugation and reconstituted in 100 µL of 50 mM ammonium bicarbonate pH 8.3 solution (Sigma-Aldrich), 0.1% deoxycholic acid. Cysteine disulfides were reduced by addition of Tris (2-carboxyethyl)-phosphine (10 mM, Thermo Scientific) and incubated at 50°C for 1h then adjusted to room temperature. Free cysteines were alkylated with iodoacetamide (20mM, Sigma-Aldrich) for 30 min at 37°C in the dark. Trypsin (500ng/µL 50 mM ammonium bicarbonate, sequencing grade, Promega) was added to CSF samples at a 1:25 enzyme to protein ratio and digested for 16-18h or overnight at 37°C. Samples were acidified with 5% formic acid (v/v) and centrifuged at 13,000 rpm to pellet deoxycholic acid precipitates. The supernatant was then transferred to a separate microcentrifuge tube and dried by vacuum centrifugation.

CSF tryptic digests were reconstituted in 100µl of 0.1% formic acid, 3% acetonitrile for LC-MS/MS. Samples were desalted using a C18 trap column connected to C18 PepMap reversed phase HPLC column for peptide separation. CSF samples were analyzed using an LTQ-Orbitrap or Q-Exactive Orbitrap mass spectrometer (Thermo). Peptide separation was done in a 120min gradient from 5-35% of mobile phase (100% acetonitrile, 0.1% formic acid). Analysis on the Q-Exactive was performed in the positive ion mode with settings: resolution –70,000; m/z range–300-2000; maximum MS1 injection time–50ms; MS automatic gain control (AGC) target–$1x10^6$. Acquisition was set to record up to 10 confirmatory product ion spectra (MS2) per full scan spectrum by selecting precursor ions of decreasing signal intensity with 30sec dynamic and charge state exclusions to exclude signals with unassigned charge, charge +1 and charges >+5. MS2 instrument settings were: resolution – 35,000; maximum MS2 injection time – 100ms; MS2 AGC target – $2x10^5$; fixed first mass m/z–100.

The data was searched using Mascot (Matrix Science) against the human subset of the SwissProt database. Oxidation of methionine was set variable carbamidomethylation of cysteine was set as fixed. Enzyme specificity was set to C-terminal cleavage at arginine and lysine with up to 2 mixed cleavages allowed. Strict m/z error tolerances were set to 15 ppm in MS mode and 0.01 Dalton in MS2 mode. Peptide spectral matches were validated against a decoy database using the Percolator algorithm at a 5% false discovery rate.

Immunoblotting, sub-saturated densitometry and technical variance

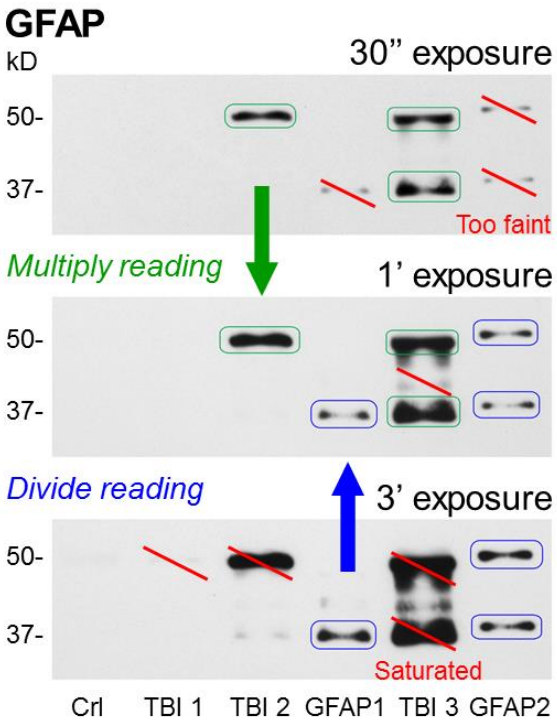
Sample preparation: Protease inhibitors (Roche) and dithiothreitol (5mM, Calbiochem) were added to conditioned media and fluids were concentrated by ultrafiltration to one-twentieth of the original volume (Vivaspin, VWR). Thawed CSF and blood samples were supplied with EDTA (1mM, pH7.4) and protease inhibitors bestatin (40µM), pepstatin A (10µM) and phosphoramidon (10µM). Samples were centrifuged for 10min at 16,060g at 4ºC to remove lipids. Plasma and serum samples were depleted of albumin and immunoglobulins (IgGs) by immunoaffinity columns (ProteoPrep, PROTIA Sigma) and concentrated by ultrafiltration (Vivaspin, VWR). Depletion removed ~ 85% of original abundant protein content.

Immunoblotting: Samples were denatured (5min at 100ºC followed by ice), reduced by adding 1% β-mercaptoethanol and adjusted to Laemmli Sample Buffer (2%SDS, 125mM TrisHCl pH6.8, 10% glycerol, 0.6% bromphenol blue). Proteins were separated in 200mM glycine, 25mM Tris, 0.1% SDS for 30min at 100V followed by 1-2h at 120V and transferred onto nitrocellulose (Hybond-ECL, Amersham) with 20% methanol in the same buffer. Proteins were reversibly stained using 0.1% Ponceau-S in 5% acetic acid. Protein concentrations were determined by assay (Pierce 660nm) against bovine serum albumin (BSA) dilutions: interquartile concentration range for control CSF was 0.23 to 0.4mg/ml, while the range for TBI CSF was 0.45 to 2.24mg/ml. Proteins above 30kD were separated on 10%, 10-25kD sized proteins were separated on 15% polyacrylamide 2% SDS Tris-based handcast gels. A molecular weight standard (Precision Plus Kaleidoscope, Bio-Rad) and His-tagged pure proteins ALDOC, BLBP, PEA15 (EnCor Biotech. Inc) and GFAP full size, 37 and 20kD fragments (Abbott Diagnostics) at various concentrations were diluted in 0.5% BSA and then analyzed in parallel with biofluids (see Supplementary Table 3). Blots were blocked for 30min with 10% non-fat milk in Tris-buffered saline with 0.05% Tween-20 (TBST) before overnight incubation at 4ºC with primary antibodies diluted in 5% BSA in TBST (Supplementary Table 2, [1]). Washed blots were incubated for 1hr at room temperature with peroxidase-conjugated secondary antibody (Thermo, Supplementary Table 4). Washed blots were incubated for 5min in enhanced chemiluminescence substrate (West Pico ECL, Thermo Scientific). Multiple films (Denville) with the same sequence and same exposure lengths (10sec, 30sec, 1min, 3min, 5min, 20min) were used consistently.

Densitometry: Immunoblot signal levels were measured from scanned films using a bio-imaging and analysis system with background correction (Autochemie Systems, UVP). Post-hoc signal normalization of non-saturated and above background signals from multiple exposures extended the linear range and provided signal measurements covering two to four orders of magnitude. Analysis variance was 22±10%. Each experiment included positive controls (purified proteins or replicates with biomarker signal) and negative controls (healthy subject biofluids, see Supplementary Table 1A). Two subjects with very low (0.09, 0.32mg/ml) total CSF protein concentrations lacked CSF signal for most of the biomarkers, but their values were not excluded Wet experimental replicates produced signals that varied from the sample mean by 16±13% (Replicates are listed in Supplementary Table 1A, B). Overall, analysis variance fell lower than one order of magnitude below significant differences. In the trauma model, variation in release of each

biomarker due to base astroglial expression varied no more than 1-2 z-scores. We controlled for the use of clinical serum versus plasma samples by comparing marker signals in both samples in the same subject, which resulted in similar results for all blood-compatible biomarkers.

S1: Immunoblot measurement approach



Three exposures (30 seconds, 1min and 3min) of an immunoblot show GFAP signals in CSF samples from three TBI patients (TBI 1: 68 year old female; TBI 2: 44-year old male; TBI 3: 34-year old male) and one healthy subject (Crl: 54-year old female, negative control). GFAP purified from human brain (GFAP1, Millipore) and 10ng recombinant GFAP (GFAP2; 50 and 37kD, Supplementary Table 3) were analyzed as positive controls. Faint signal that was not significantly elevated above background was excluded (cutoff optical density, OD ~0.01 on a scale to 1, red lines). Further, saturated signal defined as not showing an increase with longer exposure was also excluded (cutoff OD: ~0.6, red lines). Included signals had ODs within the linear range of change (boxed). Measurements were background-corrected. Most samples were measured on 2-3 different exposures. Measurements were normalized to one common exposure, selected for the most measured bands (here 1min). This was done by converting from a shorter exposure to the common one by multiplying each reading by an average scaling factor of these two exposures (green arrow). The scaling factors were determined from all included signals across the entire sample cohort. To convert a reading from a longer exposure to the common one, each reading was divided by an average scaling factor determined in the same manner (blue arrow). This standardized conversion was performed across all exposure pairs and introduced an analytical error of 22 ± 10%, depending on the biomarker. The approach enabled the inclusion of an extended range of signals over two to four orders of magnitude and accommodated the broad heterogeneity of biomarker release after TBI.

Quantitation of Biomarkers in CSF using multiple reaction monitoring mass-spectrometry

Peptides specific to each biomarker were selected and the sequences are provided in Supplementary Table 5. The corresponding synthetic standard peptides with heavy isotope labeled arginine ($6C_{14}4N_{15}$) and lysine ($6C_{13}2N_{15}$) were purchased (Thermo Scientific). Peptide standards were prepared in 5% acetonitrile (5pmol/µl) and spiked into CSF samples to concentrations between 25-75pmol per ml of CSF. CSF samples were then reduced, alkylated and digested as described above. Digested CSF peptides were dried by vacuum centrifugation and reconstituted in 0.1% formic acid, 3% acetonitrile in water. Samples were desalted

using an on-line C18 trap column before LC-MS/MS analysis. Peptides were separated on a 5%-35% gradient of mobile phase B (0.1% formic acid in acetonitrile) over 40 min on a C18 PepMap reversed phase HPLC column. Samples were analyzed using either a Q-Exactive Orbitrap MS or a 4000 QTRAP triple quadrupole MS (AB Sciex). MRM-MS analysis was performed with the Q-Exactive by parallel reaction monitoring targeting an inclusion list of precursor peptide ions listed under 'MRM transition' (Supplementary Table 5). The following parameters were used for MS2 analysis: resolution 17500, AGC target $2x10^5$, maximum ion injection time 50ms, isolation window 3.0Da, fixed first mass 100, normalized collision energy 27.

Biomarker-specific precursor peptide ions were fragmented by higher energy collisional dissociation or collision activated dissociation depending on the MS instrument, into their component product ions. Biomarker abundance was calculated based on the area under the curve (AUC) of the precursor to product ion transitions for each peptide using Pinpoint (Thermo) and Skyline. Three transitions were summed per biomarker specific peptide, and the ratio of the endogenous peptide signal to its heavy labeled counterpart was determined. Biomarker concentrations were calculated based on each peptide's endogenous to heavy standard signal ratio, heavy standard concentration, protein molecular weight (MW) and a dimensional conversion factor according to the formula:

Endogenous protein concentration (ng/ml) = $\frac{endogenous}{standard} ratio \times \frac{25 fmol}{\mu l} \times protein\ MW \times \frac{1}{1000}$.

Human astroglial trauma model

Primary human astrocytes were prepared from donated, de-identified human fetal cerebral neocortices at 16-19 gestational weeks as described[2]. Dissociation with a scalpel was done in calcium and magnesium-free Hank's buffered saline solution (HBSS) followed by filtration through 70 µm and 10 µm nylon meshes (Nitex) into the culture medium (DMEM-F12) with 10% fetal bovine serum (FBS, Atlanta Biol.). Neural progenitor cells were removed by 30min centrifugation at 30,000 g in an HBSS-buffered 33% Percoll gradient (Sigma). The top fraction was washed and cells were diluted in DMEM/F12, 10% FBS and astrocytes were cultured in T150 cell culture-treated plastic flasks (Corning). Confluent cultures were shaken for four days at 200rpm on a shaker in an incubator to remove residual proliferating cells. Astrocytes were passaged onto stretch plates by mild loosening of cell-substrate contacts with 0.25% trypsin/EDTA followed by gentle mechanical dissociation, washes and cells were counted for seeding defined numbers onto collagen I-coated silastic membrane culture plates (6 well Bioflex, Flexcell Intl.) at a density of ~135,000 human cells/962mm^2. Upon confluence, the medium was replaced with DMEM/F12 with 10% heat-inactivated horse serum (Atlanta Biol.) that was then stepwise reduced. Serum-free astrocytes in 2ml DMEM/F12 were used for stretch-injury experiments described in the manuscript.

Cell permeability, viability assays and immunocytochemistry protocols

Cells were incubated with 0.025µg/ml propidium iodide (PI) in Leibowitz' L15 (Gibco) for 10min at 37°C followed by four rinses in L15. Dye was crosslinked to DNA of permeable cells by 5min exposure to UV light. Cells were fixed in freshly

depolymerized 4% paraformaldehyde in Tris-buffered saline for 30min at 4°C. Rinsed cells were permeabilized with 0.3% Triton in the same buffer and blocked with 5% normal donkey serum in same buffer, followed by overnight incubation with primary antibody at 4°C diluted in blocking solution (Supplementary Table 2). After rinsing, secondary antibodies (Supplementary Table 4) were applied in blocking solution for 1h at room temperature. Cultures were rinsed and stained with a bisbenzimide nuclear dye (Hoechst, 1:75 in distilled water) for 5min, rinsed, dried and coverslipped (Fluorogel, Biomedia). Hardened cultures were mounted on slides[1, 2].

Statistical Analyses

Replicates: Multiple replicates of each MRM sample were measured (analytical replicates). Same day patient samples were independently prepared three times, assuring experimental MRM-MS and immunoblotting consistencies (experimental replicates). Same day patient replicates with different draw times were analyzed in parallel and averaged for graphs and statistical analyses. Replicates are given in Supplementary Table 1.

TABLE 1: TBI patient's and control subject's data and replicate experimental analyses

Subject			Sample		Analyses			
Gender	Age	Source	CSF collection	Blood preparation	Biomarker panel measurements			CSF Proteome
					IB, CSF	IB, blood	MRM-MS, CSF	LC-MS/MS
M	24	Donor	lumbar drain	Serum	5	1	3	2
M	22	Donor	lumbar drain	Serum	1	1	3	N/A
M	45	unruptured aneurysm	lumbar drain	N/A	1	N/A	3	1
F	21	Donor	lumbar drain	Serum	1	1	3	3
M	43	Donor	lumbar drain	Serum	2	1	3	3
M	20	Donor	lumbar drain	Serum	2	2	3	3
F	47	unruptured aneurysm	lumbar drain	Plasma	2	1	N/A	N/A
F	37	Donor	N/A	Plasma	N/A	1	N/A	N/A
M	33	Donor	lumbar drain	Serum	1	1	3	5
M	24	Donor	lumbar drain	Serum	1	1	3	N/A
M	25	Donor	lumbar drain	N/A	1	N/A	3	N/A
F	54	unruptured aneurysm	lumbar drain	N/A	3	N/A	3	2
M	24	Donor	N/A	Plasma	N/A	1	N/A	N/A
F	67	Donor	N/A	Plasma	N/A	1	N/A	N/A
M	79	Donor	N/A	Plasma	N/A	1	N/A	N/A
F	60	Donor	lumbar drain	N/A	1	N/A	3	3
F	40	Donor	lumbar drain	N/A	1	N/A	3	3

Table 1 (A): Control subjects: Figure IDs refer to signals of control subject CSF and blood samples shown in Figures 1 to 3. Listed are 17 healthy subjects who donated CSF obtained by lumbar drain, blood or both. The average age was 39 years old among control subjects. 59% of control subjects were male. Sample measurements and replicates are listed for immunoblotting (IB), MRM-MS and proteome analyses (LC-MS/MS).

Figure Labels			Subject		Clinical Data				Samples			Analyses			
Fig. 1 CSF	Fig. 2 MRM	Fig. 4 Blood	Gender	Age	Injury cause	GCS	Survival	CT scan report	CSF post-injury day	Blood preparation	Blood post-injury day	Biomarker panel measurements IB, CSF	Biomarker panel measurements IB, blood	Biomarker panel measurements MRM-MS, CSF	CSF Proteome LC-MS/MS
TBI 1	TBI 1	TBI 1	M	54	Fall	3	Survived	SDH, SAH, Is	i	Serum	i	12	4	24	2
TBI 1	TBI 1	TBI 1							i+1	Serum	i+1	3	4	6	2
TBI 1	TBI 1								i+2	Serum	i+2	12	4	18	2
TBI 1	TBI 1								i+3	Serum	i+3	9	3	9	N/A
TBI 1	TBI 1								i+4	Serum	i+4	6	1	6	2
TBI 1	TBI 1								i+5	Serum	i+5	3	1	3	N/A
TBI 2	TBI 2		M	21	MVA	6	Survived	SDH, SAH	i +1	Plasma	i+1	1	1	3	N/A
TBI 3			M	N/A	MVA	N/A	Died	N/A	i	Plasma	i	2	1	3	N/A
TBI 4			M	28	MVA	6	Survived	SDH, SAH	i	Plasma	i	2	2	3	N/A
TBI 4									i+1	Plasma	i+1	1	1	3	4
TBI 5			F	19	MVA	8	Survived	SAH, Edm	i	Plasma	i+2	2	1	3	4
		TBI 2	M	18	MVA	4.5	Died	DAI, Is	i	Plasma	i	3	1	20	6
		TBI 2							i+1	Plasma	i+1	1	1	3	1
TBI 6		TBI 2							i+2	Plasma	i+2	2	1	9	1
		TBI 2							i+3	Plasma	i+3	1	1	3	1
		TBI 2							N/A	Plasma	i+4	N/A	1	N/A	N/A
		TBI 3	F	36	MVA	4	Survived	EDH, DAI	i+4	Plasma	i	2	1	1	6
		TBI 3							N/A	Plasma	i+1	N/A	1	N/A	N/A
		TBI 3							N/A	Plasma	i+2	N/A	1	N/A	N/A
		TBI 3							N/A	Plasma	i+3	N/A	1	N/A	N/A
TBI 7		TBI 3							N/A	Plasma	i+4	N/A	1	N/A	N/A
TBI 8			M	41	Blunt	N/A	Survived	EDH, SDH, SAH, Edm,	i+4	N/A	N/A	2	N/A	6	5
		TBI 4	F	49	Assault, bat	9.5	Survived	Cnts, SDH	i	Plasma	i	2	1	5	1
		TBI 4							i+1	Plasma	i+1	1	1	4	1
		TBI 4							i+2	Plasma	i+2	3	2	21	3
			M	16	MVA	3	Survived	SAH, DAI	i+4	Plasma	i+4	5	2	3	N/A
			M	43	Fall	3	Survived	EDH, Cnts, SDH, SAH	i+4	N/A	N/A	1	N/A	6	4
			M	21	MVA	6	Survived	SDH, Edm	i+1	Plasma	i+1	1	2	N/A	N/A
			M	17	Football	4	Survived	Cnts, SDH	i	Plasma	i	2	1	N/A	N/A
			M	45	GSW	8	Survived	-	i	Plasma	i	1	1	N/A	N/A
			M	36	MVA	3	Survived	SDH, SAH, Edm	i	Plasma	i	1	1	3	4
			M	33	MVA	3	Survived	Cnts, SDH, SAH, Edm	i+1	Plasma	i+1	2	2	N/A	N/A
									i+3	Plasma	i+3	2	1	3	3
			F	19	MVA	4	Survived	Cnts, Edm	i+1	Plasma	i+1	1	2	N/A	4
			F	23	MVA	3	Survived	ICH	i+2	N/A	-	1	N/A	3	8
			M	23	Fall	7	Survived	SDH, SAH,DAI	i+2	Plasma	i+2	2	1	3	N/A
			M	25	Fall	6	Survived	EDH, Ctns, SDH, mdls, DAI	i+2	N/A	-	1	N/A	3	3
			M	54	Fall	8	Died	SAH, SDH,DAI	i+2	Plasma	i+2	2	4	3	N/A
			M	44	Fall	9	Survived	Cnts, SDH, SAH, Edm, DAI	i+2	Plasma	i+2	3	1	3	3
			M	47	Blunt	9	Survived	SDH, SAH, IVH, DAI	i+3	Plasma	i+3	2	1	3	3
			M	18	Fall	3	Survived	SDH, SAH,Edm, DAI	i+4	Plasma	i+4	2	1	3	3
			F	68	MVA	5	Died	SDH, SAH, Edm	i+4	Plasma	i+4	3	1	3	3
			M	34	Fall	3	Survived	EDH, Cnts, SDH, SAH, DAI	i+5	Plasma	i+5	3	1	3	N/A

Table 1 (B) TBI patients: Figure labels "TBI 1-8" are used in Figures 1, 2 and 4 and distinguished by fluid type. Average TBI patient age was 33.3 years old and 77% were male. Injury causes are listed for each patient and include motor vehicle accidents (MVA, 50%), falls (30%), gunshot wounds (GSW), football and assaults. Post-resuscitation GCS scores, survival and CT findings are given. CT scan reports document presence of intracerebral hemorrhage (ICH, 92%) including one or more findings of contusion (Cnts, 31%), subdural hematoma (SDH, 62%), subarachnoid hemorrhage (SAH, 62%) or intraventricular hemorrhage (IVH, one case). Further, presence of epidural hematoma (EDH, 19%), diffuse Axonal Injury (DAI, 42%), ischemia (Is, two cases) and edema or midline shift (Edm, 35% mdls, 4%) are reported. Post-injury days are given for each sample and multiple same-day samples were averaged. CSF and blood analyses are listed with replicates for IB, MRM and LC-MS/MS measurements.

Figure label		Subject		Clinical Data			
		Gender	Age	Injury cause	GCS	CT scan report	Hours Post-mTBI
CT+ mild TBI	1	F	71	Fall	15 15	CT+: Vertebral Fracture, SAH	1h 17h
	2	M	76	MVA	15 15	CT+: 5 Cnts, skull fracture	1h 31h
	3	F	68	Fall	15 15	CT+: SDH	2h 22h
CT- mild TBI	4	M	20	-	15	Mouth lesions, muscle lesion; CT-	17h
	5	M	35	Fall	15	normal, CT-	13h
	6	M	20	MVA	15	Amnesia; CT-	2h
	7	M	75	Fall	15	Nasal fracture; CT-	2h
		M	50	MVA	15	CT+: 1 lesion	10h
		M	48	Fall	15	CT-	1h
		F	73	Fall	14	CT-	2h
		M	65	MVA	14	Skull and wrist fractures, CT+: frontal Cnts	15h
		F	72	Fall	14	CT+: Frontal Contusions, SDH, SAH	19h
		F	70	Fall	15	normal, CT-	3h30m
		F	59	MCA	15	normal, CT-	4h
		M	63	Fall	15	normal, CT-	2h

Table 1 (C) Mild TBI patients: Figure labels from Figure 4H are given on the left. Average age of the listed 15 mild TBI patients was 58 years old and 60% were male. Two thirds of injuries were caused by falls and one third by motor vehicle accidents (MVA). GCS scores are listed. CT status is given as normal (negative (CT-)) or abnormal (positive (CT+)). Scan findings are also presented. Serum draw times are listed as hours after mild TBI accident.

TABLE 2: List of used primary antibodies with epitopes.

Marker	Antibodies (Company, Catalog #)	Epitope	Dilution		
			CSF IB	Blood IB	ICC
GFAP	Rabbit (rb) polyclonal anti-GFAP (DAKO, Z0334)	Whole cow GFAP; recognizes full size GFAP and large and small breakdown products (BDPs).	rb: 1:150,000 (large) rb: 1:15,000 (small)	1:2,000	1:500
	Chicken (ch) polyclonal anti GFAP (ThermoFisher Scientific, PA1-10004)	Whole bovine GFAP; Recognizes full size GFAP, large and small BDPs	ch: 1:30,000	N/A	1:700
ALDOC	Rabbit affinity purified polyclonal anti-ALDOC (Genetex, GTX102284)	Recombinant ALDOC fragment amino acids 10-163 (P09972)	Genetex rb: 1:5,000		
	Rabbit Serum 88 (Encor)	Recombinant whole ALDOC and BDP	rb Serum88: 1:4,000		
	Monoclonal ALDOC antibodies (Encor): IgG1 mab 1A1 (MCA-1A1) IgG1 mab E9 (MCA-E9) IgG1 mab 4A9 (MCA-4A9), IgG1 mab 5C9	mab 1A1: C-terminal peptide mab E9: Recombinant whole protein mab 4A9: N-terminal peptide (MPHSYPALSAEQKKELS) mab 5C9: N-terminus	mab 1A1: 1:1,000 mab E9: 1:2,000 mab 4A9: 1:1,000	mab E9: 1:2,000 mab 4A9: 1:1,000 mab 5C9: 1:10 (5C9: hybridoma supernatant)	mab E9: 1:300
PEA15	Rabbit polyclonal affinity purified anti PEA15 (Cell Signaling)	Human PEA15 peptide surrounding Leu60	rb: 1:1,000	1:1,000	1:100
BLBP = FABP7	Affinity purified rabbit polyclonal anti – FABP7 (Millipore)	GST-tagged recombinant full size human FABP7, specific to BLBP	rb: 1:1,000	1:1,000	1:300
	Affinity purified rabbit polyclonal anti-FABP7 clone RB22973(Abgent)	C-terminal human FABP7 peptide amino acids 104-132, specific to BLBP	rb: 1:1,000	N/A	N/A
GS	Rabbit IgG fraction polyclonal anti GS (Sigma, G2781)	GS peptide amino acids 357-373,	rb: 1:10,000	N/A	N/A
	Mouse mab IgG2A to GS clone 6 (BD Transduction, 610517)	Full size GS	mab: 1:3,000	N/A	
APOB	Rabbit affinity purified polyclonal IgG anti-APOB (PTGlab, 20578-1-AP)	Unspecified APOB peptide APOB 120-130 kD observed band, full size 516 kD	rb: 1:3,000	N/A	N/A
PTGDS	Rabbit affinity purified IgG anti-PTGDS (USBiological, P9053-24D)	Synthetic human PTGDS peptide amino acids 120-190	rb: 1:50,000	N/A	N/A

Primary antibodies for immunblotting (IB) and immunocytochemistry (ICC), their sources, epitopes, and dilution ranges used for CSF and blood IB and for ICC. Full size GFAP plus large GFAP-BDPs were detected using rabbit anti-GFAP 1:150,000. Small GFAP-BDPs were detected using rabbit anti-GFAP 1:15,000. Antibodies with different epitopes were used to unambiguously specify GS, BLBP, ALDOC and ALDOC-BDP in biofluids; however, readings were standardized using same-sample replicates.

TABLE 3: List of pure proteins

Name	Sources (Company, Cat# where available)
GFAP	Recombinant human full-length 50kD (Abbott, gift) Recombinant human 38kD BDP (Abbott, gift) Recombinant human 20kD BDP (Abbott, gift) Purified from human brain (Merck-Millipore, Cat# 345996)
ALDOC	Recombinant human ALDOC (EnCor, gift)
PEA15	Recombinant human PEA15 (EnCor, gift)
BLBP	Recombinant human BLBP (EnCor, gift)

Recombinant proteins of GFAP and proteolytic breakdown products (BDPs) of 38 and 20kD molecular weight were provided by Abbott Diagnostics, Chicago. GFAP purified from human brain was purchased (Merck-Millipore). Recombinant isoform-specific proteins aldolase C (ALDOC) and brain lipid binding protein (BLBP, also known as FABP7) as well as astrocytic phosphoprotein 15 (PEA15) were provided by EnCor Biotechnology Inc., Gainesville. These proteins were used in known concentrations as positive controls and to estimate concentration ranges of biomarkers (Figure 2F). All three recombinant GFAP proteins were used to create standard curves for estimating total GFAP concentrations in CSF. The 20kD recombinant GFAP-BDP was used for a standard curve to estimate small GFAP-BDP amounts in blood samples.

TABLE 4: List of secondary antibodies used.

Application	Host, target, conjugate	Company	Catalog #	Dilution
Immuno-blotting	Goat anti-rabbit IgG, HRP	Thermo Fisher	31460	1:10,000
	Goat anti-mouse IgG, HRP	Thermo Fisher	31430	1:10,000
	Goat anti-chicken IgY, HRP	Thermo Fisher	SA1-72012	1:10,000
Immunocytochemistry	Donkey anti-rabbit IgG, AlexaFluor 488	JacksonImmuno	711-545-152	1:150
	Donkey anti-rabbit IgG, AlexaFluor 647	JacksonImmuno	711-605-152	1:150
	Donkey anti-rabbit IgG, Cy 3	JacksonImmuno	711-165-152	1:250
	Donkey anti-mouse IgG, AlexaFluor 488	JacksonImmuno	715-545-151	1:200
	Donkey anti-mouse IgG, Cy 3	JacksonImmuno	715-165-151	1:150
	Donkey anti-chicken IgY, AlexaFluor 647	JacksonImmuno	703-605-155	1:80
	Donkey anti-goat IgG, AlexaFluor 488	JacksonImmuno	705-545-003	1:100
	Donkey anti-rat IgG, AlexaFluor 594	JacksonImmuno	712-585-150	1:250

Secondary detection antibodies used for western blots and fluorescence immunocytochemistry with their dilution and sources.

Table 5: List of multiple reaction monitoring-mass spectrometry, MRM-MS, peptides and ion transitions

Name	Peptide Sequence	Measured MRM Transition
GFAP	ALAAELNQLR(Heavy)	554.821 (2+) --> 924.514 (1+, y8) 554.821 (2+) --> 853.477 (1+, y7) 554.821 (2+) --> 782.439 (1+, y6)
	ALAAELNQLR(Light)	549.816 (2+) --> 914.505 (1+, y8) 549.816 (2+) --> 843.468 (1+, y8) 549.816 (2+) --> 722.431 (1+, y8)
	LADVYQAELR (Heavy)	594.758 (2+) --> 1003.508 (1+, y8) 594.758 (2+) --> 789.413 (1+, y6) 594.758 (2+) --> 626.350 (1+, y5)
	LADVYQAELR (Light)	589.314 (2+) --> 993.500 (1+, y8) 589.314 (2+) --> 779.405 (1+, y6) 589.314 (2+) --> 616.341 (1+, y5)
ALDOC	TPSALAILENANVLAR (Heavy)	831.974 (2+) --> 1193.688 (1+ y11) 831.974 (2+) --> 1122.651 (1+ y10) 831.974 (2+) --> 1009.566 (1+ y9)
	TPSALAILENANVLAR (Light)	826.970 (2+) --> 1183.679 (1+, y11) 826.970 (2+) --> 1112.642 (1+, y10) 826.970 (2+) --> 999.558 (1+, y9)
GS	DIVEAHYR (Heavy)	506.758 (2+) --> 784.398 (1+, y6) 506.758 (2+) --> 685.329 (1+, y5) 506.758 (2+) --> 556.287 (1+, y4)
	DIVEAHYR (Light)	501.753 (2+) --> 774.389 (1+, y6) 501.753 (2+) --> 675.321 (1+, y5) 501.753 (2+) --> 546.278 (1+, y4)
BLBP = FABP7	ALGVGFATR (Heavy)	451.260 (2+) --> 717.392 (1+, y7) 451.260 (2+) --> 660.370 (1+, y6) 451.260 (2+) --> 561.302 (1+, y5)
	ALGVGFATR (Light)	446.256 (2+) --> 707.384 (1+, y7) 446.256 (2+) --> 650.362 (1+, y6) 446.256 (2+) --> 551.294 (1+, y5)
APOB	SPAFTDLHLR (Heavy)	389.545 (3+) --> 764.429 (1+, y6) 389.545 (3+) --> 663.381 (1+, y5) 389.545 (3+) --> 491.771 (2+, y8)
	SPAFTDLHLR (Light)	386.208 (3+) --> 754.421 (1+ y6) 386.208 (3+) --> 653.373 (1+ y5) 386.208 (3+) --> 486.767 (2+ y8)
PTGDS	APEAQVSVQPNFQQDK (Heavy)	897.449 (2+) --> 1297.663 (1+, y11) 897.449 (2+) --> 1198.594 (1+, y10) 897.449 (2+) --> 884.435 (1+, y7)
	APEAQVSVQPNFQQDK (Light)	893.442 (2+) --> 1289.648 (1+, y11) 893.442 (2+) --> 1190.580 (1+, y10) 893.442 (2+) --> 876.421 (1+, y7)

Human CSF biomarker-specific peptide precursor ions were selected for MRM-MS based on the above peptide and ion transition list.

MRM-MS was operated in positive ion mode. Mass over charge (m/z) and charge state (CS [z]) values for each peptide are listed.

SUPPLEMENTARY METHODS REFERENCES

1. Levine J, Kwon E, Sondej M, et al. Traumatically injured astrocytes release a proteomic signature modulated by STAT3 dependent cell survival. *Glia*. 2016; 64: 668-94.
2. Wanner IB. An in vitro trauma model to study rodent and human astrocyte reactivity. *Methods in molecular biology*. 2012; 814: 189-219.