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(54) Title: ASTROCYTE TRAUMATOME AND NEUROTRAUMA BIOMARKERS

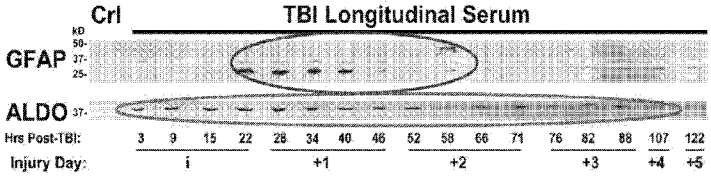

FIGURE 16

(57) Abstract: A method for detection or monitoring status of traumatic brain injury (TBI) or spinal cord injury (SCI) in a subject is provided. In one embodiment, the method comprises contacting a specimen of bodily fluid obtained from the subject with reagents for assaying for a marker of TBI selected from aldolase C (ALDOC) and brain lipid binding protein (BLBP), or a trauma-specific break down product (BDP) of ALDOC or BLBP. The method further comprises measuring the amount of marker present in the specimen as compared to a control sample, and determining the presence of TBI or SCI when an elevated amount of marker is present in the specimen compared to the control sample. The method can comprises measuring the amount of glutamine synthetase (GS), astrocytic phosphoprotein PEA-15 (PEA15), αB-crystallin (CRYAB/HSP27), a cleavage product of ALDOC, GS, PEA15, or CRYAB, or a combination of two or more thereof.

ASTROCYTE TRAUMATOME AND NEUROTRAUMA BIOMARKERS

[0001] This application claims benefit of United States provisional patent application number 62/157,389, filed May 5, 2015, the entire contents of which are incorporated by reference into this application.

REFERENCE TO A SEQUENCE LISTING SUBMITTED VIA EFS-WEB

[0002] The content of the ASCII text file of the sequence listing named "UCLA217_SL" which is 3 kb in size was created on May 5, 2016, and electronically submitted via EFS-Web herewith the application is incorporated herein by reference in its entirety.

ACKNOWLEDGEMENT OF GOVERNMENT SUPPORT

[0003] This invention was made with Government support under NS072606, awarded by the National Institutes of Health. The Government has certain rights in the invention.

[0004] This invention was made with Government support of Grant No. FSECSTS EMS# 12339079, awarded by the Dept. of Defense USAMRMC CDMRP award. The Government has certain rights in the invention.

TECHNICAL FIELD OF THE INVENTION

[0005] The present invention relates to compositions of matter, including antibodies, probes, kits and related materials, and their use for detection, early prediction of severity and outcome, monitoring of progression and of treatment of neurotrauma, including traumatic brain injury (TBI), mild TBI (concussion) and traumatic spinal cord injury (SCI) and their distinction from chronic neurodegenerative diseases.

BACKGROUND OF THE INVENTION

[0006] Each year, 1.7 million individuals sustain a TBI in the US. Between 1.6 and 3.8 million often unreported concussions occur annually, making TBI a silent epidemic of great significance. An additional one million patients are evaluated for spinal injuries every year in the US emergency departments, with 2-3 percent thereof suffering spinal cord injury. Life-saving treatment decisions for neurotrauma patients require rapid diagnosis and repeated accurate risk assessment due to evolving injury progression, as a brain trauma victim's condition typically changes each day after a TBI. Assessing moderate and severe traumatic brain and spinal cord injured patients is critical for safe urgent care, monitoring injury evolution to be ready for responding to secondary adverse events and for predicting outcome that is an early evaluation of the recovery potential for neurotrauma patients. Neurotrauma patient assessment is challenged by a broad heterogeneity in severity among patients. Identifying individual concussion victims at risk of complications, these are mild TBI patients with persistent symptoms or positive CAT scan finding, is a priority for urgent care responders as well as

sports and military arena operations. Infants, children and juvenile brain injuries comprise the leading cause of death and disability in children worldwide, yet the diagnosis is challenging because signs and symptoms of TBI are absent or overlap with common childhood illnesses. Since the developing brain is more sensitive to the ionizing radiation of CAT scanning, it is imperative to reduce unnecessary CT scans by providing objective biomarker testing. In addition to the youth, the elderly are a common target group for TBI due to falls, making it desirable to distinguish TBI biomarker signals from chronic neurodegenerative marker profiles.

[0007] Diagnosis and monitoring of TBI victims is critical for assessing severity of brain disturbance and assessing the risk level accurately to respond with the appropriate preventative care. For severe TBI patients, timely surgical intervention could be life-saving. For mild TBI patients, the identification of concussion patients at risk for developing chronic pain and cognitive or psychological deficits will help to provide treatment options, guidance in coping strategies and prevent exposing the recovering brain to a second impact. Current severity evaluations rely mainly on depth and duration of coma using the Glasgow Coma Scale, which varies daily with the patient's progressive injury course and is subject to medications that may be needed to maintain a coma (Iankova, 2006). Mild TBI is evaluated by time of unconsciousness, cognitive or psychological and pain symptoms that are subjective and may be motivationally influenced. Neuroimaging tools, especially advanced modalities, are difficult to be repeatedly administered for intensive care patients and have diverse readout values that lack standardization, are not everywhere available, and are of limited use for mild TBI and pediatric patients.

[0008] Measuring blood levels of surrogate chemical biomarkers can provide a simpler, objective and more easily standardized tool as a diagnostic starting point to classify risk and needs for TBI patients. Neurotrauma biomarkers should be acutely released from traumatized brain cells, be brain and mechanical trauma specific, readily pass the blood-brain barrier and show no or consistent low levels in healthy subjects.

[009] Currently, there is no sensitive, objective, standardized diagnostic test in clinical use for concussion patients, who are the majority of TBI patients, nor for pediatric patients with suspected TBI. These are both target populations particularly in need of objective risk assessment to prevent repeated hits putting the vulnerable brain at risk for suffering lasting brain damage. Intensive care unit head trauma patients are another target group who can benefit from repeated noninvasive blood sample analysis for monitoring, instead of, or supplementing time and cost consuming imaging because trauma progression is known for secondary deterioration on consecutive post-injury days that might require informed intervention. In addition, there remains a need for repeated biofluid sample analysis of brain injury biomarkers to determine short-term post-acute severity assessment and to determine efficacy of drug or other treatment paradigms administered to TBI patients.

SUMMARY OF THE INVENTION

[0010] The invention meets these needs and others, by providing a method for detection or monitoring status of traumatic brain injury (TBI) across the entire severity spectrum including diagnosis of mild TBI or/and determining mild TBI patients at risk of complications, and/or detection or monitoring status of spinal cord injury (SCI) in a subject. In one embodiment, the method comprises contacting a specimen of bodily fluid obtained from the subject with reagents for assaying for a marker of TBI selected from aldolase C (ALDOC) and brain lipid binding protein (BLBP/ FABP7), or a trauma-specific break down product (BDP) of ALDOC or BLBP/FABP7. The method further comprises measuring the amount of marker present in the specimen as compared to a control sample, and determining the presence of TBI or SCI when an elevated amount of marker is present in the specimen compared to the control sample. In one embodiment, the marker of TBI is ALDOC and/or a BDP thereof, and BLBP and/or a BDP thereof. Optionally, the method further comprises measuring the amount of glutamine synthetase (GS), astrocytic phosphoprotein PEA-15 (PEA15), αB-crystallin (CRYAB/HSP27), a trauma-specific proteolytic cleavage product of ALDOC, GS, PEA15, or CRYAB, or any combination of two or more thereof. In one embodiment, the method further comprises measuring the amount of glial fibrillary acid protein (GFAP), or of a 20-30 kDa BDP of GFAP.

[0011] Representative examples of the trauma-specific proteolytic cleavage product of ALDOC include a 38 kDa major fragment, or a 35 kDa fragment, a 30 kDa fragment, and a 25 kDa fragment. An example of the trauma-specific proteolytic cleavage product for BLBP/FABP7 is a 3 kDa breakdown product. Examples of trauma-specific proteolytic cleavage product of GS include a 37+35 kDa doublet, a 32 kDa fragment, a 23 kDa fragment, a 20 kDa fragment, and 18 kDa fragment. Examples of the trauma-specific proteolytic cleavage product of PEA15 include a 12+13 kDa doublet and a 8 kDa fragment. Examples of the trauma-specific proteolytic cleavage product of αB-crystallin is selected from the group consisting of a 18+19 kDa doublet, a 17 kDa fragment, a 15+14 kDa doublet and a 8 kDa fragment.

[0012] In one embodiment, the method further comprises measuring the amount of a blood specific protein in a cerebrospinal fluid (CSF) sample obtained from the subject. The detection and monitoring of such markers can be used to determine the status of intraventricular brain bleeding post-injury. In one embodiment, the blood specific protein is apolipoprotein B (APOB). In another embodiment, the method further comprises measuring the amount of prostaglandin synthase (PTGDS) in a cerebrospinal fluid (CSF) sample obtained from the subject. PTGDS, also known as beta trace protein, is abundant in control, non-TBI CSF positively correlated with a healthy CSF composition. The presence of TBI is determined when the amount of PTGDS is reduced, and rises with recovery. The detection and monitoring of such markers as an elevated blood-specific protein or a reduced CSF protein after TBI can therefore be used to determine the status of recovery to control, or normal, levels after injury.

[0013] In some embodiments of the method, no additional markers are assayed beyond those recited herein. In other embodiments, only markers recited herein are assayed. In some embodiments, additional markers known to those skilled in the art are assayed in combination with markers recited herein. In other embodiments, only a subset of possible markers is assayed. For example, the method can comprise assaying for 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 markers. In one particular embodiment, no more than 4 markers are assayed.

[0014] The reagents for use in the method of the invention can comprise antibodies or other molecules that specifically bind the marker of TBI. In one embodiment, the measuring comprises immunoassay. Examples of immunoassays include western blotting, immunofluorescence, immunoluminescence, radioimmunoassay, and enzyme linked immuno-sorbent assay (ELISA).

[0015] In another embodiment, the reagents comprise protein-sequence and -fragment-specific peptides. Such reagents are useful for methods in which the measuring comprises targeted quantitative mass spectrometry. In one embodiment, the measuring comprises quantitative signal detection of endogenous (in the sample) proteo-typic peptides that are compared to added ('spiked in') labeled (e.g., heavy isotope labeled) known amounts of the same proteo-specific peptides (internal standards) using multiple or parallel reaction monitoring mass spectrometry.

[0016] In one embodiment, the control sample is a pre-injury sample obtained from the subject. In another embodiment, the control sample is representative of normal, healthy subjects, such as an average value obtained from a control cohort of healthy subjects.

[0017] Representative examples of a specimen of bodily fluid for use in the invention include, but are not limited to, plasma, serum, cerebrospinal fluid (CSF), nasal fluid, cerumen, urine, saliva, lacrimal tears, and brain microdialysate.

[0018] The invention additionally provides a kit comprising agents that specifically bind a set of biomarkers. In one embodiment, the biomarkers comprise aldolase C (ALDOC) and brain lipid binding protein (BLBP). The agents are typically polynucleotides or antibodies, and optionally labeled with a detectable marker. The kit optionally further consists of at least one container for housing the agents and/or instructions for use of the agents for determining status of traumatic brain injury in a test sample. In some embodiments, the kit comprises agents that specifically bind astrocytic phosphoprotein PEA-15 (PEA15) and/or a 20-30 kDalton fragment of glial fibrillary acid protein (GFAP-BDP), either alone or together with additional markers described herein or known in the art. In one embodiment, the antibodies are monoclonal antibodies. In one embodiment, the set of biomarkers consists of up to 3, 4, 5, 6, 7, 8, 9, or 10 biomarkers.

[0019] The invention further provides a method of determining the expression of the biomarkers ALDOC and BLBP in a sample of serum obtained from a subject. In one

embodiment, the method comprises contacting the serum sample with a kit of the invention and measuring the binding of the agents to the biomarkers.

[0020] Also provided is a method of determining the status of traumatic brain injury in a sample of serum obtained from a subject. In one embodiment, the method comprises contacting the serum sample with a kit of the invention and measuring the binding of the agents to the biomarkers, and comparing the binding to a control sample. TBI is then determined to be present if the binding of the agents to ALDOC and BLBP is increased in the serum sample from the subject relative to the control sample. The invention further provides a method of detecting TBI in a subject. In one embodiment, the method comprises assaying a specimen of bodily fluid from the subject for an elevated amount of ALDOC and BLBP compared to a control sample. An elevated amount of ALDOC and/or BLBP is indicative of TBI. In one embodiment, the assaying is performed within 24 hours of a suspected injury, and up to one week post-injury. In some embodiments, the assaying is performed within 1-3 hours, or as early as within 15-30 minutes, of a suspected injury. In some embodiments, the subject is an infant or child, including, for example, a subject suspected of having experienced shaken baby syndrome. Suitable for this use is a biomarker expressed in the early developing brain, such as ALDOC or BLBP. In another embodiment, the subject is elderly, and the method is used to distinguish between TBI and chronic neurodegenerative disease, by measuring a ratio of ALDOC to its breakdown product.

[0021] The invention additionally provides a method of predicting outcome of TBI and/or recovery of ambulation after SCI in a subject. In one embodiment, the method comprises assaying a specimen of bodily fluid from the subject for an elevated amount of PEA15 or small BDPs of GFAP compared to a control sample or to a sample of a TBI survivor. wherein an elevated amount of PEA15 or small BDPs of GFAP is predicative of mortality. Also provided is a method of treating TBI in a subject. In one embodiment, the method comprises assaying a sample, obtained from the subject at multiple time points after injury (a longitudinal sample series) for a marker of TBI as described herein; and treating the patient for TBI if the assay indicates presence of TBI. This method can be used to monitor the status of the patient over time, and determine drug treatment efficacy, or whether an interventional treatment of the TBI patient would be indicated. Those skilled in the art will appreciate that each of the methods described herein can be performed with any one of the markers due to their very early postinjury release and prolonged detection window as well as variable biofluid clearance kinetic: ALDOC, BLBP, GS, PEA-15, CRYAB, a BDP of any of the foregoing; alone or in combination with one or more additional markers.

[0022] The markers ALDOC and BLBP, as well as PEA15 and CRYAB, are released from wounded, that is transiently compromised human brain cells and can therefore be used to track a concussion-relevant pathophysiological process, which is the brain's vulnerable state after

injury. This association of these markers to a potentially reversible injury state provides patho-mechanistic information that can aid in making the diagnosis of mild TBI more sensitive, and can be valuable for pharmacokinetic monitoring of TBI patients beyond and in addition to tracking cell death released markers that reflect tissue loss.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] Figure 1: Human neocortical astrocyte cell fates after mechanical trauma illustrated with bar graphs and photomicrographs of live-stained cells depicting nuclei of viable, leaky and dead cells.

[0024] Figure 2: Mechanically traumatized human astrocytes show prolonged endurance in a compromised state after wounding versus mouse astrocytes, as shown with a bar graph, a series of photomicrographs from time-lapse videos of live cells imaged on a temperature and gas controlled stage via confocal microscopy, and a schematic illustration of the protocol.

[0025] Figure 3: Mechanical trauma of human astrocytes causes significant release of astroglial markers into surrounding fluids, as illustrated with immunoblots (A), and bar graphs showing amounts of marker at various time points after injury (B-G).

[0026] Figure 4: Biplots showing that neurotrauma biomarkers are associated with cell fates of human traumatized astrocytes.

[0027] Figure 5: Schematic illustration of astrocyte injury biomarker selection strategy.

[0028] Figures 6A-6K: Immunoblots (6A-6C) and scatter-plots plotted jointly with box and whisker plots (6D-6K) with interquartile ranges (90th and 10th percentiles), median (line) and geometric mean (dashed line) showing logarithmic scaled optical densities measured from immunoblot signals using scaled densitometry (see Figure 3) in CSF of 20-25 TBI patients on injury day and subsequent 5 post-injury days and 8-11 Controls (n: subjects numbers per day), showing that astroglial injury markers are elevated in CSF of TBI patients versus controls in a retrospective observational cohort on injury day and consecutive 5 days post-injury.

[0029] Figure 7: TBI patient outcome correlation of biomarker CSF amounts.

[0030] Figure 8: Assessing the spectrum of TBI using factors of grouped astroglial injury markers.

[0031] Figure 9: Cell wounding over cell death – ratio of BLBP over GFAP differentiates trauma severity in human traumatized astrocytes and TBI patients.

[0032] Figure 10: Correlation between ALDOC levels and TBI patient outcome.

[0033] Figure 11: Acute astroglial marker release correlated with histopathological severity measures, tissue loss and hemorrhage, after swine spinal cord injury.

[0034] Figure 12: Outcome correlation of acutely elevated ALDOC and GFAP after swine spinal cord injury.

[0035] Figure 13: Quantitative mass spectrometry, multiple reaction monitoring, documents concentration of astroglial TBI markers and allows marker amount comparison independent of antibodies.

[0036] Figure 14: Quantitative antibody-based evaluation of ALDOC and BLBP amounts and concentration ranges in TBI CSF and blood using known amounts of pure proteins.

[0037] Figure 15: Blood-compatible astroglial biomarkers in blood samples of severe TBI patients, as shown by immunoblots (A) and graphs of amount measured over time after injury (B).

[0038] Figure 16: Immunoblots of longitudinal severe TBI serum sample show extended detection window of ALDOC versus GFAP.

[0039] Figure 17: Immunoblots showing BLBP breakdown product in CSF and plasma after TBI.

[0040] Figure 18: Evidence for acute circulatory appearance of astroglial injury markers due to direct passage across damaged blood-brain barrier, as shown by immunoblots (A) and graphs of measured amounts over time after injury.

[0041] Figure 19: Immunoblot data showing top tier astroglial injury markers and PEA15 are detected robustly and early in serum of mild TBI patients after concussions with or without complication (CT+: with complication – positive CAT scan; CT-: without complication, no CAT scan finding).

[0042] Figure 20: Immunoblot data showing acute and robust detection of serum ALDOC in pediatric TBI, infants.

[0043] Figure 21: Immunoblot data showing that full size ALDOC is present in greater amounts than the 38 kDa BDP in acute TBI, whereas the two sizes of ALDOC are present in different ratios (given in Table 2) in the chronic neurodegenerative condition of Alzheimer's disease.

[0044] Figure 22: Partitioning illustration of Table 3 thresholds.

[0045] Figure 23: Partitioning illustration of Table 4 thresholds.

[0046] Figure 24: Increased levels of glial trauma-release markers after repeated mild injury in the human trauma culture model, as indicated by percent astrocytes with acute membrane wounding and delayed cell death (A), and Conditioned medium (CM) levels of GFAP and AldoC after stretching (B).

[0047] Figure 25: Two film exposures of GFAP, showing that calpain and caspase activation generated GFAP upper and lower breakdown products after trauma.

DETAILED DESCRIPTION OF THE INVENTION

[0048] The invention provides several new TBI biomarkers that were initially tested on CSF, plasma and serum from TBI patients and controls. New neurotrauma markers are defined by their release mechanisms to associate with cell wounding and/or cell death of human brain astroglia in a trauma model. Data presented herein demonstrate that select biomarkers show highly interesting kinetics and stability in body fluids. Immunological detectability, sensitivity and specificity is shown and suitable monoclonal antibodies have been selected. The timing of appearance of markers in CSF and serum during the first hours and days after TBI are presented in the accompanying Examples and Appendix. The results show that markers described herein and detectable in patient serum or plasma can be used to identify moderate and severe TBI, as well as mild TBI, and patterns indicative of fatal TBI. The markers are summarized in Table 1.

Definitions

[0049] All scientific and technical terms used in this application have meanings commonly used in the art unless otherwise specified. As used in this application, the following words or phrases have the meanings specified.

[0050] As used herein, "major", as in "major BDP", refers to the most frequently and consistently observed breakdown product, for example the 38 kDa BDP of ALDOC is the major BDP of ALDOC.

[0051] As used herein, "acute" refers to an early time post-injury time, typical the biofluid sample was collected on injury day for it to be considered acute. For example, 15-30min after injury in trauma models, 1-2 hours post-injury in mild TBI patients, 3 hours to 24 hours post-injury in moderate and severe TBI patients.

[0052] As used herein, complicated mild TBI is used for concussion patients with positive computed tomography, CT / CAT scan finding, or more broadly with lasting symptomology, based on Buki et al., 2015.

[0053] As used herein, a "significant difference" means a difference that can be detected in a manner that is considered reliable by one skilled in the art, such as a statistically significant difference, or a difference that is of sufficient magnitude that, under the circumstances, can be detected with a reasonable level of reliability. In the Examples provided, herein, log-transformed data followed Gaussian distribution, and were used for statistical analyses by an independent statistician. One can use repeated measures analysis of variance with non-constant variance, mixed model (Crowder and Hand, 1990). As data are linear when log-transformed, significant changes are typically manifold, even by orders of magnitude. In one example, increase or decrease between TBI and controls that range between 80 fold to 13,000 fold are observed and found to be significant. In another example, changes across different post-injury days between

6 to 32 fold are considered significant. In yet another example, changes between survivors and non-survivors of TBI are between 4 fold and 1,400 fold are observed and found to be significant. In yet another example, an increase of two-fold relative to a reference or control sample is considered significant.

[0054] As used herein, "control" or "control sample" refers to a sample that is representative of either normal levels, or obtained from a subject known to be healthy.

[0055] As used herein, "a" or "an" means at least one, unless clearly indicated otherwise.

[0056] Table 1: Astroglial injury markers

Name	ID, molecular weight	Breakdown products (BDPs) with size	Release mechanism, marker class Biomarker properties
Top tier markers			
Glial fibrillary acidic protein	**GFAP, 50 kDa**	Lower GFAP BDPs 29, **25**, (23) kDa 19 + **20** doublet, sequence defined by mass spectrometry of traumatized astrocytes, TBI patient CSF and serum	**Cell death marker,** strong correlation to human astroglial death, not cell wounding. Small fragments are calpain and caspases activity dependent. **Delayed presence in TBI blood** **Fast clearance from biofluids** (CSF, blood). Brain specific and abundant Associates with TBI and SCI outcome and predicts SCI severity.
Fructose-1,6-bisphosphate aldolase C	**ALDOC, 40 kDa**	ALDOC BDPs **38** + 37 kDa doublet 35 + 30 kDa **25 kDa**	**Cell leak marker,** strong correlation to human astroglial cell wounding, moderate correlation to cell death. **Fast release & presence in TBI blood** **Long-lived in biofluids** (CSF, blood) Has higher levels than GFAP in CSF and blood on later post-injury days. **Highly brain enriched** and abundant. Strong predictive association with SCI severity and outcome; trend to relate with TBI outcome. ALDOC BDPs present in AD patients. More robust than GFAP in infants with TBI and mild TBI patients.
Brain lipid binding protein	**BLBP, 15 kDa** **FABP7**	BLBP BDP **3 kDa**	**Cell leak and cell death marker** Moderate correlations to both in traumatized human astrocytes.

Fatty acid binding protein 7			**Fast release & presence in TBI blood** **Short-lived in biofluids** (CSF, blood) Brain enriched; Suitable for TBI progression monitoring; BLBP/GFAP ratio differentiates TBI severity. Moderate correlation with SCI severity.
Second tier markers			
Astrocytic phosphoprotein 15	**PEA15, 15 kDa**	PEA15 BDPs 13 + 12 kDa doublet 8 kDa	**Cell leak marker,** strong correlation with human astrocyte cell wounding. **Fast release & presence in TBI blood** **Short-lived in biofluids** (CSF, blood) Suitable for TBI progression monitoring Trend to relate with TBI mortality
Glutamine synthetase	**GS, 45 kDa**	GS BDPs 37, 35, 32 kDa 23, 20, 18 kDa triplet	More stable in biofluids as BLBP and PEA15, but less stable than ALDOC Predictive of SCI severity
α Crystallin, B chain = Heath shock protein 27	**CRYAB, 21 kDa HSP27**	CRYAB BDPs 19+18, 17 kDa triplet 15 + 14 kDa doublet 8 kDa	**Cell leak marker,** strong correlation with human astroglial cell wounding, moderate correlation with cell death. Short-lived in biofluids (CSF, blood) CRYAB differentiates trauma severity
Standards, indicators for CSF samples			
Apolipoprotein B	**APOB, 120-130 kDa**	N/A	**Bleeding indicator** Secreted into blood from intestine & liver; absent from healthy CSF Associates with bleeding in TBI CSF.
Prostaglandin (D2) Synthase =β trace protein	**PTGDS, 22 kDa**	N/A	**Healthy CSF standard** Most abundant CSF protein. Secreted enzyme; Associates post-TBI with survival

Methods of the Invention

[0057] The invention provides a method for detection or monitoring status of traumatic brain injury (TBI), mild TBI, and/or spinal cord injury (SCI) in a subject. The method can be used to determine the presence, progression, prediction, and discrimination of severity of TBI or SCI in a subject. In one embodiment, the method comprises contacting a specimen of bodily fluid obtained from the subject with reagents for assaying for a marker of TBI selected from aldolase C (ALDOC) and brain lipid binding protein (BLBP/ FABP7), or a trauma-specific break down product (BDP) of ALDOC or BLBP/FABP7. The method further comprises measuring the amount of marker present in the specimen as compared to a control sample, and determining

the presence of TBI or SCI when an elevated amount of marker is present in the specimen compared to the control sample. In one embodiment, the marker of TBI is ALDOC and/or a BDP thereof, and BLBP and/or a BDP thereof. Optionally, the method further comprises measuring the amount of glutamine synthetase (GS), astrocytic phosphoprotein PEA-15 (PEA15), αB-crystallin (CRYAB/HSP27), a trauma-specific proteolytic cleavage product of ALDOC, GS, PEA15, or CRYAB, or any combination of two or more thereof. In one embodiment, the method further comprises measuring the amount of glial fibrillary acid protein (GFAP), or of a 20-30 kDa BDP of GFAP.

[0058] The monitoring of elevation of BLBP and/or PEA15 on subsequent days post-TBI informs on secondary adverse events post-injury. For example, the detection of elevated levels of ALDOC, BLBP, GS and PEA15 can be used to calculate Factor A, and levels of GFAP, S100beta and APOB can be used to calculate Factor B, based on marker loadings to each factor. Factor A and Factor B combined can be used to partition patients by severity. Factor A and B thresholds provide boundaries between TBI survivors, non-survivors and controls. A patient assessment within a clinical trial or study can be more robust by using a kit that provides multiple biomarker readings and performing factor analysis. This provides a simplified approach to track individual patients within a highly variable cohort, as opposed to requiring very large cohort sizes that may not be financially and otherwise feasible. Each clinical trial or study cohort biomarker panel data can be entered into a database, standardized and each patient is assessed based on tissue demise/bleeding versus tissue compromise/wounding Factors. Using Factors representative of these two classes makes the assessment more robust, as one zero reading will not prevent the entire factor analyses from providing a relative status output for any given patient. Boundaries pin out the severity spectrum of each cohort within which each patient will have a unique status at a given time post-injury

[0059] In one embodiment, the method further comprises calculating a ratio between amounts of BLBP, an example of a cell leak marker, and GFAP, a cell death marker. The amounts can be measured using optical densities. Ratios between amounts of BLBP and GFAP in the trauma model range from 0.6-1.2 correspond to mild/moderate trauma, while ratios between 0.1-0.4 correspond to severe trauma. This reflects the finding in the human culture trauma model that, in severe trauma, there is proportionally more GFAP found than after mild trauma. BLBP/GFAP ratios in moderate TBI patients range between 0.4-0.3, whereas the range in severe TBI patients is between 0.01 and 0.05, again expressing a proportional larger GFAP to BLBP amount in severe versus moderate TBI patients. As such, using this ratio provides a more robust patient severity classification. By including two markers, significance is reached, whereas one marker alone would require a much larger cohort size. Use of marker combinations can thereby help in assessing TBI status and monitoring TBI progression, by reducing minimum required patient enrollment sizes when used as an evaluation tool in clinical studies or trials.

[0060] The method for detecting and monitoring status of TBI in a subject can be used to identify a subject at risk for complications after mild TBI or concussion. This identification is made by using acute presence, such as using samples obtained within 1-2 hours and up to 17 hours post-injury of BLBP and/or PEA15, in addition to ALDOC in serum samples. ALDOC elevation alone can identify a concussion, and injury day elevation of ALDOC and BLBP and/or PEA15 is associated with risk for complications.

[0061] Representative examples of the trauma-specific proteolytic cleavage product of ALDOC include a major 38 kDa fragment that was found most consistently, a 35 kDa fragment, a 30 kDa fragment, and a 23 kDa fragment. Examples of the trauma-specific proteolytic cleavage product of GS include a 37+35 kDa doublet, a 32 kDa fragment, a 23 kDa fragment, a 20 kDa fragment, and 18 kDa fragment. Examples of the trauma-specific proteolytic cleavage product of PEA15 include a 12+13 kDa doublet and a 8 kDa fragment. Examples of the trauma-specific proteolytic cleavage product of αB-crystallin is selected from the group consisting of a 18+19 kDa doublet, a 17 kDa fragment, a 15+14 kDa doublet and a 8 kDa fragment.

[0062] The ratio of amount of ALDOC full size (40 kDa) to amount of ALDOC cleavage product (38 kDa) is indicative for time post-injury, as well as distinction of acute versus subacute versus chronic brain injury or neurodegenerative brain disease, including Alzheimer's disease (AD). Thus, in one embodiment, the method of detecting and/or monitoring TBI or SCI comprises determining the ratio of 40 kDa ALDOC to 38 kDa ALDOC levels in the specimen obtained from the subject. A ratio larger than 1, ranging from 3.6 - 8.6, is indicative of TBI (acute and sub-acute, over post-injury days 1-5), as full size ALDOC is much more abundant than the 38 kDa ALDOC BDP. A ratio smaller than 1, ranging 0.4-0.6 is indicative of Alzheimer's disease, as the 38 kDa ALDOC BDP was similar or more abundant than the full size ALDOC optical signal density because a chronic degenerative condition allows for long-term partial degradation and accumulation of the fragment than an acute injury condition.

[0063] In one embodiment, the method further comprises measuring the amount of a blood specific protein in a cerebrospinal fluid (CSF) sample obtained from the subject. The detection and monitoring of such markers can be used to determine the status of intraventricular brain bleeding post-injury. In one embodiment, the blood specific protein is apolipoprotein B (APOB). In another embodiment, the method further comprises measuring the amount of prostaglandin synthase (PTGDS) in a cerebrospinal fluid (CSF) sample obtained from the subject. PTGDS, also known as beta trace protein, is positively correlated with a healthy CSF composition. The presence of TBI is determined when the amount of PTGDS is reduced, and rises with recovery. The detection and monitoring of such markers can therefore be used to determine the status of recovery to healthy levels after injury. In some embodiments, recovery of acute trauma-reduced PTGDS levels is monitored over subsequent post-injury days and is predictive of survival, while sustained reduced levels of PTGDS predict mortality.

[0064] In some embodiments of the method, no additional markers are assayed beyond those recited herein. In other embodiments, only markers recited herein are assayed. In some embodiments, additional markers known to those skilled in the art are assayed in combination with markers recited herein. In other embodiments, only a subset of possible markers is assayed. For example, the method can comprise assaying for 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, or 20 markers. In one particular embodiment, no more than 4 markers are assayed.

[0065] The reagents for use in the method of the invention can comprise antibodies or other molecules that specifically bind the marker of TBI. In one embodiment, the measuring comprises immunoassay. Examples of immunoassays include western blotting, immunofluorescence, immunoluminescence, radioimmunoassay, and ELISA. ALDOC isoform specific antibodies are available as monoclonal antibodies clones 4A9, 1A1, 5C9 and E9 from EnCor Biotechnology Inc. (Gainesville, Florida). BLBP specific monoclonal antibodies are also available from EnCor Biotech Inc.

[0066] In another embodiment, the reagents comprise protein-sequence and -fragment-specific peptides. Such reagents are useful for methods in which the measuring comprises targeted quantitative mass spectrometry. In one embodiment, the measuring comprises quantitative signal detection of endogenous (in the sample) proteo-typic peptides that are compared to added ('spiked in') labeled (e.g., heavy isotope labeled) known amounts of the same proteo-specific peptides (internal standards) using multiple or parallel reaction monitoring mass spectrometry.

[0067] In one embodiment, the control sample is a pre-injury sample obtained from the subject. In another embodiment, the control sample is representative of normal, healthy subjects, such as an average value obtained from a control cohort of healthy subjects.

[0068] Representative examples of a specimen of bodily fluid for use in the invention include, but are not limited to, plasma, serum, cerebrospinal fluid (CSF), nasal fluid, cerumen, urine, saliva, lacrimal tears, and brain microdialysate.

[0069] The invention further provides a method of determining the presence of the biomarkers ALDOC and BLBP in a sample of serum or plasma obtained from a subject. In one embodiment, the method comprises contacting the serum or plasma sample with a kit of the invention and measuring the binding of the agents to the biomarkers.

[0070] Also provided is a method of determining the status of traumatic brain injury in a sample of serum or plasma obtained from a subject. In one embodiment, the method comprises contacting the serum/plasma sample with a kit of the invention and measuring the binding of the agents to the biomarkers, and comparing the binding to a control sample. TBI is then determined to be present if the binding of the agents to ALDOC and BLBP is increased in the serum sample from the subject relative to the control sample. For moderate to severe TBI

patients, amounts and concentration ranges for ALDOC and BLBP are given in Figure 14 and Example 13, and biomarker comparisons are given with concentrations in Figure 13 and Example12. The invention further provides a method of detecting TBI, in a subject. In particular, it can be used to detect mild TBI, where diagnosis is not otherwise obvious. In one embodiment, the method comprises assaying a specimen of bodily fluid from the subject for an elevated amount of ALDOC and BLBP compared to a control sample. An elevated amount of ALDOC and/or BLBP is indicative of TBI. In one embodiment, the assaying is performed within 24 hours of a suspected injury. In some embodiments, the assaying is performed within 1-3 hours, or as early as within 15-30 minutes, of a suspected injury. In one embodiment, the assaying is performed up to 7 days after a suspected injury. In some embodiments, the subject is an infant or child, including, for example, a subject suspected of having experienced shaken baby syndrome. The method allows for injury severity to be assessed, and outcome predicted in a subject, acutely after a spinal cord injury.

[0071] The invention additionally provides a method of predicting outcome of TBI and/or recovery after SCI in a subject. In one embodiment, the method comprises assaying a specimen of bodily fluid from the subject for an elevated amount of PEA15 and/or 20-30 kDa (small) GFAP fragments compared to a control sample, wherein an elevated amount of PEA15 and/or small (i.e. lower) GFAP fragments is predicative of mortality. Also provided is a method of treating TBI in a subject. In one embodiment, the method comprises assaying a sample obtained from the subject for a marker of TBI as described herein; and treating the patient for TBI if the assay indicates presence of TBI. The invention further provides a method of monitoring for treatment guidance in a subject being treated for TBI. In one embodiment, the method comprises assaying a sample obtained from the subject for a marker of TBI as described herein; and initiating a treatment of the patient for TBI if the assay indicates concerning deterioration of the patients status during the days post-injury, i.e. showing secondary elevated levels of any of the markers described herein. The methods of the invention additionally provide pharmacokinetic (theragnostic) applications, that is use in monitoring drug or other patient treatment for early evaluation of treatment effects and to monitor TBI progression post-injury. Those skilled in the art will appreciate that, given the different release and clearance kinetics of the markers described herein,the benefit of using multiple markers described herein as a panel. Thus the patients' assessment can include any one of the markers: ALDOC, BLBP, GS, PEA-15, CRYAB, a BDP of any of the foregoing; alone or in combination with one or more additional markers.

[0072] Some embodiments contemplated by the invention include use of a combination of TBI markers, including aldolase C (ALDOC), glutamine synthetase (GS), astrocytic phosphoprotein PEA-15 (PEA15), αB-crystallin (CRYAB), or brain lipid binding protein (BLBP/ FABP7), a trauma-specific proteolytic cleavage product of ALDOC, GS, PEA15, CRYAB, or BLBP/FABP7, or any combination of two or more thereof. For example, embodiments include those in which

the marker of TBI is GS and aldolase C, the marker of TBI is GS and PEA15, the marker of TBI is GS and αB-crystallin, the marker of TBI is GS and BLBP, the marker of TBI is aldolase C and PEA15, the marker of TBI is aldolase C and αB-crystallin, the marker of TBI is aldolase C and BLBP, the marker of TBI is PEA15 and αB-crystallin, the marker of TBI is PEA15 and BLBP, the marker of TBI is αB-crystallin and BLBP, the marker of TBI is GS, aldolase C, and PEA15, the marker of TBI is GS, BLBP, and PEA15, the marker of TBI is GS, αB-crystallin, and PEA15, the marker of TBI is GS, αB-crystallin, and BLBP, the marker of TBI is GS, αB-crystallin, and aldolase C, the marker of TBI is GS, BLBP, and aldolase C, the marker of TBI is aldolase C, PEA15, and αB-crystallin, the marker of TBI is aldolase C, PEA15, and BLBP, the marker of TBI is aldolase C, αB-crystallin, and BLBP, and the marker of TBI is PEA15, αB-crystallin, and BLBP. In the above examples, the TBI may be the recited protein, a breakdown product thereof, or both.

Kits

[0073] The invention additionally provides a kit comprising agents that specifically bind a set of biomarkers. In one embodiment, the biomarkers comprise aldolase C (ALDOC) and brain lipid binding protein (BLBP). The agents are typically polynucleotides or antibodies, and optionally labeled with a detectable marker. The kit optionally further consists of at least one container for housing the agents and/or instructions for use of the agents for determining status of traumatic brain injury in a test sample. In some embodiments, the kit further comprises agents that specifically bind astrocytic phosphoprotein PEA-15 (PEA15) and/or a 20-30 kDalton fragment of glial fibrillary acid protein (GFAP-BDP). In one embodiment, the antibodies are monoclonal antibodies. In one embodiment, the set of biomarkers consists of up to 3, 4, 5, 6, 7, 8, 9, or 10 biomarkers.

EXAMPLES

[0074] The following examples are presented to illustrate the present invention and to assist one of ordinary skill in making and using the same. The examples are not intended in any way to otherwise limit the scope of the invention.

Example 1: Human neocortical astrocyte cell fates after mechanical trauma

[0075] This Example shows population scores of human astrocytes 30 minutes and 48 hours after abrupt pressure-pulse traumatic stretching using different severities (Figure 1; PSI ranges from 2.6-4.0 for milder injury and 4.4-5.3 for severe injury). Human astrocytes were isolated from 16-18 week donated fetal neocortical brain specimen, purified and then differentiated on deformable membranes (Wanner, 2012). Cell membrane wounding / compromise, mechanoporation (Barbee, 2005), was determined using Propidium iodide (PI) uptake in living cultures accompanied by nuclear shape assessment (middle picture, stained nuclei, Hoechst stained after fixation, with little pink dots, nucleoli that are PI-stained). Cell death was

determined by PI uptake accompanied by condensed nuclei (pyknotic nuclei with compacted chromatin and bright Hoechst and PI stains). Significant percent of cell integrity compromise is shown 30min post-injury (left graph) while significant cell death was present two days post-injury (bars on the right).

Example 2: Mechanically traumatized human astrocytes show prolonged endurance in a compromised state

[0076] This Example demonstrates that mechanically traumatized human astrocytes show prolonged endurance in a compromised state after wounding versus mouse astrocytes (Figure 2). Dye uptake (0.05 mM 10 kDa dextran rhodamine) during stretch as well as cell death by apoptosis (caspase activation using 10 μM caspase 3 substrate) was monitored using time-lapse imaging on a temperature and humidity controlled spinning disc confocal microscope (Levine et al., 2016). Human traumatized wounded and resealed human astroglia show prolonged endurance after integrity compromise.

[0077] As shown in Figure 3, mechanical trauma of human astrocytes causes significant release of astroglial marker into surrounding fluids. Figure 3A shows immunoblots of concentrated, denatured conditioned media (Levine et al., 2016; Sondej et al., 2011) from traumatized astrocytes show different release profiles of glial fibrillary acidic proteins (GFAP) and its known and newly-identified breakdown products (BDPs) versus aldolase C (ALDOC), brain lipid binding protein (BLPB), astrocytic phosphoprotein (PEA15) and α crystallin (CRYAB). In order to capture the full range of signal intensity, signals from multiple exposures of immunoblots were densitometrically measured (optical densities) and scaled to match a single exposure. Scaled densities of signals from indicated number of donors are plotted on log-spaced axis in the graphs shown in Figure 3B-G. Known upper GFAP BDPs (between 37-50kDa) showed increased release with time (triangles) and severity (circle at 5 hours post-injury). New, lower GFAP BDPs were significantly elevated only one and two days post-injury, associating them with cell death (see Figure 1). ALDOC, BLBP and PEA15 were already significantly elevated 30min post-injury over two orders of magnitude and such elevated levels remained in fluids at all post-injury times measured. CRYAB levels showed severity distinction at 5 hours and two days post-injury.

Example 3: Neurotrauma biomarkers associated with cell fates of human traumatized astrocytes

[0078] Shown in Figure 4A are biplots of trauma-released astroglial marker levels (see Figure 3) for ALDOC, BLBP, PEA15 and CRYAB over the percent membrane permeable cells (% wounded, red data, left) as well as correlated to percent of dead human traumatized astrocytes using PI-dye-update assay and nuclear morphology (see Figures 1+2). Regression lines (R2-value) and p-values indicate correlation significance. ALDOC, PEA15 and CRYAB show best

correlation with human astroglial cell wounding. CRYAB, BLBP and ALDOC also correlated with extend of cell death after trauma.

[0079] Figure 4B shows biplots of GFAP trauma-release, which show correlation of GFAP with cell death inflicted by mechanical trauma and weak / no correlation with cell wounding. Plots are separated by grouped GFAP fragment sizes (upper bands: 50-37 kD, lower bands: 25-19 kD).

Example 4: Astrocyte Injury Biomarker Selection Strategy

[0080] Candidate astrocyte injury biomarkers were selected by the following strategy (Figure 5). A TBI CSF proteome was compiled by bottom-up mass spectrometry using CSF samples from 19 severe TBI patients and compared with the CSF proteome from 9 control subjects (Crl). This TBI CSF proteome (483 proteins) overlapped with 252 proteins from the control CSF proteome (402 proteins), leaving 231 unique TBI CSF proteins. Sixty percent of the TBI CSF proteins were also present in the published plasma proteomes (Omenn et al., 2005; Schenk et al., 2008) and, if abundant, may not be suitable as TBI biomarkers. To select candidate brain injury markers, we determined proteins released from traumatized astrocytes using a proteomic approach in a simple trauma model 79 differentially released proteins were identified after pressure-pulse stretching mouse astrocytes (Levine et al., 2015; Sondej et al., 2011).

[0081] From this "traumatome" 48 proteins (62%), were present in TBI CSF. Selecting for highly astrocyte-enriched proteins (>/= 5 fold enrichment over other cell types, Cahoy et al., 2008) yielded a small candidate pool of 11 injury biomarker proteins (black outlined enclosed 3 fields). These included 3 proteins GFAP and peroxiredoxin 6 (Prdx6, both present in plasma) and N,N-dimethyl arginine dimethyl aminohydrolase (DDAH1, center field of 3). Additional 3 proteins were astrocyte enriched, "traumatome" proteins present in TBI CSF and also in control CSF that were aldolase C (ALDOC), clusterin (CLUS) and apolipoprotein E (APOE, lower field of 3). Clusterin and APOE are secreted by astrocytes and their levels decreased in fluid after trauma (arrow). Additional 5 trauma-released proteins that were highly astrocyte enriched were not listed in the shotgun mass spectrometry-based TBI CSF proteome list. These 5 were ezrin (Ezr), F-box only protein 2 (FBX2), Glutamine synthetase (GS), astrocytic phosphoprotein 15 (PEA15) and brain-lipid binding protein (BLBP, upper field of 5, dashed outline). GFAP, ALDOC, GS, BLBP, PEA15 and CRYAB were included in subsequent immunological and mass spectrometry testing.

Example 5. Astroglial injury markers are elevated in CSF of TBI patients on injury day and 5 consecutive days post-injury

[0082] This Example demonstrates that astroglial injury markers are elevated in CSF of TBI patients versus controls in a retrospective observational cohort on injury day and for five consecutive days post-injury. Figure 6A shows immunoblots of GFAP (50 kDa with BDPs 37,

25, 20 and 18 kDa) and S100□ and ALDOC (40 kDa), GS (45 kDa), BLBP (15 kDa) and PEA15 (15 kDa) of a longitudinal set of 30 µl CSF samples from injury day (i) and subsequent 5 days post-injury (i+1 to i+5) of a 54 year old male severe TBI patient (1a.-1f.) alongside with 30 µl control CSF (Crl). BLBP and PEA15 showed strongest signals on injury day and second rise on 3rd post-injury day, that associated with this patient's secondary ischemic episode. Bleeding indicator APOB (130 and 250 kDa) had variable intensity over time post-injury and was absent from healthy CSF; CSF marker PTGDS (22 kDa) had robust signal in Crl CSF but was absent acutely after TBI and one day post-injury and stepwise recovered signals on subsequent post-injury days.

[0083] Figure 6B shows six CSF samples (30 µl/lane) from four TBI patients (2.-5.), illustrating variable signal intensities of GFAP and "upper" BDPs between 50 and 37kDa and "lower/new" GFAP BDPs including 25/23 kDa doublet, 20 kDa and 18 kDa small BDPs on injury day (patients 2, 3, 4a) and one day post-injury (4b, 5) alongside control CSF from a healthy 43 year old male. Individual bands within upper GFAP BDPs have similar intensities to each other, while relative abundance between lower GFAP BDPs was distinctly different across patients.

[0084] Figure 6C shows CSF immunoblots (30µl/lane), demonstrating full size ALDOC (40 kDa) in five TBI patients (6.-10.) and 38 kDa ALDOC BDP of variable intensity on four days post-injury in three TBI patients (8-10). A healthy Control subject showed no ALDOC.

[0085] Scatter-plots (Figures 6D-K, same day and patient replicates averaged) are plotted jointly with box and whisker plots with interquartile ranges (90th and 10th percentiles), median (line) and geometric mean (dashed line) showing logarithmic scaled optical densities measured from immunoblot signals using scaled densitometry (see Figure 3) in CSF of 20-25 TBI patients on injury day and subsequent 5 post-injury days and 8-11 Controls (n: subjects numbers per day). (Fig. 6D) Upper GFAP signals (50-37 kDa) were elevated on all TBI days versus Crl ($P < 0.06$) and declined over time as indicated ($P < 0.05$, repeated measures ANOVA, see Methods). (Fig. 6E) Lower GFAP BDPs (25-18 kD) were elevated in TBI versus Crl ($P < 0.03$) and declined between first post-injury day later post-injury days ($P < 0.05$). (Fig. 6F) ALDOC ($P < 0.004$) and (Fig. 6G) GS ($P < 0.001$) were elevated on each day in TBI CSF versus Crls without decline. (Fig. 6H) BLBP ($P < 0.03$) and (Fig. 6I) PEA15 ($P < 0.004$) had elevated levels in TBI versus Crls on indicated days with larger signal ranges. Serum protein APOB (Fig. 6J) was elevated in TBI versus Crl CSF ($P < 0.005$), whereas CSF standard PTGDS was stronger in Crl versus TBI ($P < 0.004$) with levels acutely depleted to various extents followed by outcome dependent recovery (see Figure 7).

Example 6: TBI patient outcome correlation of biomarker CSF amounts

[0086] This Example demonstrates that CSF levels of new, cell death associated lower GFAP breakdown products were two orders of magnitude more elevated in non-survivors versus

survivors of TBI (Figure 7). PEA15 levels were over 2-3 orders of magnitude elevated in non-survivors versus survivors after TBI, with p-value on the third post-injury day p=0.07. Levels of health marker PTGDS, which decreased acutely post-injury, showed significant recovery by the third post-injury day in survivors of TBI while levels remained decreased in non-survivors.

Example 7: Assessing the spectrum of TBI using factors of grouped astroglial markers

[0087] This Example demonstrates the grouping of astroglial trauma markers to create factors useful in the assessment of TBI across the spectrum of injury. To combine the diverse data encompassed by the marker panel, multivariate analysis of variance that employed an unsupervised learning algorithm based on Spearman correlation coefficients was used (factor analysis, Fabrigar and Wegener, 2012; Tucker, 1997). This approach is new to the neurotrauma biomarker field, and reveals underlying neurotrauma conditions by grouping markers here based on their TBI CSF signals. Known astroglial marker S100β, cell death markers GFAP with known and new BDPs, and bleeding indicator APOB were grouped into Factor A (Fig. 8A, gray, with combined loading using Cronbach's coefficient $\alpha = 0.921$). New 'cell leak', cell wounding markers ALDOC with BDP, BLBP, GS and PEA15, were grouped into Factor B ($\alpha=0.879$). As shown in Fig. 8B, Factor A's temporal profile significantly decreased over days post-injury, while Factor B's trajectory did not. Factor A data showed significant difference between survivor and non-survivors on several post-injury days (Fig. 8C). Factor B data did not show survival differences (Fig. 8D). Standardized marker density readings from 12 TBI patients with signal present for all markers were converted into the Factors A and B using the loadings shown in Fig. 8A, and then plotted against each other (Fig. 8E). Heterogeneity among the TBI spectrum is reflected in the spread of the combined panel data in this biplot. Classification tree analysis (Fig. 8F) determined boundaries that partitioned controls, survivors and non-survivors of TBI according to given Factor thresholds (Breiman, 1984). Patient partitioning thresholds using the biomarker levels grouped into factors are illustrated by lines in Fig. 8E.

[0088] This mathematical multivariate unsupervised learning approach combines the markers of this panel with each marker given a weight (loading) that is derived from correlation coefficients and expresses how much variance in the patient cohort is captured by this marker's contribution. Overall high loading of the listed markers (0.8-0.95) documents robust categorization into the two factors. Factor A reflects markers associated with cell death, hemorrhage and tissue loss. Factor B reflects markers associated with cell leak, wounding and tissue compromise (see Figure 8A). The resulting values document a unique position of each patient based on its biomarker panel readings within a given cohort. This approach reliably covers the majority of patient variation observed in this TBI cohort. Factor analysis provides a simplified framework for capturing a large patient heterogeneity observed in TBI using only few biomarker readings, (compared to more complex principal component analysis that used many

different entries). In a clinical trial, these biomarker panel readings from a test kit can be entered into a growing database that can provide valuable monitoring information of individual TBI patients compared to other patients. This tool can simplify patient assessment and strengthen the robustness of ongoing patient evaluation using biofluid signature of tissue compromise and tissue demise.

Example 8: Differentiating trauma severity in human traumatized astrocytes and TBI patients

[0089] This Example demonstrates that the ratio of BLBP to GFAP levels in a subject's specimen can be used to differentiate trauma severity in human traumatized astrocytes and TBI patients. Significant differences in fluid level ratios of 'cell leak' marker BLBP over 'cell death' marker GFAP are shown in vitro (Fig. 9, CM, conditioned medium, left) and in TBI patients (CSF, right panel of Fig. 9). Human astrocytes from 6 donors were traumatized with different severities using indicated PSI pressure-pulses. Moderate versus severe TBI patients are distinguished by 14 fold different BLBP/GFAP ratio in CSF (n=9). Moderate TBI patients are defined by post-resuscitation Glasgow coma scale (GCS) > 8 while severe TBI patients had GCS<8.

Example 9: Correlation between ALDOC levels and TBI patient outcome

[0090] Data presented in Figure 10 show ALDOC and GFAP levels plotted against outcome of severe TBI patients, assessed using extended Glasgow outcome score (GOSe) at 6 months post-TBI. Data from 12 (11) TBI patients show ALDOC elevation early (white, i, i+1, +2) and later post-injury (black, i+3, +4 and +5 post-injury days) in patients with unfavorable outcome, while GFAP levels did not as well maintain elevated levels on later post-injury days.

Example 10: Correlation between TBI markers and severity and outcome of spinal cord injury

[0091] This Example demonstrates the correlation between astroglial trauma markers and severity of spinal cord injury in a swine animal model. Figure 11: Acute UCLA astroglial marker release correlated with histopathological severity measures, tissue loss and hemorrhage, after swine spinal cord injury. **A)** Acute (15-30 min post-injury) CSF signal densities of UCLA astroglial injury markers ALDOC, BLBP and glutamine synthetase (GS) as well as GFAP are plotted against rostro-caudal cavity diameters in the Yucatan swine spinal cord one week after spinal cord contusion injury using an established injury weight drop model (Lee et al., 2013). Spearman correlations indicate acute elevation of these astroglial CSF marker levels associated significantly with increasing tissue loss. Histopathology: Rostro-caudal extension of the cavity was measures in Sudan black stained horizontal spinal cord sections (p-values and R^2 values given, n=10 animals). **B)** Acute (15-30min post-injury) CSF signal densities of ALDOC and GS significantly associated with white matter interstitial tissue bleeding one week post-injury that was measured using swine immunoglobulin (IgG) fluorescence in injured

horizontal spinal cord sections and normalized by parallel stained uninjured horizontal spinal cord sections. Such normalized white matter fluorescence signals of rostro-caudal serial images were plotted for each animal (n=8 animals) and areas under the curves (AUC) were determined over distances with above uninjured signal levels.

[0092] Figure 12 shows the correlation of acutely elevated ALDOC and GFAP with outcome after swine spinal cord injury. Recovery of walking associates with acute CSF levels of ALDOC and GFAP after swine spinal cord injury. Plotted are acute post-injury CSF levels (15-30 min post-injury) of ALDOC (black dots) and GFAP (white dots) that predicts recovery of walking one week post-injury using the porcine thoracic injury behavioral scale (PTIBS), an established test for recovery of ambulation in the swine indicating the level of walking recovery after spinal cord injury in the Yucatan swine (Lee et al., 2013). A significant inverse correlation existed with higher ALDOC (R^2 -0.88) and GFAP (R^2 -0.89) levels in poorly ambulating animals, who were dragging their hind limbs and crawled at best, and low acute ALDOC and GFAP levels in animals who walked by one week post-injury. Preliminary biomarker thresholds are indicated by background color, showing feasibility of very early partitioning of recovering animals.

Example 11: Quantitative mass spectrometry of astroglial TBI markers

[0093] This Example demonstrates that quantitative mass spectrometry confirms the increased levels of astroglial TBI markers following injury, providing objective marker amount comparisons that is limited when using immunological methods as these are not standardized. Multiple reaction monitoring, an antibody independent, simultaneous and quantitative mass spectrometry approach, was used for the first time in the neurotrauma biomarker field to compare abundance of known and new astroglial markers in CSF of TBI patients. Marker-specific peptides are measured in parallel with defined amounts of added, isotope-labeled peptides (see Table 6). Figure 13A shows that ALDOC and GFAP had the highest concentrations in TBI CSF on injury day, and both differed significantly from levels of other markers shown. In addition, BLBP levels were significantly higher than GS levels on injury day. As shown in Figure 13B, by 3 days post-TBI, ALDOC levels significantly out-performed GFAP concentrations, differing by an order of magnitude.

Example 12: Quantitative immunoassay of ALDOC and BLBP in TBI CSF and blood

[0094] This Example provides a quantitative antibody-based evaluation of ALDOC and BLBP levels in CSF and blood of TBI specimens using standard curves. Box plots in Figure 14 show median and interquartile concentration ranges of ALDOC (left) and BLBP (right) and their amounts in CSF and blood (serum and plasma). Inserts show dose-response using known amounts of isoform-specific recombinant proteins ALDOC and BLBP using two concentration ranges and immunoblot detection conditions (see Table 7).

Example 13: Detection of astroglial biomarkers in blood samples of severe TBI patients

[0095] This Example demonstrates that the astroglial trauma markers are compatible with blood testing, using samples obtained from patients having severe TBI. Figure 15A shows longitudinal plasma samples from 3 different severe TBI patients alongside one control plasma sample (Crl). Abundant proteins are removed using immunoaffinity albumin and immunoglobulin depletion columns (Sigma, ProteoPrep). New GFAP breakdown product of 25 kDa was never detected on injury day (i) but appeared on subsequent post-injury days. ALDOC was consistently present at all time points in all 3 patients. Short-lived markers PEA15 and BLBP were robustly present on injury day and showed different temporal profiles over subsequent days post-injury in each patient. Plotted in Figure 15B are scaled densitometry signals for ALDOC (B1), BLBP (B2), GFAP/25 kDa BDP (B3) and PEA15 (B4) from 26 serum and 24 plasma samples derived from 22 severe TBI patients compared with up to 11 control blood samples (Crl). Longitudinal same patient data are connected by gray lines. ALDOC was significantly elevated on injury day and every post-injury day, while GFAP was significantly elevated beginning on the first post-injury day. BLBP and PEA15 had significant elevation on injury day versus control levels.

Example 14: Extended detection window of ALDOC versus GFAP

[0096] This Example demonstrates, using a longitudinal severe TBI serum sample, the extended detection window of ALDOC versus GFAP. ALDOC was detected 19 hours prior to first detection of GFAP 25 kDa BDP and ALDOC signals were present over two days beyond the last specific GFAP signal, a 37 kDa known GFAP BDP (Figure 17).

Example 15: BLBP breakdown product

[0097] In addition to the full size, 15 kDa BLBP a 3 kDa BLBP-specific fragment was detected using 2 antibodies in TBI CSF and plasma on injury day and various post-injury days. Results are shown in Figure 17.

Example 16: Acute circulatory appearance of TBI markers

[0098] Figure 18 presents evidence for acute circulatory appearance of new astroglial injury markers due to direct passage across damaged blood-brain barrier. Panel A of Figure 18 shows immunoblots for GFAP 25 kDa BDP along with ALDOC, BLBP and PEA15 in CSF and serum of the same severe TBI patient acutely after TBI (3 hours post-injury), as well as on the first post-injury day. While GFAP appeared first in the CSF and with a day delay in serum, ALDOC was present in both biofluids at both time points. BLBP and PEA15 were first present in serum and appeared with delay in CSF. Traces on log-spaced axis of all 4 astroglial markers document the switch for GFAP 25 kDa BDP from CSF into serum, steadier presence of biofluid-

stable ALDOC, and the presence of short-lived BLBP and PEA15 in serum prior to their appearance and delayed elevation in CSF (Figure 18B).

[0099] These observations suggest the direct passage of ALDOC, BLBP and PEA15 from the injury site into the circulation. All three markers are localized in astroglial processes, with fine endings known to entirely wrap capillaries and blood vessels (Mathiisen et al., 2010). Traumatic injury, even mild TBI, causes rupture of perivascular astroglial fibers, allowing these markers a direct passage into the blood (Barzo et al., 1996; Hicks et al., 1993; Korn et al., 2005). GFAP is not localized in astroglial endings, and, as shown in Figure 3, the 25 kDa GFAP BDP takes time to be generated, suggesting a delayed release during cell death leading to accumulation first in the CSF, and subsequent appearance in serum. Advantage of a direct passage of astroglial endfeet via open blood-brain barrier lays in enabling very acute post-injury blood-testing.

Example 17: Early detection of astroglial injury markers in serum of mild TBI patients

[00100] This Example demonstrates robust and early detection of top tier astroglial injury markers in serum of mild TBI patients. Shown in Figure 19 are 10 serum samples from 7 mild TBI (mTBI) patients early after injury alongside of one control serum (Crl). Samples were probed for GFAP, ALDOC, BLBP and PEA15. Specific GFAP signals were faint and limited to4 mTBI patients with one patient showing GFAP/25 kDa BDP by 31 hours post-injury (patient # II). ALDOC 38 kDa BDP was consistently and strongly elevated in all mTBI patients versus control. BLBP and PEA15 showed variable intensity and were present in 5 mTBI patients. ALDOC, BLBP and PEA15 were detected already one hour post-injury. Concussion patients received computed tomography (CT) scans and those with positive findings of a lesion/bleed were classified as CT+, potentially complicated mTBI patients, and those without visible wounds were CT-negative, or uncomplicated (Buki et al., 2015). Robust presence of ALDOC and differential signals for BLBP and PEA15 are suited to augment risk identification among concussion patients.

Example 18: Acute and robust detection of serum ALDOC in pediatric TBI

[00101] Figure 20 shows acute and robust detection of ALDOC in pediatric TBI patient serum samples. Injury day serum samples from 5 infants (1-4 months of age) suffering TBI are shown next to a control serum from a child aged 22 months. Robust ALDOC signals are detected in all infant TBI versus control cases while only two infants showed weak GFAP signal (34 kDa BDP, not previously described, cases # I, III).

Example 19: ALDOC as a CSF marker for Alzheimer's disease

[00102] This Example demonstrates that CSF samples from patients afflicted with the chronic neurodegenerative condition of Alzheimer's disease exhibit equally distinct levels of full size (40 kDa) and 38 kDa BDP ALDOC. In contrast, ALDOC detected in CSF of an acute TBI

patient shows a distinct preponderance of full size (40 kDa) versus 38 kDa BDP. Shown in Figure 21 are 7 CSF samples from 5 Alzheimer's disease patients (AD, stage 0.5, cases #II and III; stage 1, cases # IV and V; based on Fagan et al., Science Transl. Med. 2014) compared to one severe TBI patient (case # I) and two age-matched controls (cases # VI and VII) and protein stain for loading (Ponceau S). The TBI patient had mainly full size ALDOC and little 38 kDa fragment on day 4 post-injury, while the chronic degenerative AD samples showed equal presence of full size and 38 kDa ALDOC BDP. The data suggest that acute and chronic brain injury can be distinguished on the basis of ALDOC /ALDOC BDP ratio.

[00103] Table 2: Distinction between TBI and Alzheimer's disease, using ratio between full size ALDOC and its 38 kDa proteolytic fragment in patients' CSF

	ALDOC 40kD/38kD All antibodies used	**ALDOC 40kD/38kD Same antibody (E9) used**
AD	**0.61** ± *0.25*	**0.42** ± *0.14*
TBI	**3.63** ± *3.26*	**8.56** ± *0.85*
AD v TBI	p = 0.0001	p = 0.00002

[00104] The table shows average ratios of full size ALDOC (40 kDa) over its breakdown product (38 kDa) in CSF of Alzheimer's Disease (AD) patients and moderate to severe TBI patients. Different ALDOC antibodies to different epitopes of the protein resulted in varying emphasis of the 40 versus 38 kDa band signal intensities. On the left data of all ALDOC antibodies were combined averaging 20 AD samples and 25 TBI samples. The average AD ratio was 6 fold smaller than the average TBI ratio that was significant by two-tailed T-test. On the right 10 AD CSF samples and 5 TBI patient's samples were analyzed using same antibody (E9) for ALDOC detection. There was again a significant 20 x difference between TBI, showing more full size signal and AD showing more BDP ALDOC signal. Data examples are shown in Figure 21. This provides a distinction between TBI and chronic neurodegenerative disease. Both data selections result in significantly different ratios based on the increased abundance of the ALDOC major 38 kDa BDP signifying the chronic neurodegenerative condition. Acute TBI can easily be distinguished by a higher abundance of full size 40 kDa ALDOC acutely and up to 5 days post-TBI.

Example 20: Multivariate discriminant analysis

[0105] Multivariate Classification Tree Analysis (Breiman, 1984) was used to determine the markers that most accurately split the subject cohort into Control and Surviving or Non-surviving TBI patients on injury, analyzed by immunoblotting of 30 µl CSF sample each. Upon obtaining 100% accuracy with just two markers, AldoC and PTGDS (Table 3), the analysis was repeated allowing only those markers known to be detectable in the blood for future non-invasive assay (Table 4). Markers used for the data presented in Table 3 were Aldo C and PTGDS, while the markers considered, but not used, were: GFAP, PEA15, GFAP lower, S100B, BLBP, GS, APOB, PTGDS, and AldoC 38kD. Table 3 thus summarizes the detection of

TBI and survival outcome prediction using all markers in the classification tree analysis. This analysis selected ALDOC and PTGDS as the best partitioning markers. Among all inspected markers, those were utilized by the mathematical unsupervised learning approach. Groups, n=21, all Controls; and all injury day TBI of 30 TBI samples. The indicated 7ng/30µl CSF equals a concentration of 233 ng/ml CSF using immunoblotting. Accuracy was excellent, as grouping and predicted outcome match 100% correctly. Figure 22 provides a partitioning illustration of the Table 3 thresholds.

[0106] Table 3: Detection of TBI and survival outcome prediction

Group	n	Aldo C [OD] (ng/30ul CSF)	PTGDS [OD]	Prediction
A	8	>= 0.078 (7ng)	< 0.174 or	TBI survivor (severe)
C		>= 0.078 (7ng)	>= 0.202	TBI survivor (moderate)
B	2	>= 0.078 (7ng)	0.174 to 0.202	TBI non-survivor
D	11	< 0.078 (7ng)	> 0.202	Control (healthy subject)

[0107] Table 4 shows the detection of TBI and survival outcome prediction using the new blood-compatible glial markers. Markers used for Table 4 (1): Aldo C total. Markers considered but not used were: GFAP lower, BLBP, PEA 15, Aldo C 38 kD breakdown product (BDP). The markers NOT considered (omitted) were: GFAP total, S100B, GS, APOB, and PTGDS. Groups were: n=21 all Control; and injury day TBI patients of a total of 30 in entire cohort. Accuracy was 95%; the unweighted probability correct was 20/21, or 0.952. There was a 96% equal priority probability correct = 0.96 = (1.0 + 1.0 + 0.875). Figure 23 provides a partitioning illustration of the Table 4 thresholds.

[0108] Table 4: Detection of TBI and survival outcome prediction using TBI markers

Group	n	Aldo C total [OD]	Prediction
A	4	0.078 to 0.22	TBI survivor
B	3	0.22 to 0.4	TBI non-survivor
C	3	>= 0.4	TBI survivor
D	11	< 0.078	Control (healthy subject)

[0109] Classification matrix for data in Table 4:

	Predicted-control	Predicted-died	Predicted-Survivor	total
True - control	11	0	0	11
True - died	0	2	0	2

True- survivor	0	1	**7**	8

[0110] Receiver Operating Characteristic (applicable with higher n):

Group	Area	N	N correct	Prop correct
Control	**1.0000**	11	11	1.0
Died	**0.9737**	2	2	1.0
survived	**0.9904**	8	7	0.875

Example 21: Spearman Correlations between marker signal density [OD] and CAT scan imaging data

[0111] Secondary TBI progression can cause elevated intracranial pressure (ICP) which is often associated with secondary injury. Observed is a significant correlation between levels of cell death marker GFAP lower BDPs (19, 20, 25 kDa doublet) and ICP. Bleeding marker APOB is significantly correlated with extra+intraparenchymal lesion volume and midline shift. Both these CT findings are associated with brain bleeding, including epidural hematoma, subdural hematoma, subarachnoid hemorrhage and intraparenchymal lesions. Glial injury markers BLBP and PEA15, as well as cell death glial marker GFAP lower BDPs, are correlated with intra-parenchymal lesions, including brain tissue contusion, intracranial hemorrhage and diffuse axonal injury.

[0112] Table 5: Spearman Correlations between marker signal density and CAT scan data

New TBI marker panel	ICP, Intracranial pressure		Midline Shift (edema indication)		Extra+Intra Parenchymal Lesion Volume		Intra-parenchymal Lesion Volume	
	Correla-tion	p-value	Correla-tion	p-value	Correla-tion	p-value	Correla-tion	p-value
Lower GFAP BDPs	*0.834*	**0.0005** 9	0.121	0.733	*0.676*	0.136	*0.775*	0.333
ALDOC	-0.165	0.614	-0.0872	0.776	-0.126	0.72	-0.4	0.517
BLBP	0.204	0.559	-0.194	0.58	0.116	0.803	*0.6*	0.417
PEA15	0.21	0.55	0.373	0.321	*0.667*	0.233	*0.738*	0.333
APOB	-0.258	0.446	*0.684*	**0.0361**	*0.986*	**0.0028**	*0.667*	0.233

Example 22: Increased levels of glial trauma-release markers after repeated mild injury in the human trauma culture model

[0113] Figure 24 shows results obtained using a model for repeat mild injury. Human astrocytes received a single (•) or double (•-•) mild pressure pulse 30 mins apart (30') or one day apart (1D). Cell populations of acute leaky and delayed dead were not much changed by

the repeated trauma (Fig. 24A). Yet, conditioned medium (CM) fluid levels of trauma-release markers GFAP and AldoC were elevated after shortly-followed repeated mild injury versus a single mild stretch, and were only slightly elevated when the two insults were one day apart. AldoC levels nearly reached those of a single severe insult (•) after shortly-followed mild stretches, indicating its sensitivity to repeated injury.

Example 23: Calpain and caspase activation generated GFAP upper and lower breakdown products after trauma

[0114] Shown in Figure 25 are two film exposures of GFAP, upper and lower breakdown products 48 hour post-injury in conditioned medium samples using the DAKO anti-GFAP polyclonal antibody. Severely stretched and unstretched cultures received either no drug, calpain inhibitor PD150606 (100μM, "Cali") or pan-caspase inhibitor Z-VAD FMK (8.8μM, "Casi"). Trauma-released GFAP upper and lower BDPs were reduced by both calpain and caspase inhibition, suggesting trauma induced activation of both enzymes degrading GFAP after injury. Part of this enzymatic breakdown occurs in cells, part extracellular as suggested by similar analyses of cell lysate fractions of the same experiments. Minor release of lower GFAP BDPs in unstretched cultures was due to small numbers of non-traumatic cell death.

Example 24: Antibodies and proteins used for western blotting of astroglial injury markers

[0115] Table 6: Antibodies and proteins used for western blotting of markers

Name	Antibodies, recombinant proteins	Epitope	Comments
GFAP	Rabbit polyclonal anti-GFAP (DAKO, Z0334) Chicken polyclonal anti GFAP (ThermoFisher Scientific, PA1-10004)	Whole cow GFAP Whole bovine GFAP	Polyclonal Abs recognize full size GFAP, upper & lower BDP set
ALDOC	Rabbit affinity purified polyclonal anti-ALDOC (Genetex, GTX102284) Rabbit Serum 88, 89 (EnCor, Biotech) Several monoclonal ALDOC antibodies (EnCor): IgG1 mab 1A1 (MCA-1A1), IgG1 mab E9 (MCA-E9), IgG1 mab 4A9 (MCA-4A9), IgG1 mab 5C9 (still under development) Standard: His-tagged human ALDOC recombinant protein (EnCor Biotech. Inc.)	Recombinant ALDOC fragment amino acids 10-163 (P09972) Recombinant whole ALDOC, BDPs Mab 1A1: C-terminal peptide, does not detect ALDOC BDPs, no signal in blood; Mab E9: Recombinant whole ALDOC, detects ALDOC BDPs, signal in human blood (serum, plasma); Mab 4A9: N-terminal peptide sequence: MPHSYPALSAEQKKELS (SEQ ID NO: 1), signal in human and pig blood); Mab 5C9: N-terminus, signal in human blood (serum & plasma).	 Standard curves using pure recombinant ALDOC (EnCor) See Fig. 14

GS	Rabbit IgG fraction polyclonal anti GS (Sigma, G2781) Mouse mab IgG2A to GS clone 6 (BD Transduction, 610517)	GS peptide amino acids 357-373, BDPs are seen, more sensitive Full size GS, less sensitive no BDPs	
PEA15	Rabbit polyclonal affinity purified anti PEA15 (Cell Signaling) Standard: Recombinant PEA15 (EnCor)	Human PEA15 peptide surrounding Leu60	
BLBP = FABP7 = B-FABP	Affinity purified rabbit polyclonal anti –FABP7 (Millipore) Affinity purified rabbit polyclonal anti- FABP7 clone RB22973(Abgent) Mouse monoclonal IgG2b anti-FABP7 (Hycult, HM2299) Standard: His-tagged recombinant BLBP protein (EnCor Biotech.Inc.)	GST-tagged recombinant full size human FABP7, brain specific (Millipore) C-terminal human FABP7 peptide amino acids 104-132, brain specific (Abgent) Peptide derived from human B-FABP sequence (Hycult)	Standard curves using pure recombinant BLBP (EnCor) see Fig. 14
CRYAB = HSP 27	Mouse monoclonal IgG1 anti-CRYAB (Enzo, 1B61-3G4) Rabbit affinity purified polyclonal anti-CRYAB (EMD Millipore, ABN185)	Whole bovine CRYAB, recognizes full size and BDPs N-terminus	
APOB	Rabbit affinity purified polyclonal IgG anti-APOB (PTGlab, 20578-1-AP)	Unspecified APOB peptide APOB 120-130 kDa observed band, full size 516 kDa	
PTGDS	Rabbit affinity purified IgG anti-PTGDS (USBiological, P9053-24D)	Synthetic human PTGDS peptide amino acids 120-190	

Example 25: Multiple Reaction Monitoring mass spectrometry

[0116] Biofluid concentrations of the TBI injury biomarker proteins were measured by targeted multiple-reaction-monitoring (MRM) mass spectrometry. Biofluid samples were first digested using endoproteinase trypsin, cleaving all proteins into their respective tryptic peptides. Protein specific peptide signals were used as a surrogate measure for their respective proteins. In MRM-MS, peptide signals are measured by what are known as precursor → to product ion transitions as shown in Table 7 below (e.g. 554.821 (2+) --> 924.514 (1+, y8)). Selection of specific precursor ions of interest allows for increased sensitivity. By measuring signal from specific product ions from selected precursors, MRM allows for a high degree of analyte specificity. For quantitation, defined amounts of stable isotope-labeled standard (SIS) peptides containing either a heavy lysine [K(Label:13C(6)15N(2))] or heavy arginine [R(Label:13C(6)15N(4))] are spiked into biofluid samples. These heavy standard peptides are chemically identical to their endogenous (light) counterparts but display a mass shift of +8 and +10 Da (K and R respectively) for differentiation from endogenous biofluid peptides. Comparison of the peak area ratios between the light and heavy MRM transitions allows for absolute quantitation of biomarker concentrations. For our assay, trypsin digested biofluids were first separated by reversed phase liquid chromatography using a 0.1% formic acid in

water and 0.1% formic acid in acetonitrile elution system to further reduce sample complexity and improve signal sensitivity.

[0117] Table 7: Multiple Reaction Monitoring mass spectrometry peptide TBI biomarkers

Name	Peptide Sequence	Measured MRM Transition
GFAP all within core fragment	ALAAELNQLR(Heavy) SEQ ID NO: 2	554.821 (2+) --> 924.514 (1+, y8) 554.821 (2+) --> 853.477 (1+, y7) 554.821 (2+) --> 782.439 (1+, y6)
	ALAAELNQLR(Light) SEQ ID NO: 2	549.816 (2+) --> 914.505 (1+, y8) 549.816 (2+) --> 843.468 (1+, y8) 549.816 (2+) --> 722.431 (1+, y8)
	LADVYQAELR (Heavy) SEQ ID NO: 3	594.758 (2+) --> 1003.508 (1+, y8) 594.758 (2+) --> 789.413 (1+, y6) 594.758 (2+) --> 626.350 (1+, y5)
	LADVYQAELR (Light) SEQ ID NO: 3	589.314 (2+) --> 993.500 (1+, y8) 589.314 (2+) --> 779.405 (1+, y6) 589.314 (2+) --> 616.341 (1+, y5)
ALDOC	TPSALAILENANVLAR (Heavy) SEQ ID NO: 4	831.974 (2+) --> 1193.688 (1+ y11) 831.974 (2+) --> 1122.651 (1+ y10) 831.974 (2+) --> 1009.566 (1+ y9)
	TPSALAILENANVLAR (Light) SEQ ID NO: 4	826.970 (2+) --> 1183.679 (1+, y11) 826.970 (2+) --> 1112.642 (1+, y10) 826.970 (2+) --> 999.558 (1+, y9)
	LSQIGVENTEENR (Heavy) SEQ ID NO: 5	749.872 (2+) --> 1170.563 (1+, y10) 749.872 (2+) --> 1057.478 (1+, y9) 749.872 (2+) --> 901.389 (1+, y7)
	LSQIGVENTEENR (Light) SEQ ID NO: 5	744.868 (2+) --> 1160.554 (1+, y10) 744.868 (2+) --> 1047.470 (1+, y9) 744.868 (2+) --> 891.380 (1+, y7)
GS	DIVEAHYR (Heavy) SEQ ID NO: 6	506.758 (2+) --> 784.398 (1+, y6) 506.758 (2+) --> 685.329 (1+, y5) 506.758 (2+) --> 556.287 (1+, y4)
	DIVEAHYR (Light) SEQ ID NO: 6	501.753 (2+) --> 774.389 (1+, y6) 501.753 (2+) --> 675.321 (1+, y5) 501.753 (2+) --> 546.278 (1+, y4)
PEA15	DNLSYIEHIFEISR (Heavy) SEQ ID NO: 7	582.629 (3+) --> 661.354 (1+, y5) 582.629 (3+) --> 702.363 (2+, y11) 582.629 (3+) --> 658.847 (2+, y10)
	DNLSYIEHIFEISR (Light) SEQ ID NO: 7	579.293 (3+) --> 651.346 (1+, y5) 579.293 (3+) --> 697.359 (2+, y11) 579.293 (3+) --> 653.843 (2+, y10)
BLBP = FABP7	ALGVGFATR (Heavy) SEQ ID NO: 8	451.260 (2+) --> 717.392 (1+, y7) 451.260 (2+) --> 660.370 (1+, y6) 451.260 (2+) --> 561.302 (1+, y5)
	ALGVGFATR (Light) SEQ ID NO: 8	446.256 (2+) --> 707.384 (1+, y7) 446.256 (2+) --> 650.362 (1+, y6) 446.256 (2+) --> 551.294 (1+, y5)
CRYAB = HSP 27	HFSPEELK (Heavy) SEQ ID NO: 9	497.758 (2+) --> 857.450 (1+, y7) 497.758 (2+) --> 710.381 (1+, y6) 497.758 (2+) --> 623.349 (1+, y5)

	HFSPEELK (Light) SEQ ID NO: 9	493.751 (2+) --> 849.435 (1+, y7) 493.751 (2+) --> 702.367 (1+, y6) 493.751 (2+) --> 615.335 (1+, y5)
APOB	SPAFTDLHLR (Heavy) SEQ ID NO: 10 SPAFTDLHLR (Light) SEQ ID NO: 10	389.545 (3+) --> 764.429 (1+, y6) 389.545 (3+) --> 663.381 (1+, y5) 389.545 (3+) --> 491.771 (2+, y8) 386.208 (3+) --> 754.421 (1+ y6) 386.208 (3+) --> 653.373 (1+ y5) 386.208 (3+) --> 486.767 (2+ y8)
PTGDS	APEAQVSVQPNFQQDK (Heavy) SEQ ID NO: 11 APEAQVSVQPNFQQDK (Light) SEQ ID NO: 11	897.449 (2+) --> 1297.663 (1+, y11) 897.449 (2+) --> 1198.594 (1+, y10) 897.449 (2+) --> 884.435 (1+, y7) 893.442 (2+) --> 1289.648 (1+, y11) 893.442 (2+) --> 1190.580 (1+, y10) 893.442 (2+) --> 876.421 (1+, y7)

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[0136] Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to describe more fully the state of the art to which this invention pertains.

[0137] Those skilled in the art will appreciate that the conceptions and specific embodiments disclosed in the foregoing description may be readily utilized as a basis for modifying or designing other embodiments for carrying out the same purposes of the present invention. Those skilled in the art will also appreciate that such equivalent embodiments do not depart from the spirit and scope of the invention as set forth in the appended claims.

What is claimed is:

1. A method for detection or monitoring status of traumatic brain injury (TBI) and/or spinal cord injury (SCI) in a subject, the method comprising:

(a) contacting a specimen of bodily fluid obtained from the subject with reagents for assaying for a marker of TBI selected from aldolase C (ALDOC) and brain lipid binding protein (BLBP/ FABP7), or a trauma-specific break down product (BDP) of ALDOC or BLBP/FABP7;

(b) measuring the amount of marker present in the specimen as compared to a control sample; and

(c) determining the presence of TBI or SCI when an elevated amount of marker is present in the specimen compared to the control sample.

2. The method of claim 1, wherein the marker of TBI is ALDOC or a BDP thereof, and BLBP or a BDP thereof.

3. The method of claim 1, further comprising measuring the amount of glutamine synthetase (GS), astrocytic phosphoprotein PEA-15 (PEA15), αB-crystallin (CRYAB/HSP27), a trauma-specific proteolytic cleavage product of ALDOC, GS, PEA15, or CRYAB, or any combination of two or more thereof.

4. The method of claim 1, wherein the trauma-specific proteolytic cleavage product of ALDOC is selected from the group consisting of a 38 kDa fragment, a 35 kDa fragment, a 30 kDa fragment, and a 23 kDa fragment.

5. The method of claim 3, wherein the trauma-specific proteolytic cleavage product of GS is selected from the group consisting of a 37+35 kDa doublet, a 32 kDa fragment, a 23 kDa fragment, a 20 kDa fragment, and 18 kDa fragment.

6. The method of claim 3, wherein the trauma-specific proteolytic cleavage product of PEA15 is selected from the group consisting of a 12+13 kDa doublet and an 8 kDa fragment.

7. The method of claim 3, wherein the trauma-specific proteolytic cleavage product of αB-crystallin is selected from the group consisting of an 18+19 kDa doublet, a 17 kDa fragment, a 15+14 kDa doublet and a 8 kDa fragment.

8. The method of claim 1, further comprising measuring the amount of a blood specific protein in a cerebrospinal fluid (CSF) sample obtained from the subject.

9. The method of claim 8, wherein the blood specific protein is apolipoprotein B (APOB).

10. The method of claim 1, further comprising measuring the amount of prostaglandin synthase (PTGDS) in a cerebrospinal fluid (CSF) sample obtained from the subject, and the presence of TBI is determined when the amount of PTGDS is reduced.

11. The method of claim 1, further comprising measuring the amount of a 20-30 kDa BDP of glial fibrillary acid protein (GFAP).

12. The method of claim 1, wherein the reagents of step (a) comprise antibodies that specifically bind the marker of TBI, and the measuring comprises immunoassay.

13. The method of claim 12, wherein the immunoassay comprises western blotting, or ELISA.

14. The method of claim 1, wherein the control sample is a pre-injury sample obtained from the subject.

15. The method of claim 1, wherein the control sample is an average value obtained from a control cohort of healthy subjects.

16. The method of any of the preceding claims in which no additional markers are assayed.

17. The method of any of the preceding claims in which no more than 4 markers are assayed.

18. The method of any of the preceding claims in which the specimen of bodily fluid comprises plasma, serum, cerebrospinal fluid (CSF), nasal fluid, cerumen, urine, saliva, lacrimal tears, or brain microdialysate.

19. The method of claim 1, wherein the reagents comprise protein-sequence and -fragment-specific peptides, and where in the measuring comprises targeted quantitative mass spectrometry.

20. The method of claim 1, wherein the measuring comprises multiple or parallel reaction monitoring mass spectrometry.

21. A kit comprising agents that specifically bind a set of biomarkers, wherein the biomarkers comprise:

(a) aldolase C (ALDOC); and

(b) brain lipid binding protein (BLBP);

wherein the agents are polynucleotides or antibodies, the agents optionally labeled with a detectable marker, and wherein the kit optionally further consists of at least one container for housing the agents and/or instructions for use of the agents for determining status of traumatic brain or spinal cord injury in a test sample.

22. The kit of claim 21, further comprising agents that specifically bind:

(c) astrocytic phosphoprotein PEA-15 (PEA15); and/or

(d) a 20-30 kDalton fragment of glial fibrillary acid protein (GFAP-BDP).

23. The kit of claim 21, wherein the antibodies are monoclonal antibodies.

24. The kit of claim 21, wherein the set of biomarkers consists of up to 4 biomarkers.

25. A method of determining the expression of the biomarkers ALDOC and BLBP in a sample of serum obtained from a subject, the method comprising:

(a) contacting the serum sample with a kit of claim 21; and

(b) measuring the binding of the agents to the biomarkers.

26. A method of determining the status of traumatic brain injury in a sample of serum obtained from a subject, the method comprising:

(a) contacting the serum sample with a kit of claim 21; and

(b) measuring the binding of the agents to the biomarkers;

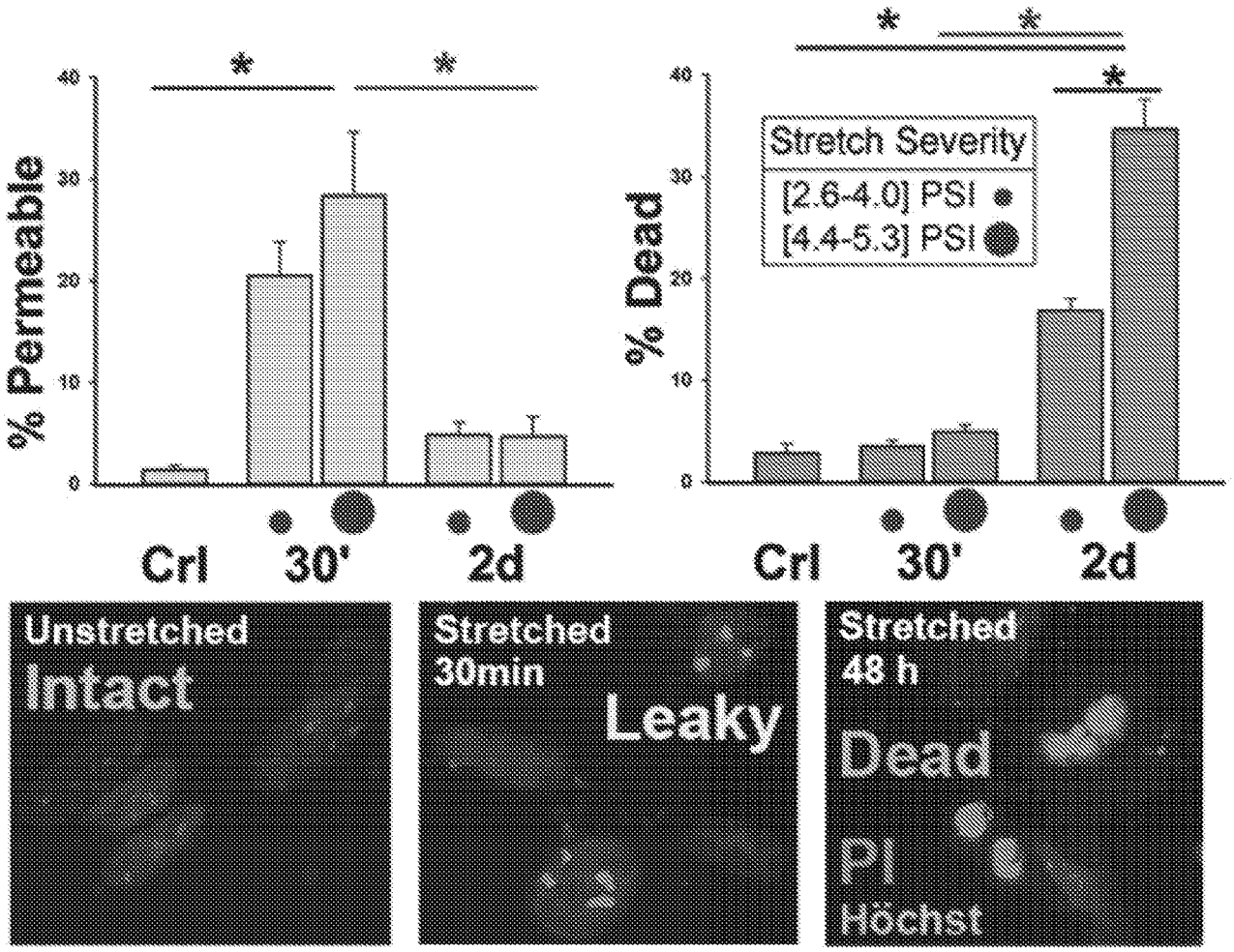
(c) comparing the binding to a control sample; and

(d) determining TBI to be present if the binding of the agents to ALDOC and BLBP is increased in the serum sample from the subject relative to the control sample.

27. A method of detecting TBI in a subject, the method comprising assaying a specimen of bodily fluid from the subject for an elevated amount of ALDOC and BLBP compared to a control sample, wherein an elevated amount of ALDOC and/or BLBP is indicative of TBI.

28. The method of claim 27, wherein the assaying is performed within 24 hours of a suspected injury.

29. The method of claim 27 or 28, wherein the subject is an infant or child.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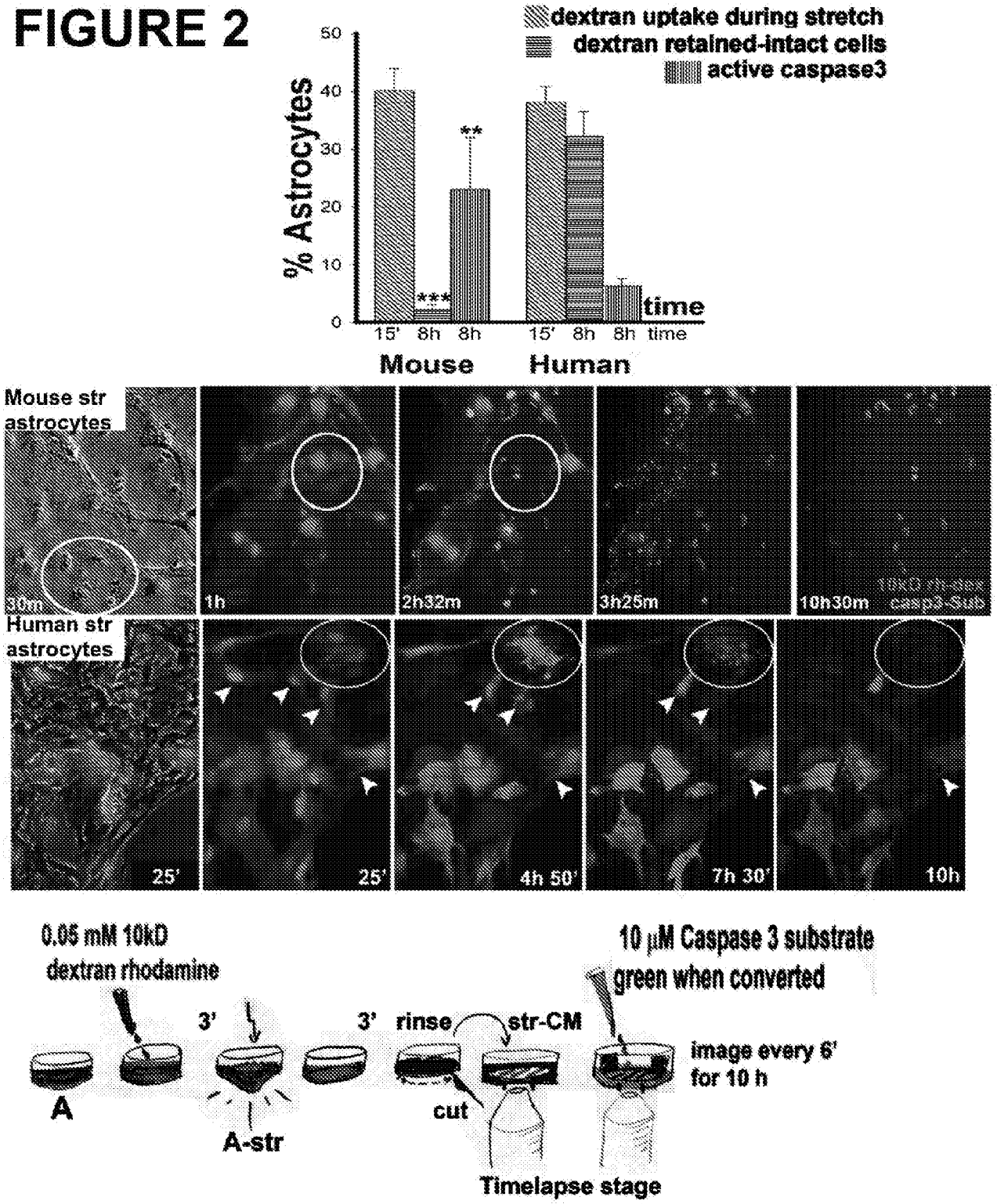
Ctrl
30'
2d
% Dead
Stretch Severity
[2.6-4.0] PSI
[4.4-5.3] PSI
Unstretched
Intact
Stretched
30min
Leaky
Stretched
48 h
Dead
PI
Höchst

FIGURE 1

FIGURE 2


dextran uptake during stretch
dextran retained-intact cells
active caspase3
% Astrocytes
50
40
30
20
10
0
**
***
time
15' 8h 8h 15' 8h 8h time
Mouse
Human
Mouse str astrocytes
30m
1h
2h32m
3h25m
10h30m
10KD dextran
casp3-Sub
Human str astrocytes
25'
25'
4h 50'
7h 30'
10h
0.05 mM 10kD dextran rhodamine
3'
3'
rinse
str-CM
10 μM Caspase 3 substrate green when converted
image every 6' for 10 h
A
A-str
cut
Timelapse stage

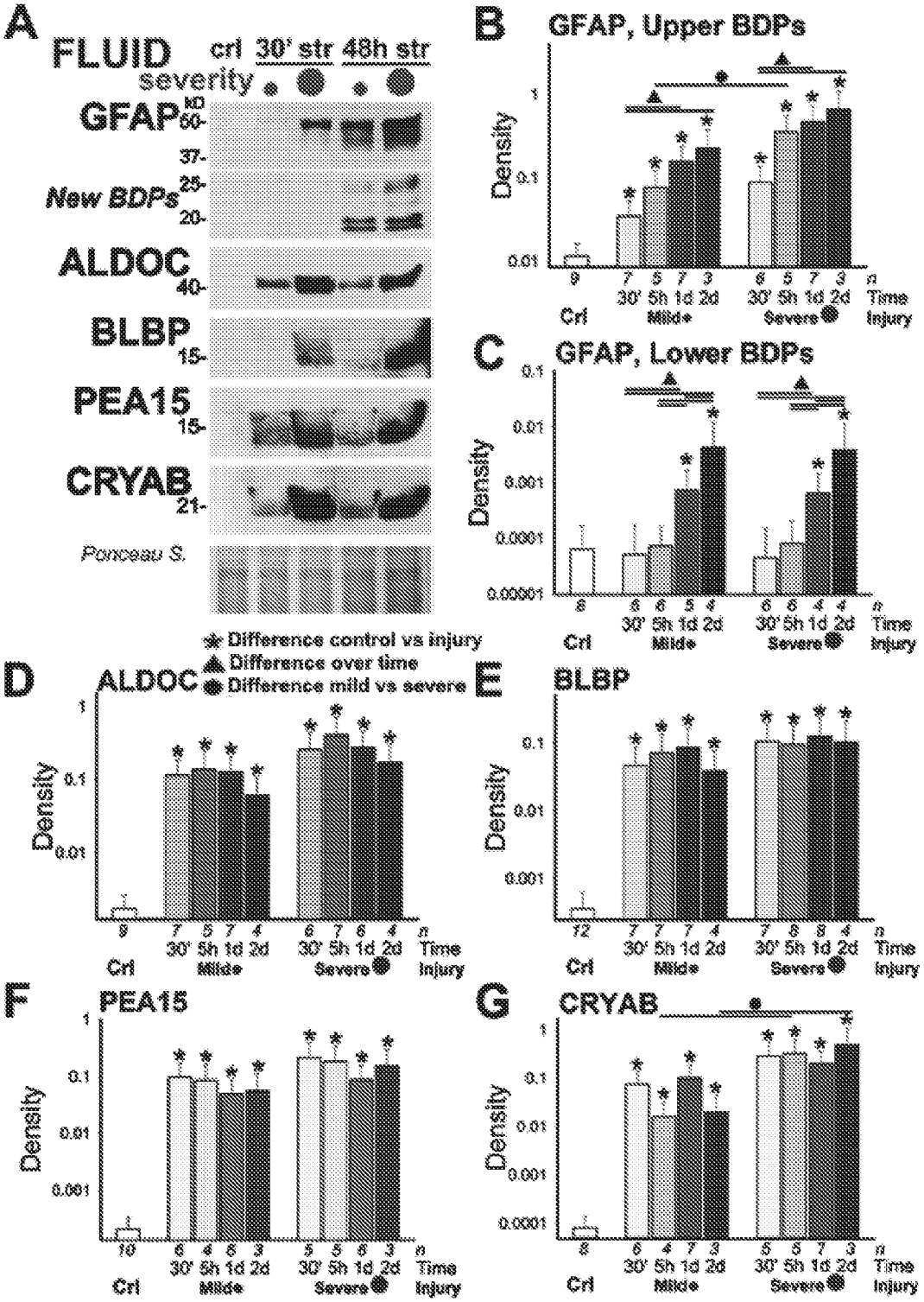

A
FLUID
severity
ctrl
30' str
48h str
GFAP
New BDPs
ALDOC
BLBP
PEA15
CRYAB
Ponceau S.
B
GFAP, Upper BDPs
C
GFAP, Lower BDPs
Difference control vs injury
Difference over time
Difference mild vs severe
D
ALDOC
E
BLBP
F
PEA15
G
CRYAB
Density
Ctrl
Mild
Severe
Time
Injury

FIGURE 3

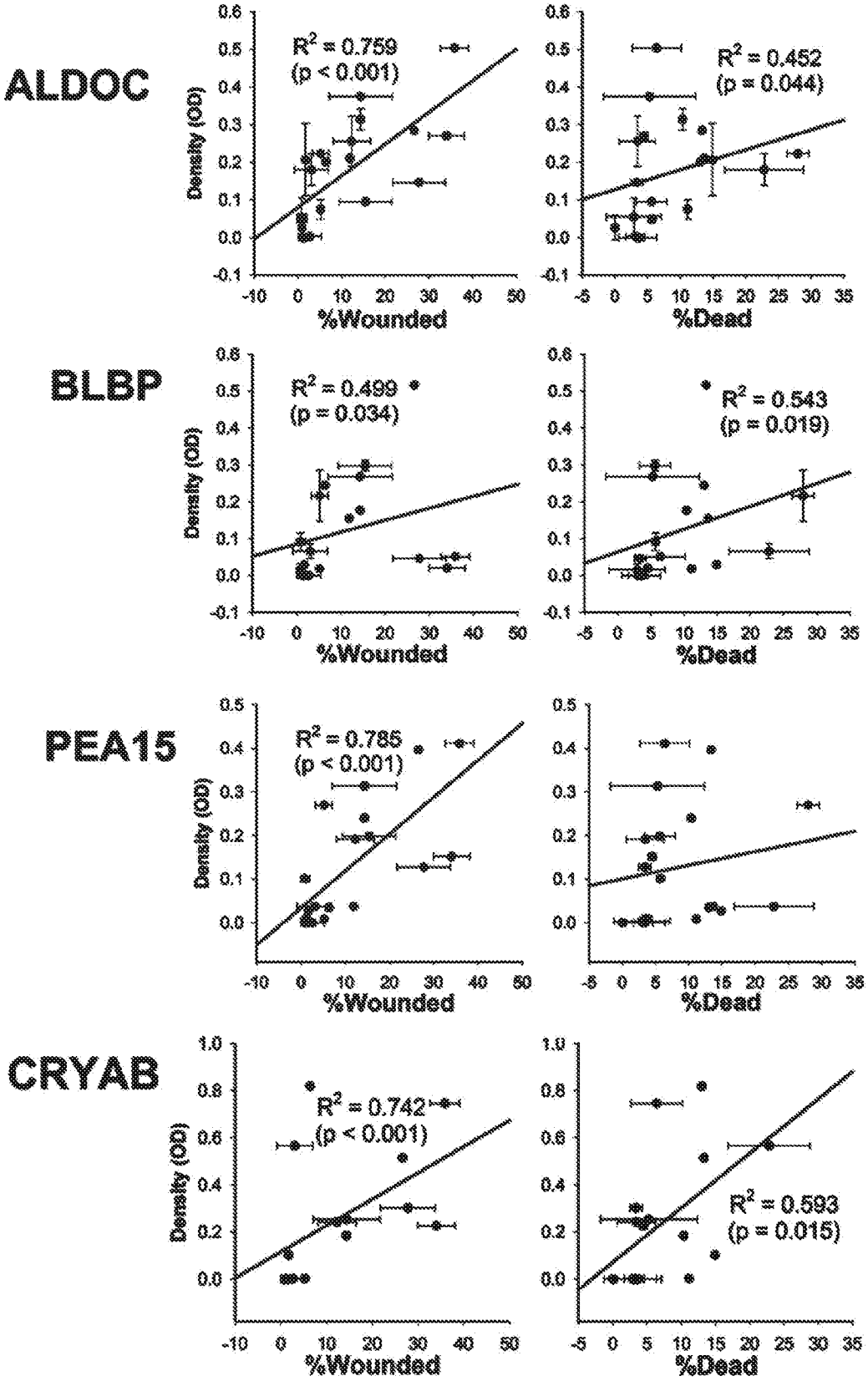

ALDOC
BLBP
PEA15
CRYAB
Density (OD)
%Wounded
%Dead
R² = 0.759
(p < 0.001)
R² = 0.452
(p = 0.044)
R² = 0.499
(p = 0.034)
R² = 0.543
(p = 0.019)
R² = 0.785
(p < 0.001)
R² = 0.742
(p < 0.001)
R² = 0.593
(p = 0.015)

FIGURE 4A

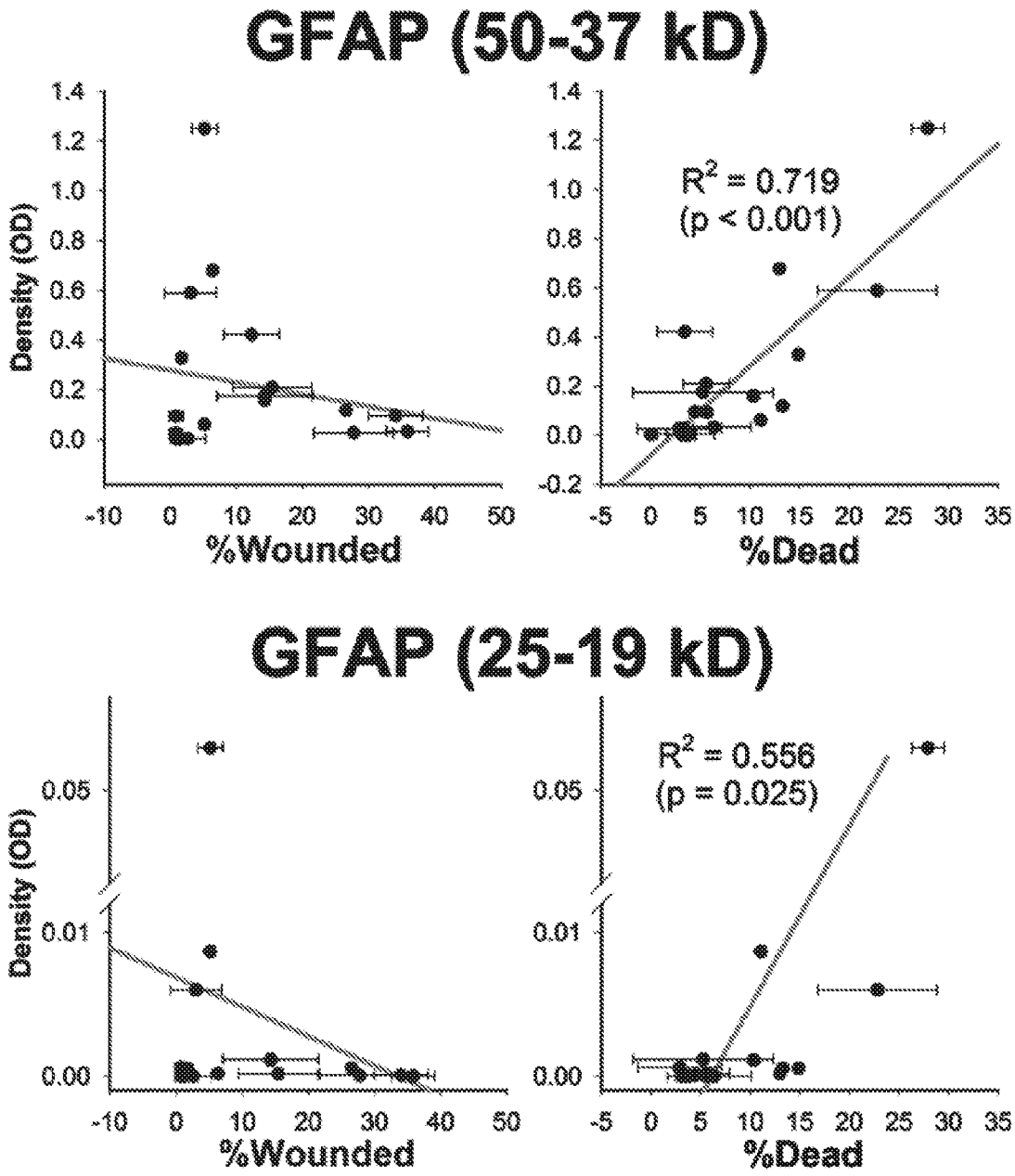

GFAP (50-37 kD)
Density (OD)
%Wounded
%Dead
R^2 = 0.719
(p < 0.001)
GFAP (25-19 kD)
Density (OD)
%Wounded
%Dead
R^2 = 0.556
(p = 0.025)

FIGURE 4B

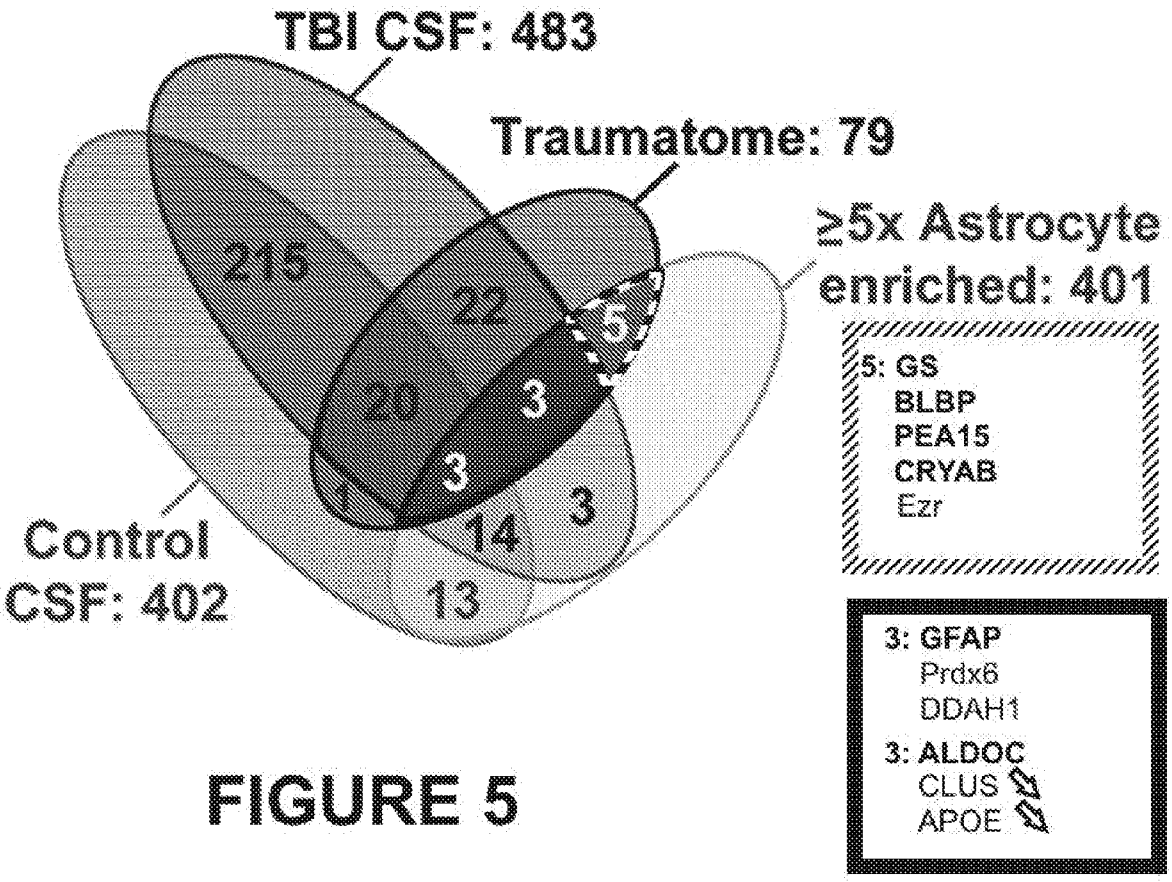
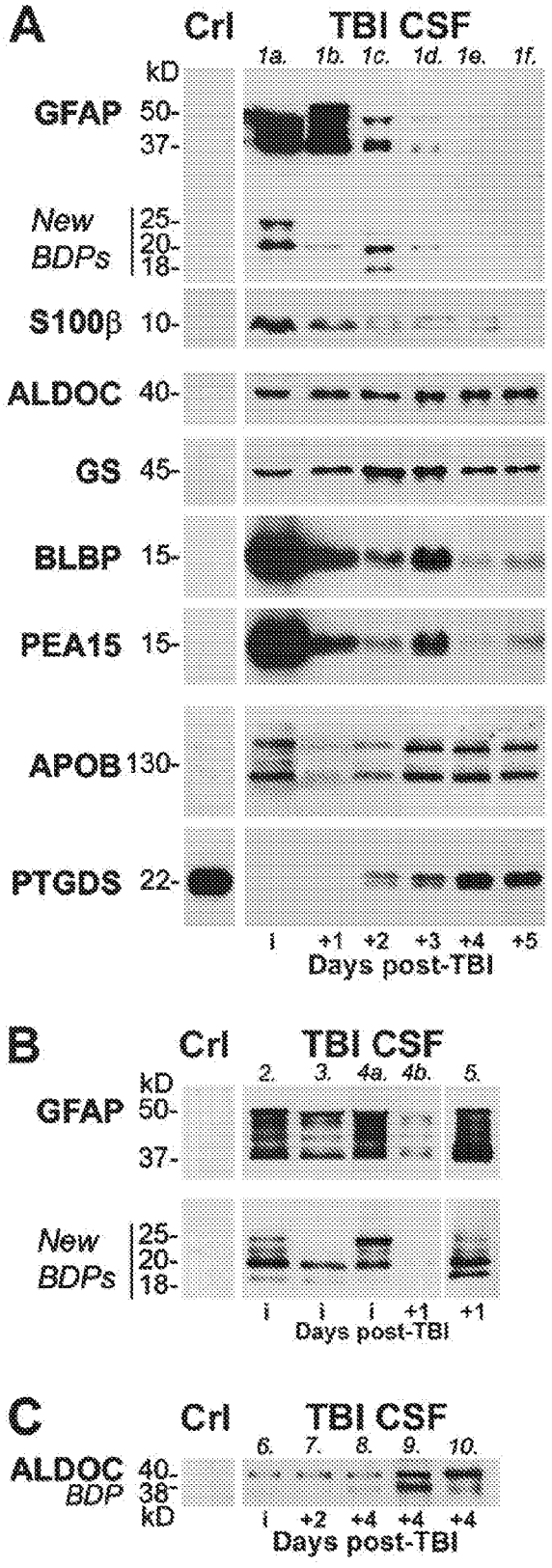
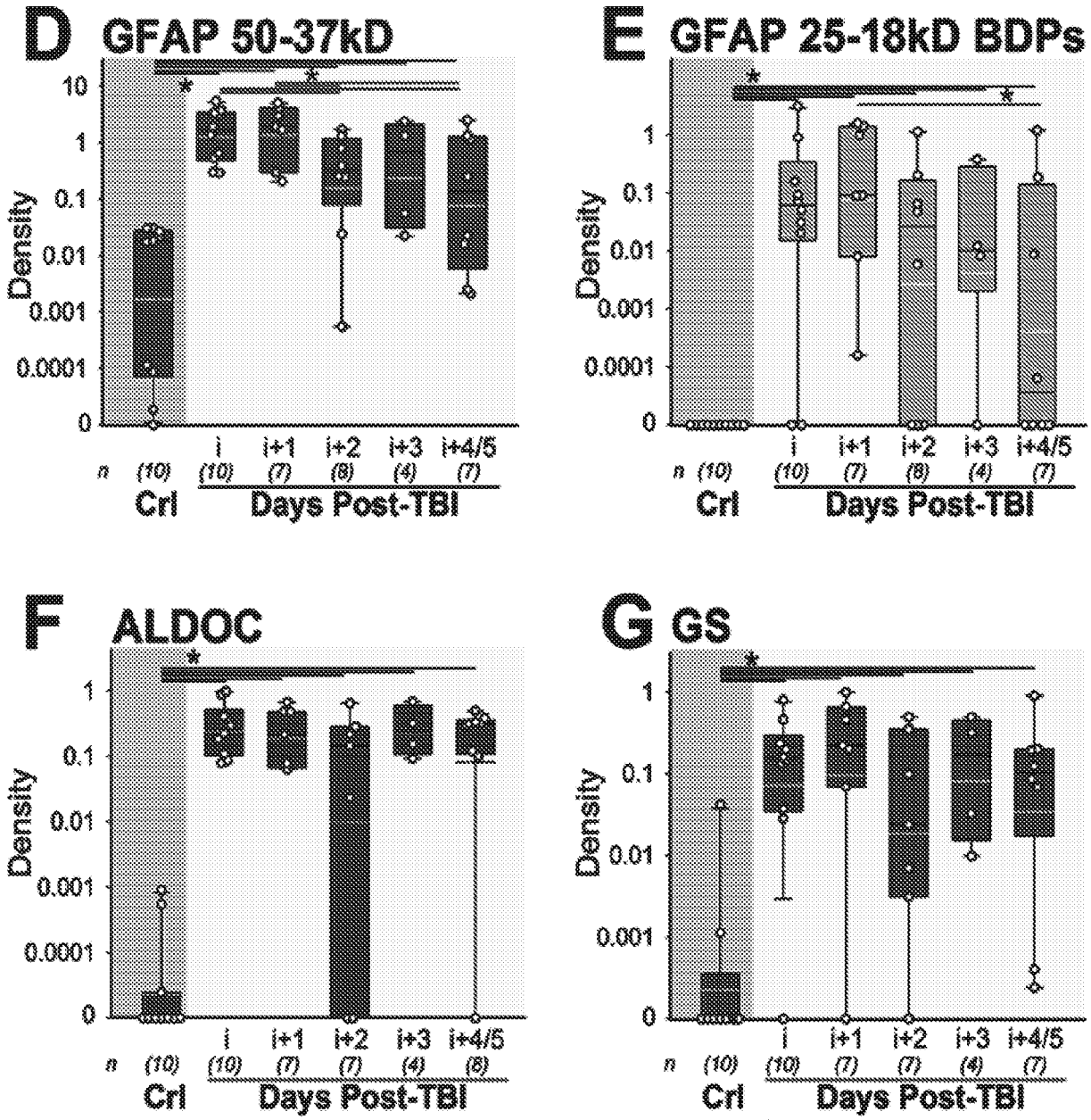

TBI CSF: 483
Traumatome: 79
≥5x Astrocyte enriched: 401
Control CSF: 402
215
22
5
20
3
3
1
14
3
13
5: GS
BLBP
PEA15
CRYAB
Ezr
3: GFAP
Prdx6
DDAH1
3: ALDOC
CLUS
APOE

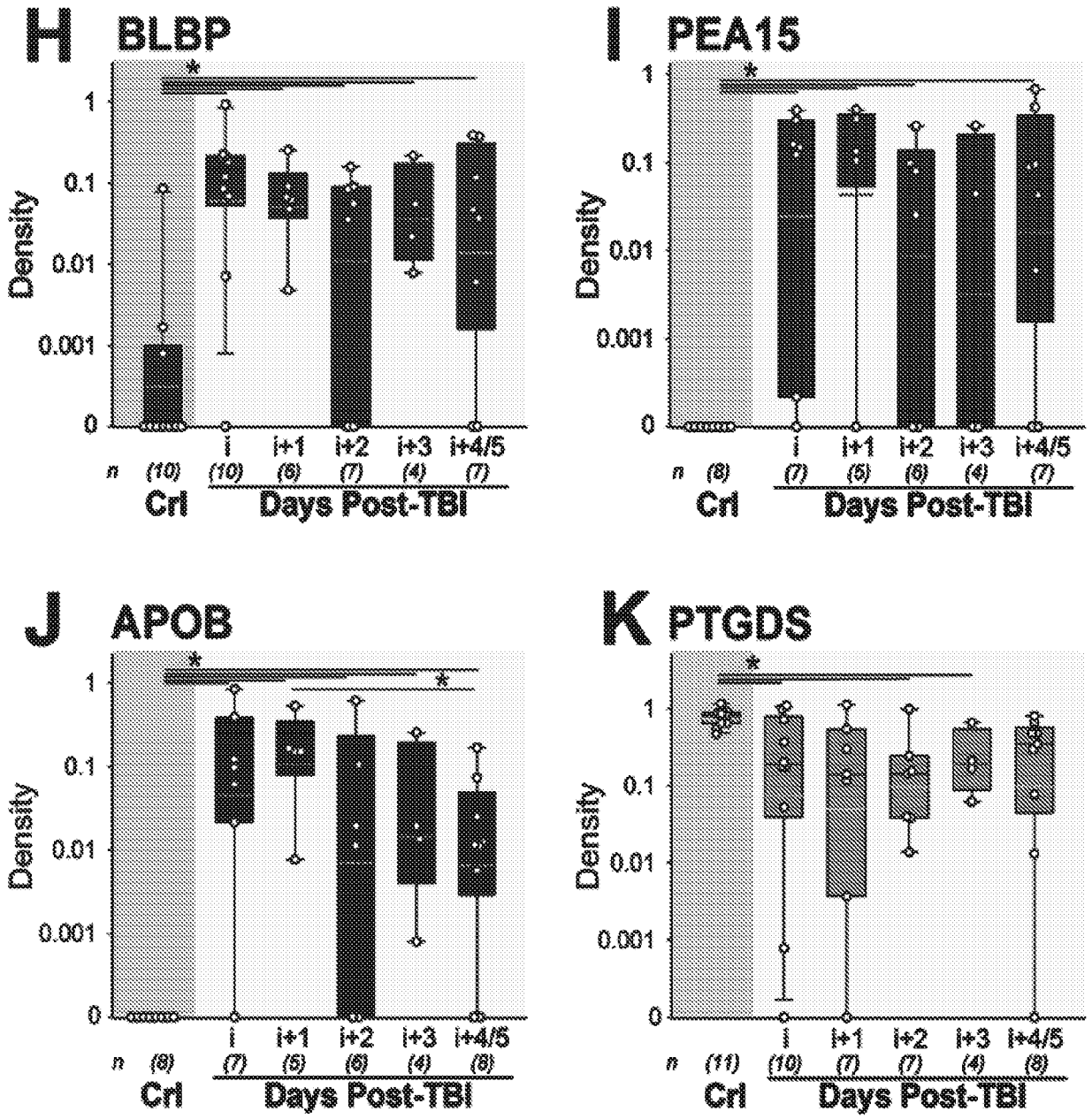
FIGURE 5



FIGURES 6A-6C



FIGURES 6D-6G



FIGURES 6H-6K

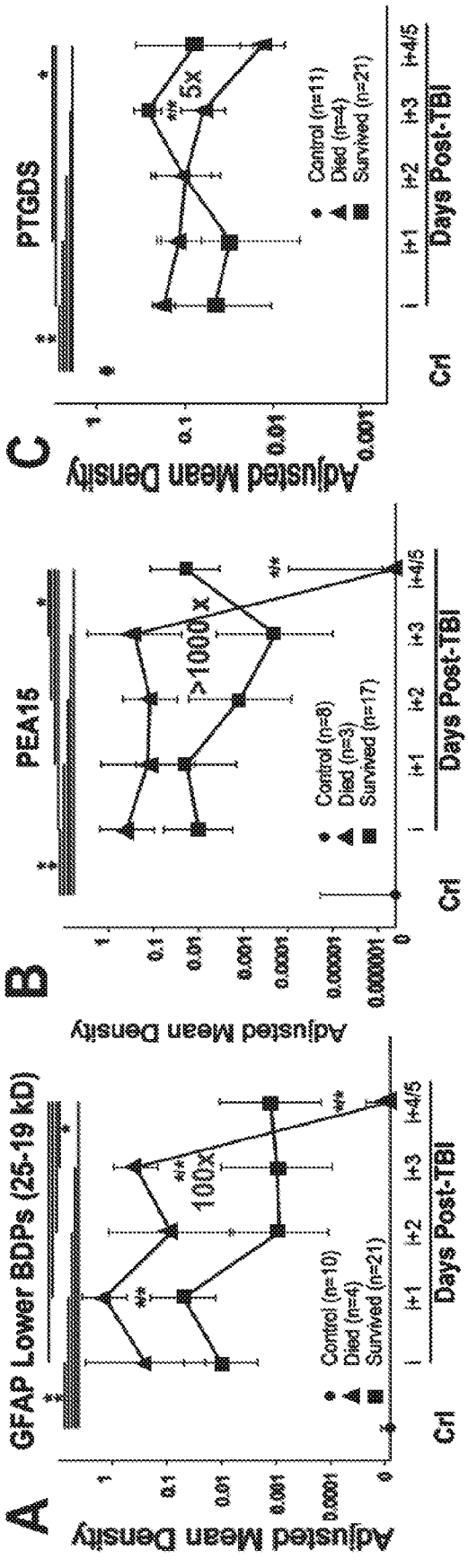
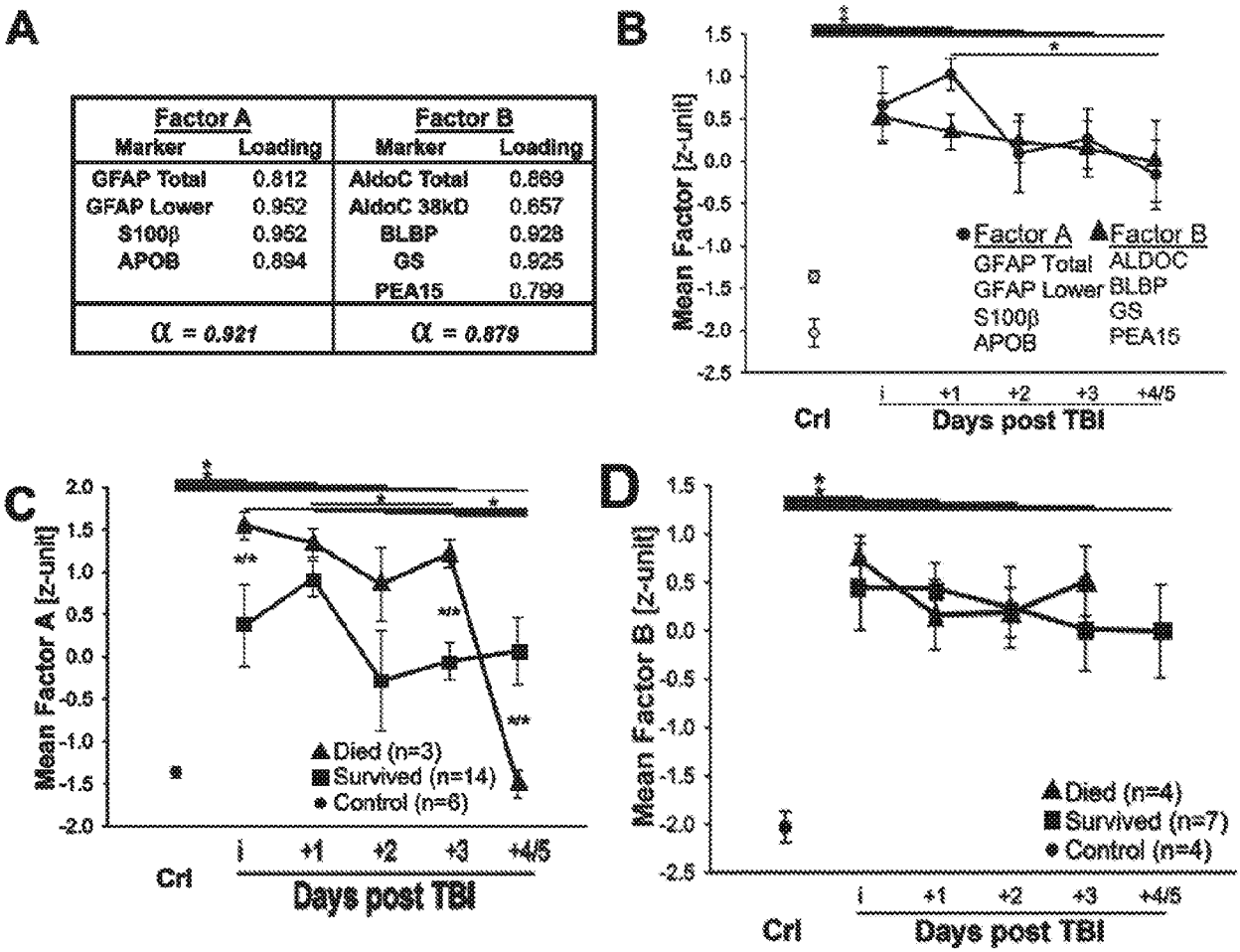

A
GFAP Lower BDPs (25-19 kD)
Adjusted Mean Density
100x
Control (n=10)
Died (n=4)
Survived (n=21)
Ctrl
i
i+1
i+2
i+3
i+4/5
Days Post-TBI
B
PEA15
Adjusted Mean Density
>1000x
Control (n=8)
Died (n=3)
Survived (n=17)
Ctrl
Days Post-TBI
C
PTGDS
Adjusted Mean Density
5x
Control (n=11)
Died (n=4)
Survived (n=21)
Ctrl
Days Post-TBI

FIGURE 7

A

Factor A		Factor B	
Marker	Loading	Marker	Loading
GFAP Total	0.812	AldoC Total	0.869
GFAP Lower	0.952	AldoC 38kD	0.657
S100β	0.952	BLBP	0.928
APOB	0.894	GS	0.925
		PEA15	0.799
α = 0.921		α = 0.879	



F

	Factor A	Factor B
Control	< -1	< -1.5
Died	> 1.2	> 0.2
Survived	< 1.2	> -1.5

FIGURE 8

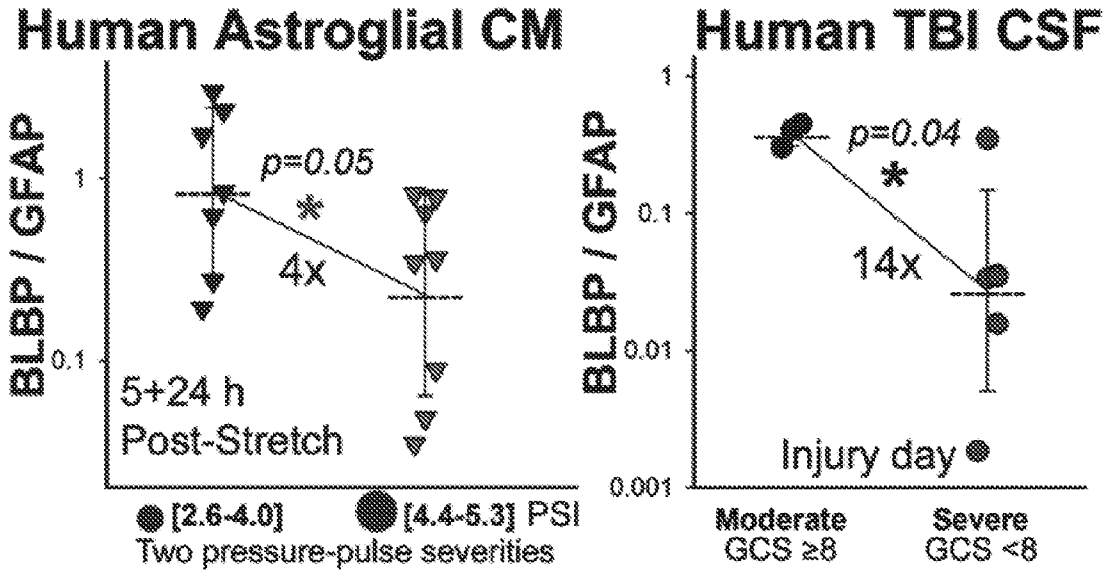


FIGURE 9

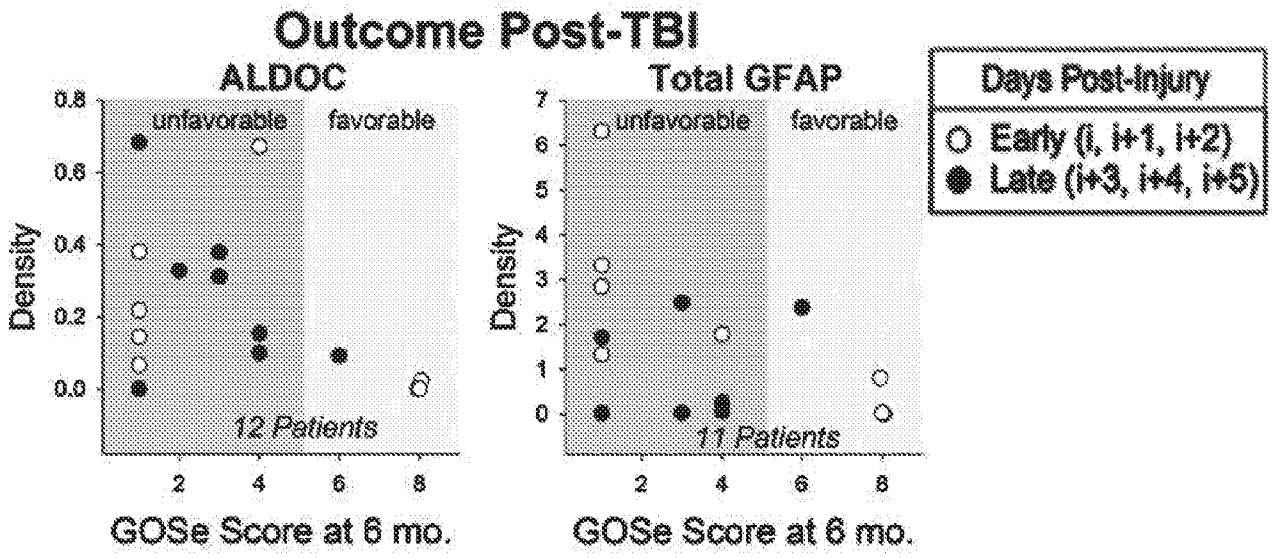


FIGURE 10

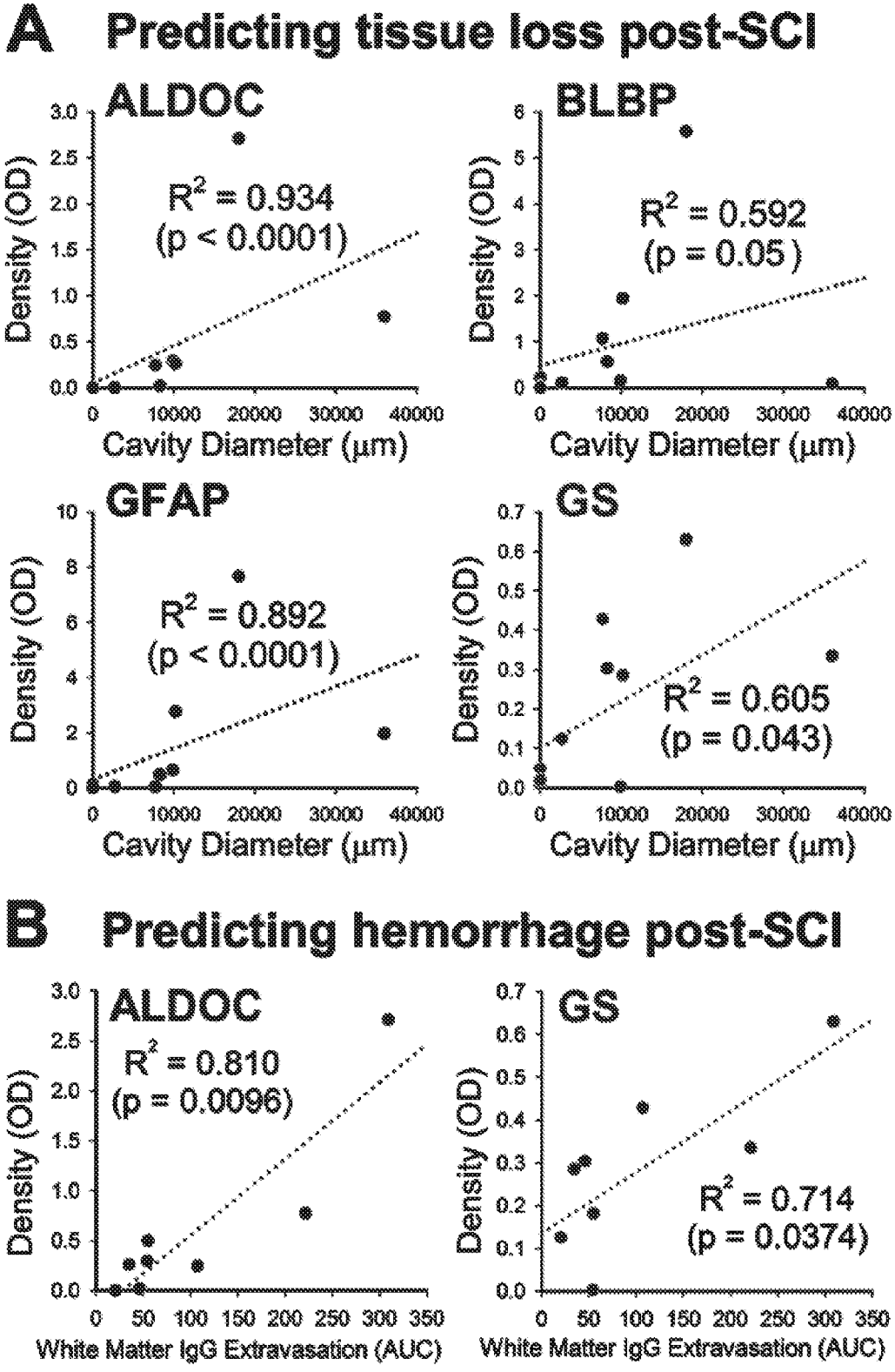

A Predicting tissue loss post-SCI
ALDOC
R² = 0.934
(p < 0.0001)
BLBP
R² = 0.592
(p = 0.05)
GFAP
R² = 0.892
(p < 0.0001)
GS
R² = 0.605
(p = 0.043)
Density (OD)
Cavity Diameter (µm)
B Predicting hemorrhage post-SCI
ALDOC
R² = 0.810
(p = 0.0096)
GS
R² = 0.714
(p = 0.0374)
White Matter IgG Extravasation (AUC)

FIGURE 11

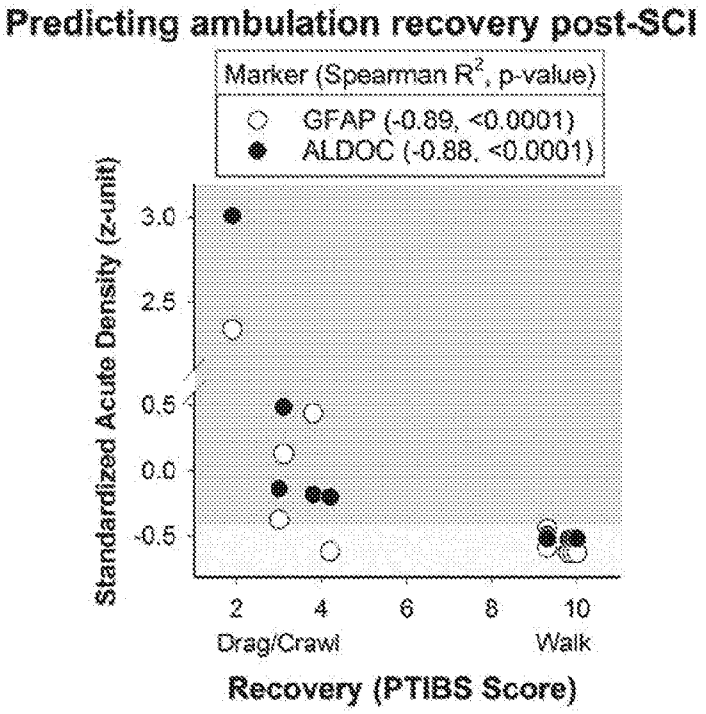


FIGURE 12

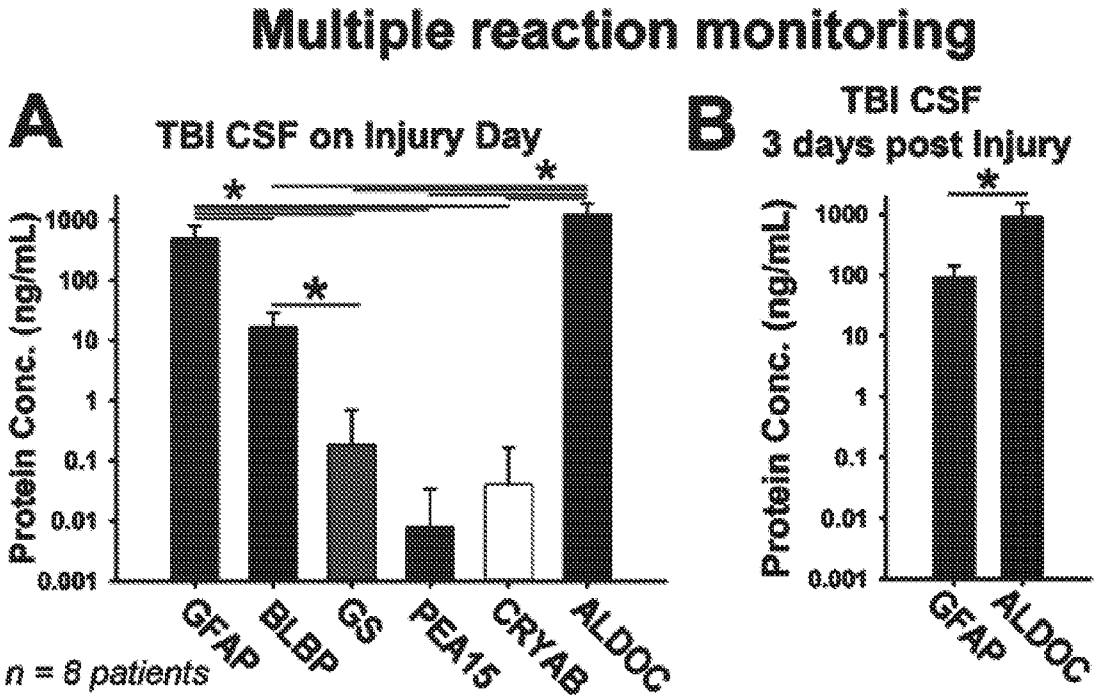


FIGURE 13

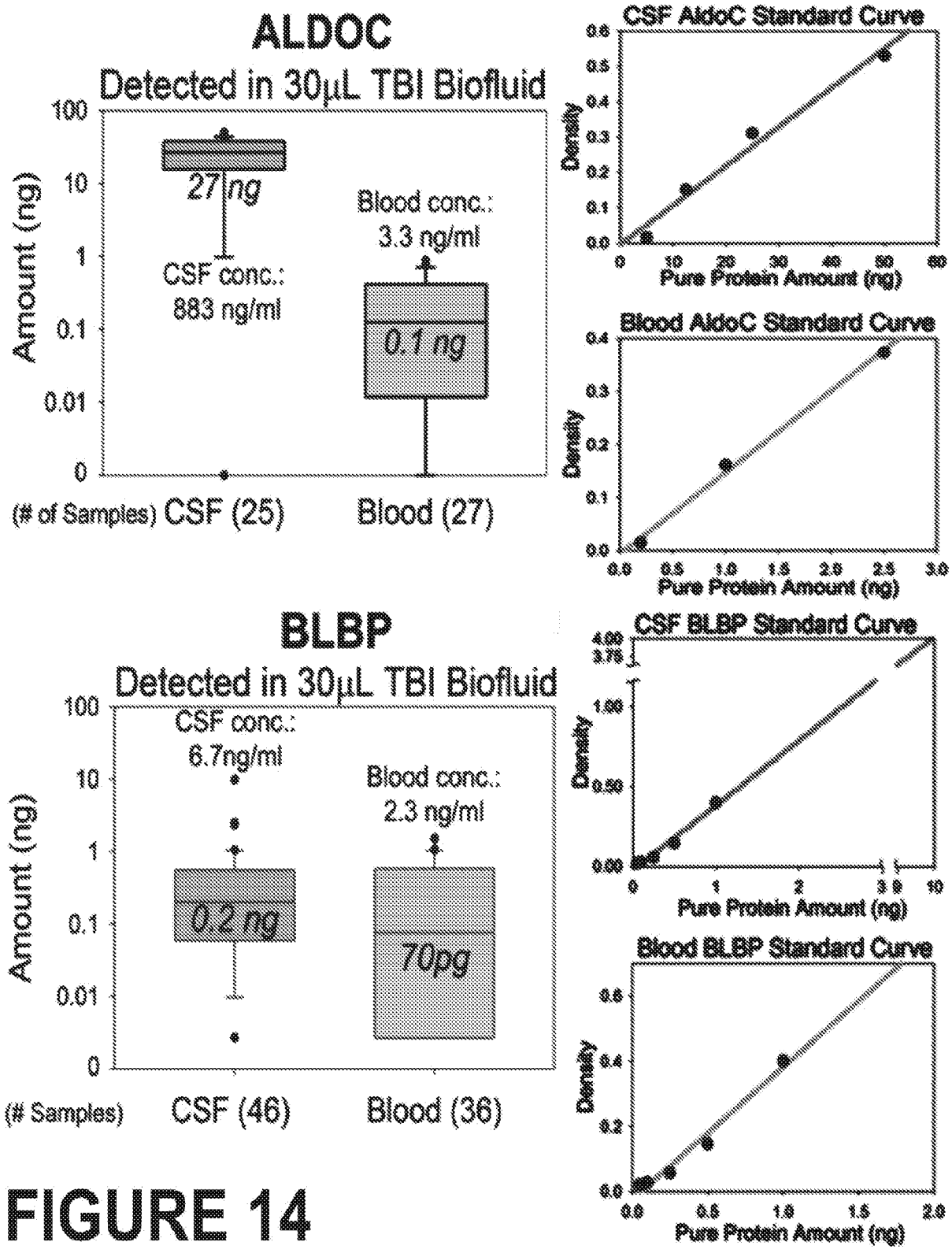

ALDOC
Detected in 30µL TBI Biofluid
Amount (ng)
100
10
1
0.1
0.01
0
27 ng
CSF conc.:
883 ng/ml
Blood conc.:
3.3 ng/ml
0.1 ng
(# of Samples) CSF (25)
Blood (27)
CSF AldoC Standard Curve
Density
Pure Protein Amount (ng)
Blood AldoC Standard Curve
Density
Pure Protein Amount (ng)
BLBP
Detected in 30µL TBI Biofluid
Amount (ng)
100
10
1
0.1
0.01
0
CSF conc.:
6.7ng/ml
Blood conc.:
2.3 ng/ml
0.2 ng
70pg
(# Samples)
CSF (46)
Blood (36)
CSF BLBP Standard Curve
Density
Pure Protein Amount (ng)
Blood BLBP Standard Curve
Density
Pure Protein Amount (ng)

FIGURE 14

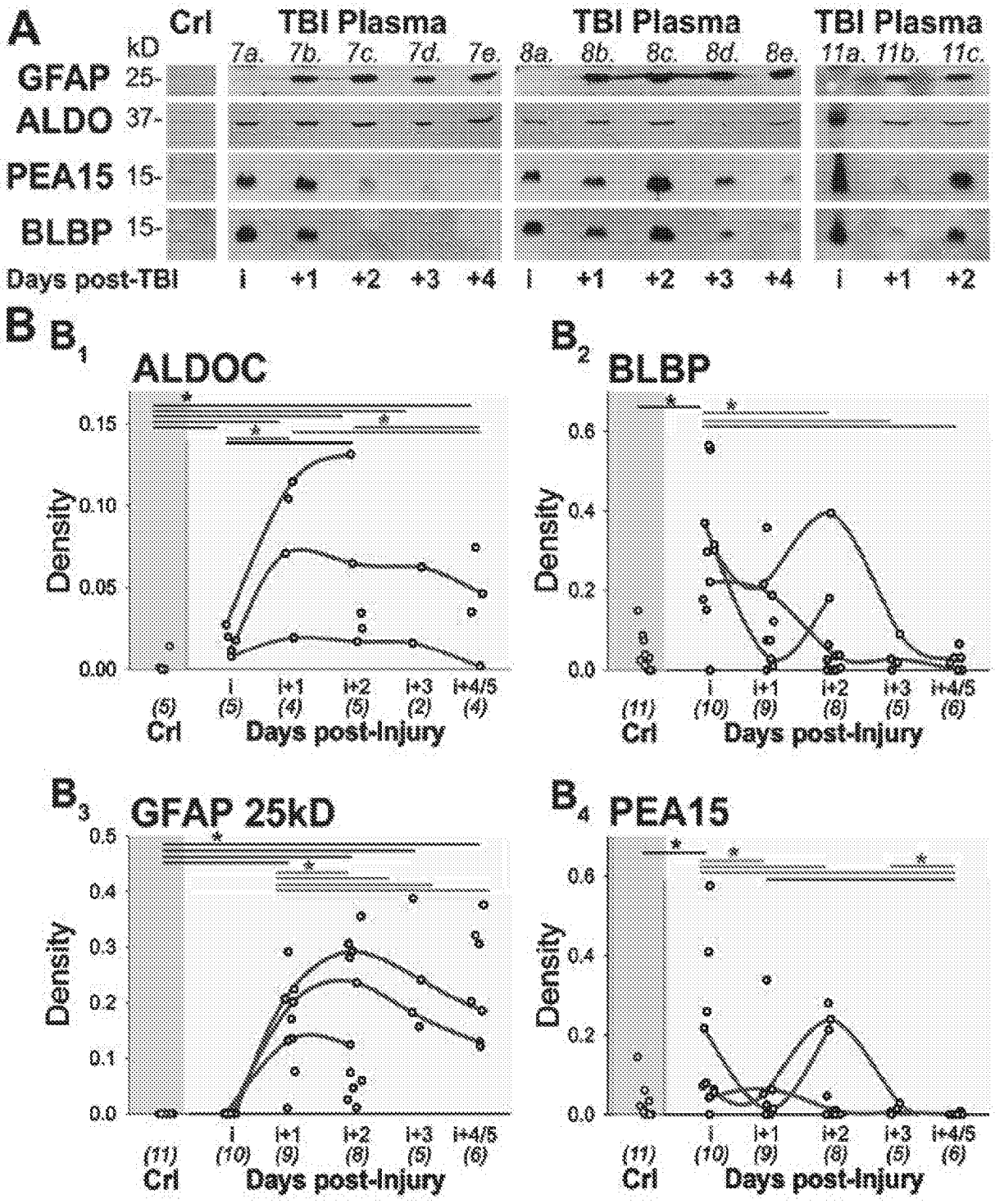

A
Crl
TBI Plasma
TBI Plasma
TBI Plasma
kD
7a. 7b. 7c. 7d. 7e.
8a. 8b. 8c. 8d. 8e.
11a. 11b. 11c.
GFAP 25-
ALDO 37-
PEA15 15-
BLBP 15-
Days post-TBI
i +1 +2 +3 +4
i +1 +2 +3 +4
i +1 +2
B
B1 ALDOC
B2 BLBP
B3 GFAP 25kD
B4 PEA15
Density
Crl
Days post-Injury
i i+1 i+2 i+3 i+4/5
(5) (5) (4) (5) (2) (4)
(11) (10) (9) (8) (5) (6)

FIGURE 15

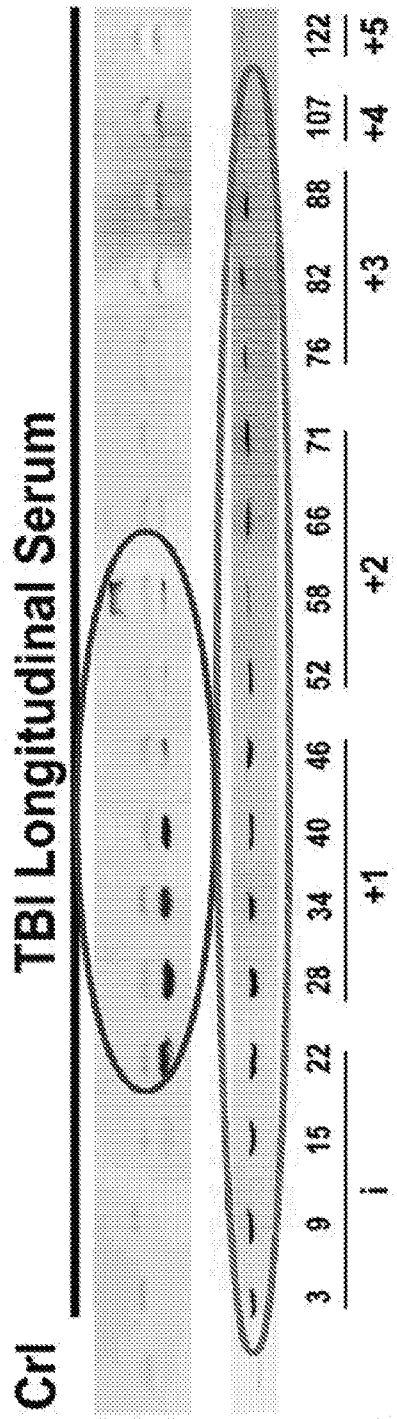


FIGURE 16

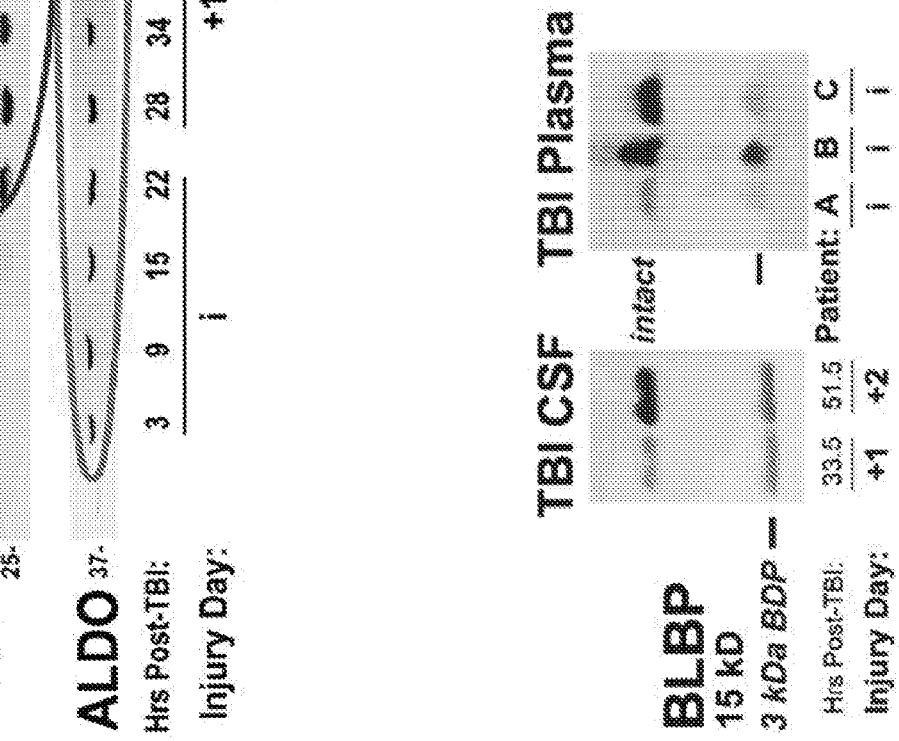


FIGURE 17

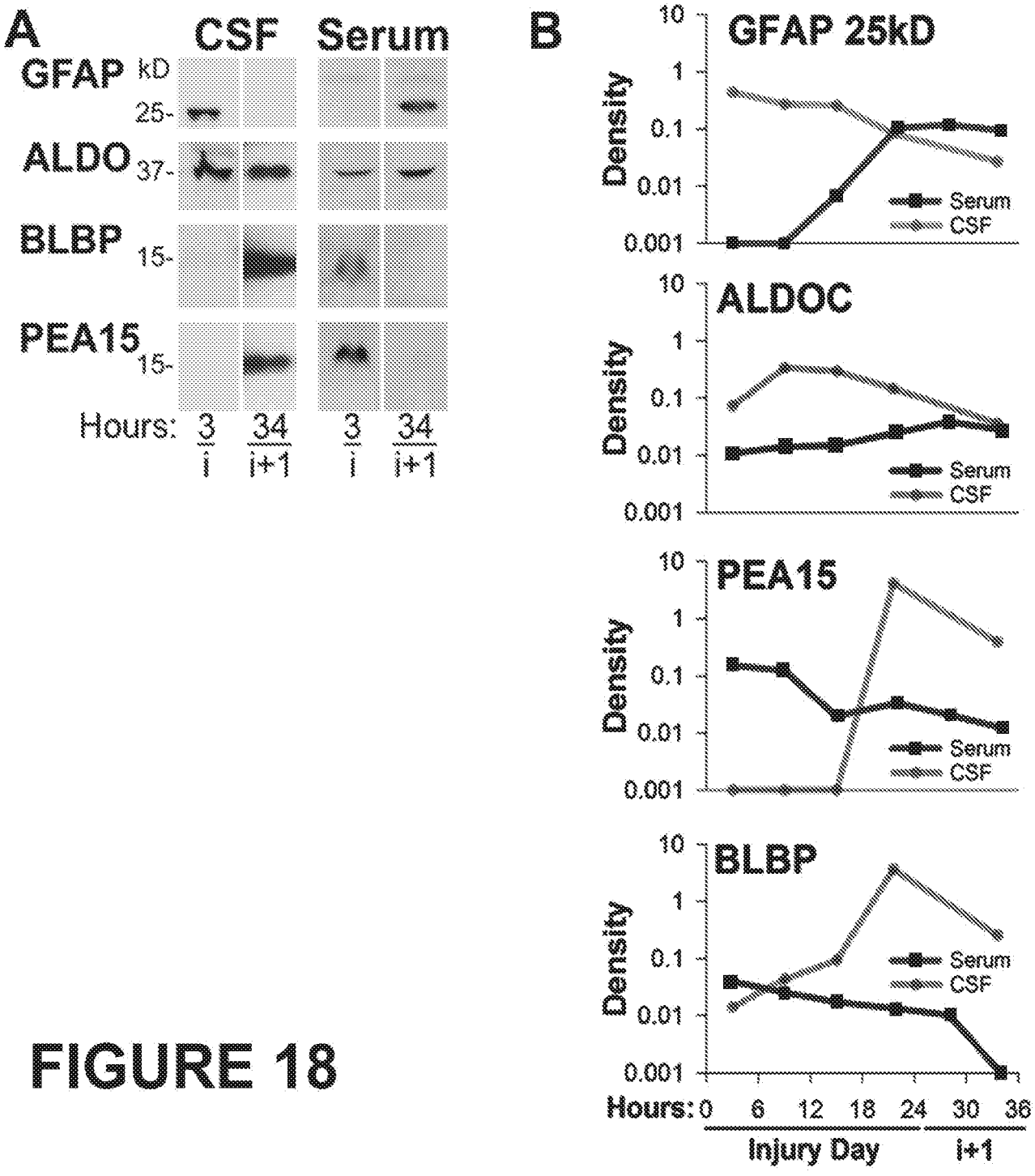

A
CSF
Serum
GFAP kD
25-
ALDO 37-
BLBP 15-
PEA15 15-
Hours: 3 34 3 34
i i+1 i i+1
B
GFAP 25kD
ALDOC
PEA15
BLBP
Density
10
1
0.1
0.01
0.001
Serum
CSF
Hours: 0 6 12 18 24 30 36
Injury Day
i+1

FIGURE 18

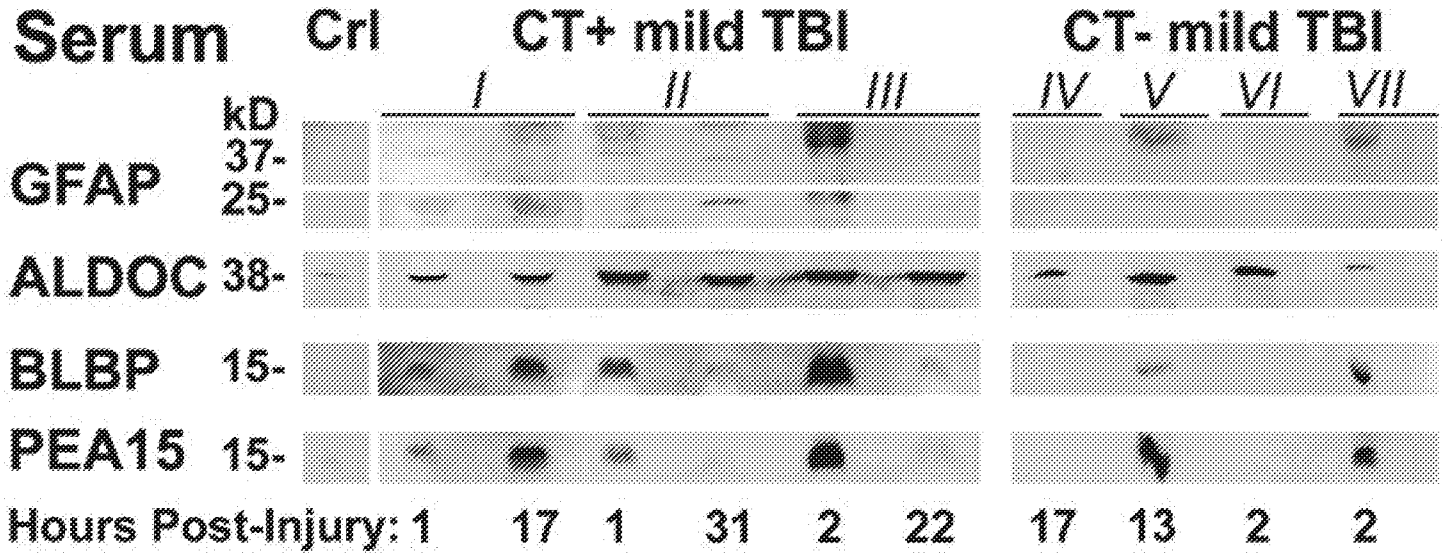


FIGURE 19

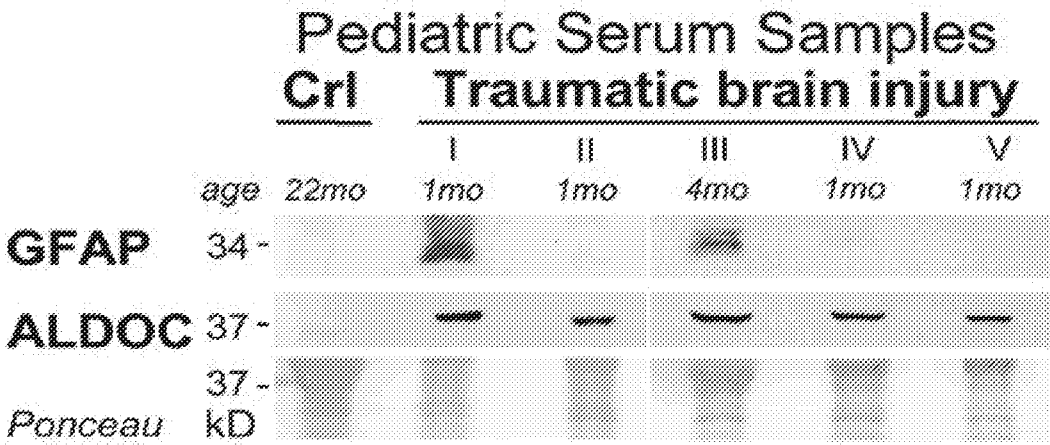


FIGURE 20

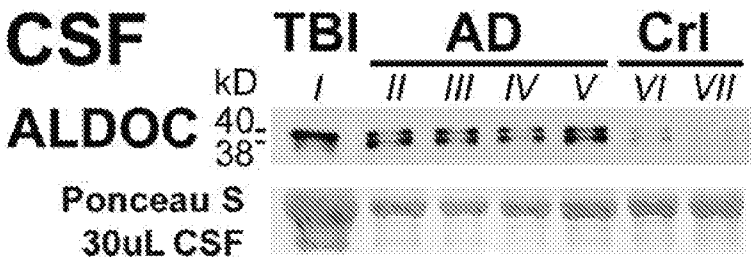
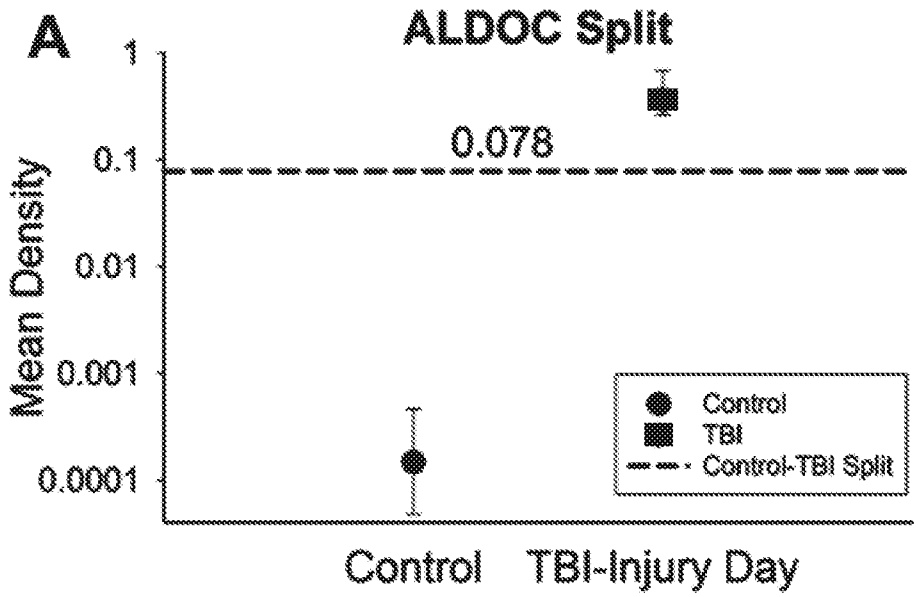


FIGURE 21



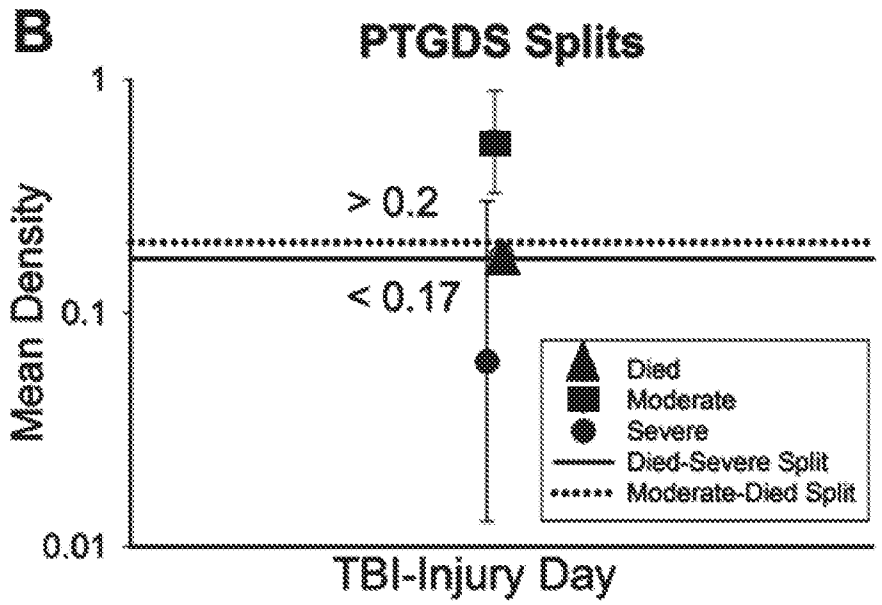


FIGURE 22

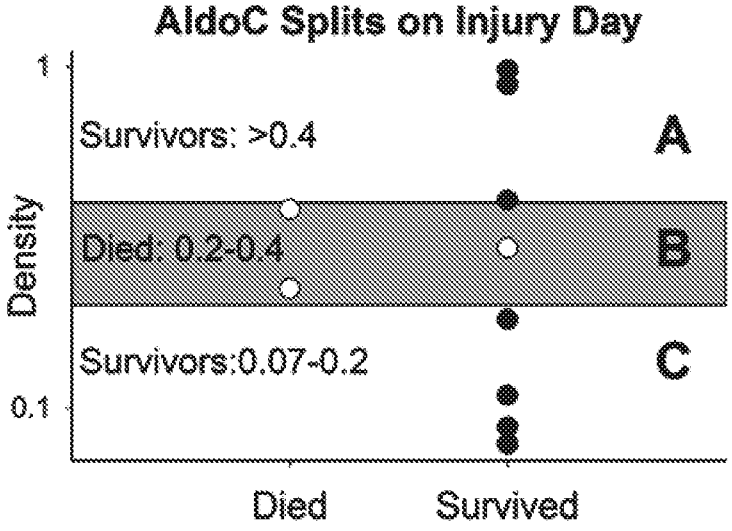


FIGURE 23

A

stretch	% leaky	% dead
(-)	1 ± 1	4 ± 4
●	5 ± 4	11 ± 4
●	6 ± 1	27 ± 1
30'	1 ± 1	14 ± 7
1d	5 ± 2	15 ± 5

B

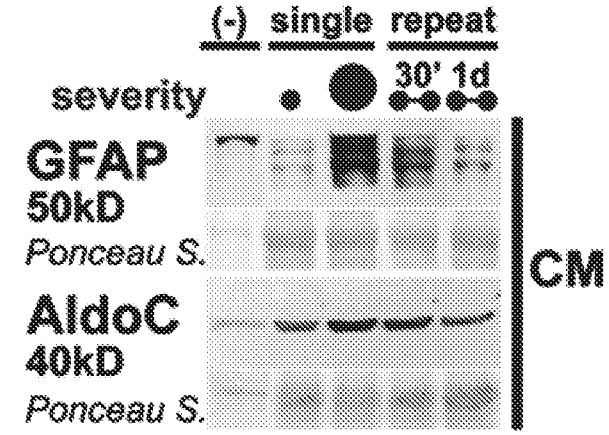
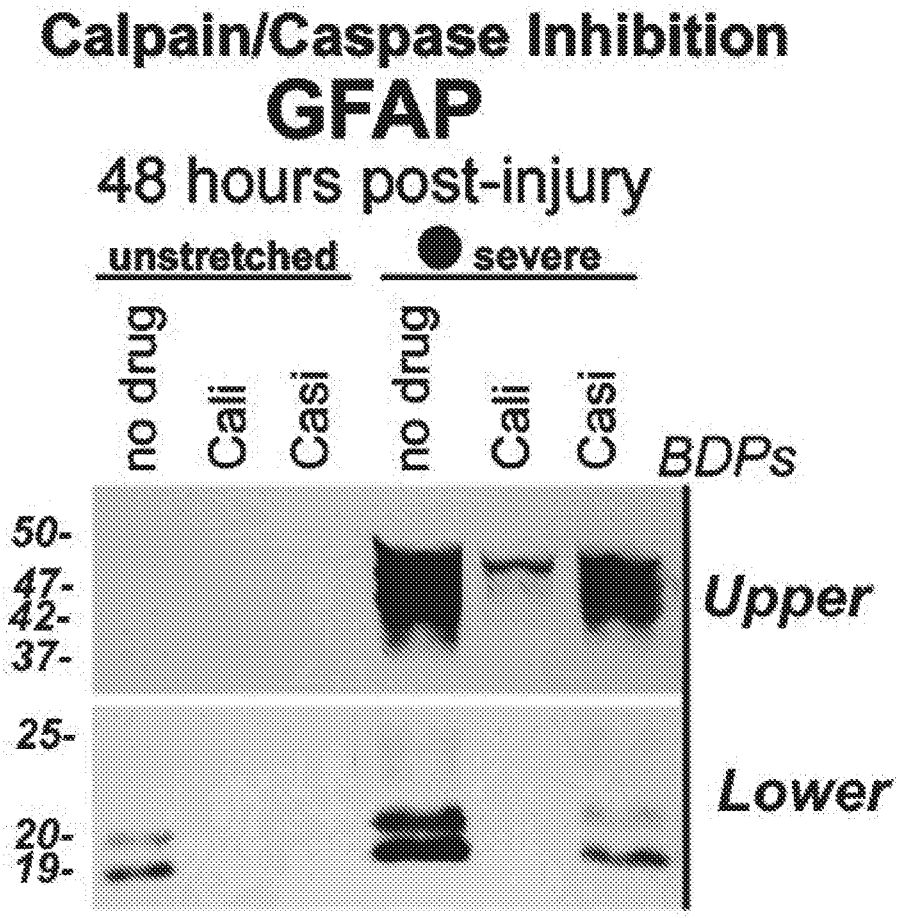


FIGURE 24

FIGURE 25


Calpain/Caspase Inhibition
GFAP
48 hours post-injury
unstretched
severe
no drug
Calli
Casi
no drug
Calli
Casi
BDPs
50-
47-
42-
37-
Upper
25-
20-
19-
Lower

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/031043

A. CLASSIFICATION OF SUBJECT MATTER

G01N 33/68(2006.01)i, G01N 33/53(2006.01)i, G01N 27/64(2006.01)i, G01N 33/577(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N 33/68; C12Q 1/44; G01N 33/53; C40B 30/10; A61K 31/215; G01N 27/64; G01N 33/577

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: traumatic brain injury, spinal cord injury, ALDOC, BLBP/FABP7, PEA15, GFAP-BDP

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2011-0082203 A1 (WANG et al.) 07 April 2011 See abstract; paragraphs [0041]-[0070]; claims 1-6.	1-2,12-16,19-20
Y		3-9,21-29
A		10-11
Y	US 2011-0177974 A1 (WANG et al.) 21 July 2011 See abstract; paragraphs [0012]-[0015].	3-7
Y	US 2014-0045713 A1 (EVERETT et al.) 13 February 2014 See abstract; paragraph [0062]; Table 1.	8-9
Y	FEALA et al., `Systems biology approaches for discovering biomarkers for traumatic brain injury` Journal of Neurotrauma, Vol.30, pages 1101-1116 (2013) See abstract; Table 1; pages 1103, 1110.	21-29
Y	KOH, `Melatonin prevents down-regulation of astrocytic phosphoprotein PEA-15 in ischemic brain injury` Journal of Pineal Research, Vol.51, pages 381-386 (2011) See abstract; pages 383-385.	22

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 July 2016 (28.07.2016)	**02 August 2016 (02.08.2016)**

Name and mailing address of the ISA/KR
International Application Division
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon, 35208, Republic of Korea
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Authorized officer
KIM, Seung Beom
Telephone No. +82-42-481-3371

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/031043

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ZHANG et al., `Human traumatic brain injury induces autoantibody response against glial fibrillary acidic protein and its breakdown products` PLoS One, Vol.9, Issue 3, e92698 (internal pages 1-16) (2014) See the whole document.	1-16,19-29

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/031043

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 17,18
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2016/031043

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2011-0082203 A1	07/04/2011	AU 2009-212463 A1	13/08/2009
		CA 2715248 A1	13/08/2009
		CN 101983337 A	02/03/2011
		EP 2245466 A2	03/11/2010
		JP 2011-511301 A	07/04/2011
		WO 2009-100131 A2	13/08/2009
		WO 2009-100131 A3	19/11/2009
US 2011-0177974 A1	21/07/2011	AU 2005-238446 A1	10/11/2005
		AU 2005-238446 B2	30/07/2009
		CA 2578670 A1	10/11/2005
		EP 1745149 A2	24/01/2007
		EP 2207033 A2	14/07/2010
		EP 2207033 A3	03/11/2010
		EP 2207033 B1	18/06/2014
		JP 2007-532915 A	15/11/2007
		JP 4659025 B2	30/03/2011
		US 2005-0260654 A1	24/11/2005
		US 2009-0087868 A1	02/04/2009
		US 2010-0317041 A1	16/12/2010
		US 7396654 B2	08/07/2008
		US 8492107 B2	23/07/2013
		WO 2005-106038 A2	10/11/2005
US 2014-0045713 A1	13/02/2014	EP 2628013 A2	21/08/2013
		EP 2628013 A4	12/03/2014
		JP 2013-545087 A	19/12/2013
		US 2015-0051115 A1	19/02/2015
		WO 2012-051519 A2	19/04/2012
		WO 2012-051519 A3	20/09/2012

专利名称(译)	星形胶质细胞损伤和神经损伤生物标志物		
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摘要(译)

提供了一种用于检测或监测受试者中的创伤性脑损伤（TBI）或脊髓损伤（SCI）的状态的方法。在一个实施方案中，该方法包括使得自受试者的体液样品与用于测定选自醛缩酶C（ALDOC）和脑脂质结合蛋白（BLBP）或创伤特异性分解产物的TBI标记物的试剂接触。（BDP）ALDOC或BLBP。该方法还包括与对照样品相比测量样品中存在的标记物的量，并且当与对照样品相比样品中存在升高量的标记物时确定TBI或SCI的存在。该方法可包括测量谷氨酰胺合成酶（GS），星形胶质细胞磷蛋白PEA-15（PEA15），αB-晶状体蛋白（CRYAB / HSP27），ALDOC，GS，PEA15或CRYAB的裂解产物或两者的组合的量。或更多。