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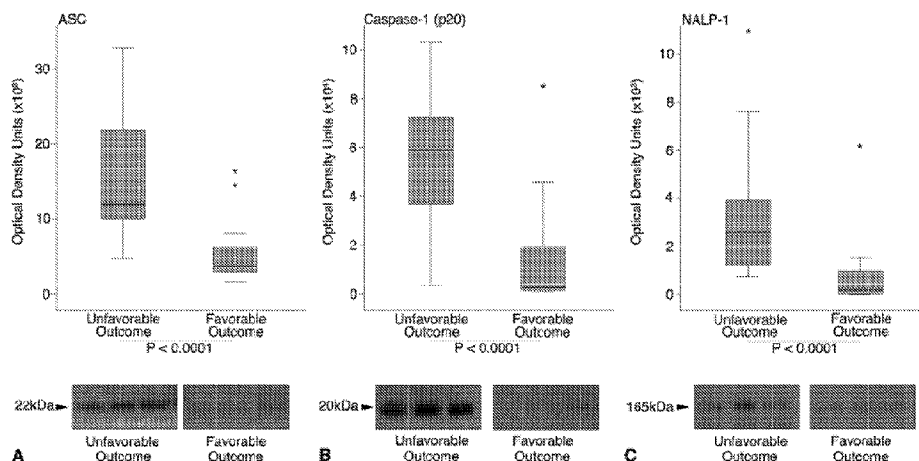
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The present invention provides novel markers of the severity of a central nervous system injury, such as spinal cord injury or traumatic brain injury, in a patient. In particular, protein components of inflammasomes in the cerebrospinal fluid that can be used to assess the severity of central nervous system injury in a patient are disclosed. Methods of using such protein biomarkers to determine a prognosis, direct treatment and rehabilitation efforts, and monitor response to treatment for a patient with a central nervous system injury are also described.

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(54) Title: INNATE IMMUNE PROTEINS AS BIOMARKERS FOR CNS INJURY

FIGURE 4
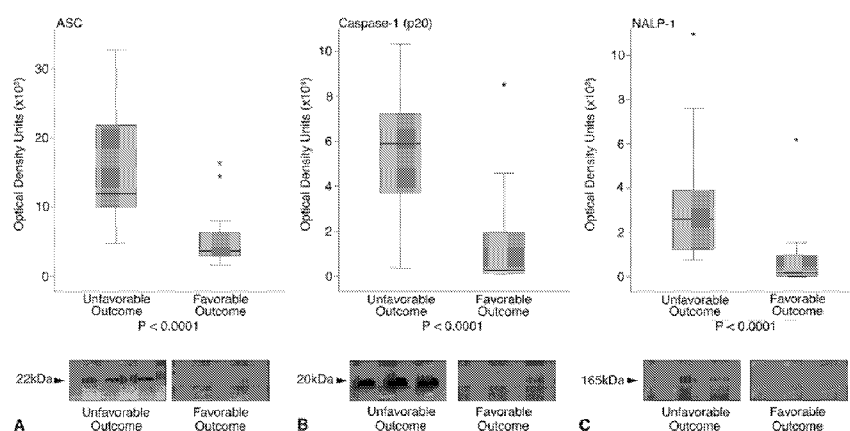
(57) Abstract: The present invention provides novel markers of the severity of a central nervous system injury, such as spinal cord injury or traumatic brain injury, in a patient. In particular, protein components of inflammasomes in the cerebrospinal fluid that can be used to assess the severity of central nervous system injury in a patient are disclosed. Methods of using such protein biomarkers to determine a prognosis, direct treatment and rehabilitation efforts, and monitor response to treatment for a patient with a central nervous system injury are also described.

INNATE IMMUNE PROTEINS AS BIOMARKERS FOR CNS INJURY

CROSS-REFERENCE TO RELATED APPLICATIONS
[0001]

FIELD OF THE INVENTION
[0002] The present invention relates generally to the fields of neurology, immunology, and diagnostics. In particular, the present invention relates to the identification of biomarkers in biological samples which can predict the severity of neuronal injury, such as spinal cord and traumatic brain injury, in patients. The identified biomarkers may also be used in determining prognosis, directing therapeutic and rehabilitation efforts, and monitoring response to treatment for patients with a central nervous system injury.

BACKGROUND OF THE INVENTION
[0003] Nucleotide-binding oligomerization domain (NOD)-containing protein-like receptors (NLRs) are a recently discovered class of innate immune receptors that play a crucial role in initiating inflammatory responses following tissue injury in the central nervous system (CNS) (Abulafia et al., 2009, Silverman et al., 2009). Previous work shows that NLRP1 (also known as NAcht leucine-rich-repeat protein 1 (NALP-1)) forms an inflammasome complex comprising NLRP1, the adaptor protein apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and the caspase-1 enzyme that orchestrate the early inflammatory processes after spinal cord injury (SCI) and traumatic brain injury (TBI) via IL-1β activation (de Rivero Vaccari et al., 2008; 2009). The formation of inflammasomes is induced by physical damage to the plasma membrane, and by certain endogenous ligands referred to as danger associated molecular patterns (DAMPs) or exogenous ligands known as pathogen associated molecular patterns (PAMPs) (Bianchi, 2007, Wakefield et al., 2010). However, the full IL-1β response also depends on the activation of Toll-like receptors (TLRs) and/or purinergic ATP-gated receptors, which induce the transcription of pro- IL-1β.

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[0004] Hyperinflammatory responses associated with tissue damage can promote pathogenesis of SCI and TBI via overproduction of IL-1β and other potentially neurotoxic products. Inflammasome-mediated IL-1β overproduction is involved in the pathogenesis of type 2 diabetes, liver damage and muscular dystrophy (Kufer and Sansonetti, 2011). Moreover, increasing genetic evidence suggests that inflammasome activation could also drive adaptive immunity in types of dermatitis, skin related allergies and asthma (Kufer and Sansonetti, 2011). In addition, inflammasome components may be secreted into the extracellular milieu via a mechanism involving the exosome pathway (Bianchi, 2007). The inflammasome therefore has a complex connection with the control of adaptive immune responses that has become the subject of intense investigation. Whether inflammasomes are associated with tissue destructive inflammatory processes after SCI and TBI in humans has not been investigated.

[0005] TBI affects an estimated 1.5 million people each year and causes one-third of injury-related deaths. Approximately 5.3 million Americans are living today with a permanent TBI-related disability. Predicting the severity and outcome of TBI and well as SCI is difficult, given the lack of objective, laboratory-based biomarkers. Currently, the Glasgow Coma Scale (GCS) score (Teasdale et al., 1974) is the best available clinical predictor of injury severity; however, its value is limited in patients undergoing pharmacological paralysis for intubation, as a motor score cannot be obtained (Brain Trauma Foundation, American Association of Neurological Surgeons, 2000). Predicting outcome is further complicated by the heterogeneity of pathology in patients with a similar GCS score. Therefore, the identification of diagnostic and prognostic biomarkers that directly reflect injury to CNS cells is imperative. Such biomarkers of TBI and SCI will enable clinicians to assess the degree of damage to the brain or spinal cord, relay prognostic information to the patient's family members, and target acute and chronic treatments to specific CNS damage mechanisms. Therefore, an early, accurate diagnostic test designed to target neuroprotective strategies would be a most desirable prognostic tool.

[0006] Although, significant progress has been made regarding the verification and testing of various biomarkers after stroke and TBI, limited data are available regarding what biomarkers are appropriate for SCI. The biomarkers S-100β, neuron-specific enolase, neurofilament light chain and glial fibrillary acidic protein are significantly increased in cases of SCI in experimental animals studies (Skouen et al., 1999, Ma et al., 2001, Nagy et al., 2002, Cornefjord et al., 2004, Loy et al., 2005, Cao et al., 2008, Pouw et al., 2009). Although some biomarkers show

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promising results, these do not yet provide a sensitive prognostic tool. Quantitative standards for determining the extent of SCI and TBI during the acute phase must be developed and validated.

[0007] A new approach for evaluating the primary cord and brain damage in the acute phase is the assessment of biomarkers in the cerebrospinal fluid (CSF). Since CSF surrounds the spinal cord and brain, damage to the cord or brain may lead to the release of proteins and molecules from central nervous system cells into the CSF that may serve as biomarkers for SCI and TBI in the CSF. Several studies have been conducted concerning S-100β, neuron-specific enolase, neurofilament light chain, and glial fibrillary acidic protein (GFAP) in CSF and serum of animal models of SCI (Pouw *et al.*, 2009). However, only one study has investigated neurofilament protein and GFAP in CSF after SCI in humans (Guez *et al.*, 2003). Thus, there is a need in the art to identify biomarkers of neuronal damage following central nervous system injury in humans that can be used to ascertain the severity of the injury and facilitate the selection of an appropriate therapeutic strategy to maximize recovery.

SUMMARY OF THE INVENTION

[0008] The present invention is based, in part, on the discovery that NLRP1 (NALP-1) inflammasome components are secreted into the cerebrospinal fluid (CSF) acutely after SCI and traumatic brain injury (TBI) in humans. Elevated inflammasome protein levels in the CSF following central nervous system (CNS) injury represent the degree of neuroinflammation in CNS tissue and reflect the extent of inflammatory-induced damage. The CSF levels of inflammasome protein following injury correlate with the degree of functional recovery in patients and thus, can be used as acute biomarkers to predict patient prognosis and direct therapeutic interventions. Accordingly, the present invention provides a method of assessing the severity of a CNS injury in a patient.

[0009] In one embodiment, the invention provides a method of evaluating a patient suspected of having a CNS injury comprising providing a biological sample from a patient presenting with clinical symptoms consistent with a CNS injury, measuring the level of at least one inflammasome protein in the biological sample, determining the presence or absence of a protein signature associated with a CNS injury or a more severe CNS injury, wherein the protein signature comprises an elevated level of said at least one inflammasome protein, and selecting patients exhibiting the presence of the protein signature as having a CNS injury or a more severe

CNS injury. In certain embodiments, said one or more inflammasome proteins are NLRP1 (NALP-1), ASC, or caspase-1. The diagnostic methods of the invention may further comprise administering a neuroprotective treatment to the patient based on the measured level of one or more inflammasome proteins, and following changes in the level of one or more inflammasome proteins as a mechanism to monitor response to treatment.

[0010] In some embodiments, the levels of one or more inflammasome proteins in the patient's sample can be used to prepare an inflammasome protein profile associated with CNS injury. The levels of inflammasome proteins in the profile may be determined relative to levels of the proteins in control samples or pre-determined reference values or ranges of reference values. The inflammasome protein profiles are, in some embodiments, indicative of the presence or severity of CNS injury in a patient. When such protein profiles are prepared from samples obtained from patients following administration of a neuroprotective treatment, the inflammasome protein profiles are indicative of therapeutic efficacy of the neuroprotective treatment in the patient.

[0011] The present invention also provides a method of determining a prognosis for a patient with a central nervous system injury. In one embodiment, the method comprises providing a biological sample, such as cerebrospinal fluid, obtained from the patient shortly after injury (e.g., within a week of injury), and measuring the level of at least one inflammasome protein in the biological sample to prepare an inflammasome protein profile, wherein the inflammasome protein profile is indicative of the prognosis of the patient. In particular embodiments, an elevated level of at least one inflammasome protein relative to a pre-determined reference value or range of reference values is indicative of a poorer prognosis or unfavorable patient outcome. For, example elevated inflammasome protein levels are predictive of the patient having a Glasgow Outcome Scale (GOS) score of 1 to 3 upon follow-up assessment. In other embodiments, a reduced level of at least one inflammasome protein relative to a pre-determined reference value or range of reference values is predictive of a favorable patient outcome (e.g. GOS score of 4 or 5 upon follow-up assessment). In certain embodiments, the method provides a prognosis for a patient with a spinal cord or traumatic brain injury.

[0012] The present invention also includes kits for preparing an inflammasome protein profile associated with CNS injury. In one embodiment, the kit comprises a labeled-binding partner, such as labeled-antibody or fragment thereof, that specifically binds to one or more

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inflammasome proteins, wherein said one or more inflammasome proteins are selected from the group consisting of NLRP1, ASC, caspase-1, and combinations thereof.

BRIEF DESCRIPTION OF DRAWINGS

[0013] **Figure 1. NLRP1, ASC, and Caspase-1 are biomarkers that predict outcome after SCI.** CSF samples were immunoblotted with antibodies against NLRP1, caspase-1 and ASC. CSF samples from uninjured patients were used as controls. Immunoblot analysis of 6 different cases of patients with SCI indicates that patients (2, 3 and 4) who express low levels of caspase-1 acutely after SCI have a better prognosis than subjects (1, 5 and 6) who have elevated levels of this protein in CSF.

[0014] **Figure 2. NLRP1 inflammasome proteins are expressed in cells of the CNS.** Spinal cord sections were obtained from decedents that had injury to the spinal cord. Immunohistochemical analysis combined with light microscopy indicates that NLRP1 is expressed in neurons of the ventral horn (black arrows) myelinated axons (black arrow heads) and oligodendrocytes (yellow arrows) (top panel). Caspase-1 is expressed in swollen axons (spheroids, blue arrows), motor neurons (black arrows) and in oligodendrocytes (yellow arrows) (central panel). ASC is present in neurons in the ventral horn (black arrows), white matter oligodendrocytes (yellow arrow) and macrophages/microglia (blue arrow heads) (bottom panel).

[0015] **Figure 3. Scatter plots of expression of inflammasome proteins in controls and patients with TBI.** Samples were immunoblotted for ASC (**A**), caspase-1 (**B**), and NALP-1 (**C**). The p values in the upper left corner represent results of a Mann-Whitney U-test. Densitometric analysis revealed a significant increase in expression of ASC, caspase-1 (p20), and NALP-1 in the CSF of patients with TBI compared with nontrauma controls. Solid lines denote mean values for each group. Different shapes correspond to patient outcomes at 5 months postinjury. Representative immunoblots are shown. Samples were run on the same gel but were noncontiguous. N = the number of TBI samples analyzed; ★ = GOS Score 5; ■ = GOS Score 4; ● = GOS Score 3; ▽ = GOS Score 1.

[0016] **Figure 4. Box plots of expression of inflammasome proteins sorted by outcome category.** The ends of the whiskers represent the lowest datum within 1.5 interquartile range of the lower quartile and the highest datum within 1.5 interquartile range of the upper quartile. The asterisks represent the outliers. Mann-Whitney U-tests indicate higher expression of ASC (**A**),

caspase-1 (p20) (**B**), and NALP-1 (**C**) are significantly associated with an unfavorable outcome 5 months after injury (p < 0.0001). Representative immunoblots for each protein are shown. Samples were run on the same gel but were noncontiguous.

[0017] **Figure 5.** Scatter plots and estimated linear regression of ASC (**A**), caspase-1 (p20) (**B**), and NALP-1 (**C**) expression in the CSF with GOS score. Probability values of the linear regression are shown in the top left of each graph. Expression of each protein correlated significantly with GOS score at 5 months post-injury. The p values on the x axis represent post hoc comparisons of a Kruskal-Wallis test. Representative immunoblots are shown. Samples were run on the same gel but were noncontiguous.

[0018] **Figure 6.** Caspase-1 levels in CSF one, two, and three days following TBI in pediatric patients receiving hypothermia treatment or no treatment (normothermia).

DETAILED DESCRIPTION OF THE INVENTION

[0019] The present invention is based, in part, on the discovery that NLRP1 inflammasomes play an important role in inflammatory responses after SCI and TBI in humans. In particular, the present inventors have surprisingly found that nucleotide-binding leucine-rich repeat pyrin domain containing protein 1 (NLRP1), the adaptor protein apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), and caspase-1 are secreted into the cerebrospinal fluid (CSF) of human patients following SCI and TBI. Thus, these inflammasome proteins represent sensitive biomarkers of the severity of central nervous system injury in human patients. Accordingly, the present invention provides a method of assessing the severity of a central nervous system injury in a patient by measuring the level of at least one inflammasome protein in a biological sample obtained from the patient, wherein the measured level of said at least one inflammasome protein is indicative of the severity of the central nervous system injury in the patient.

[0020] As used herein, the term "inflammasome" refers to a multi-protein complex that activates caspase-1 activity, which in turn regulates IL-1β, IL-18 and IL-33 processing and activation. *See* Arend *et al.* 2008; Li *et al.* 2008; and Martinon *et al.* 2002, each of which is incorporated by reference in their entireties. An "inflammasome protein" is a protein component of inflammasome complexes and can include, but is not limited to, an nucleotide binding domain, leucine-rich repeat containing (NLR) family member (*e.g.* NLRP1), ASC, caspase-1, caspase-11,

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X-linked inhibitor of apoptosis protein (XIAP), and pannexin-1. NLRP1 is also known as NAcht leucine-rich-repeat protein 1 (NALP-1). Thus, the terms "NLRP1" and "NALP-1 are used interchangeably throughout the disclosure. In certain embodiments, the method comprises measuring an inflammasome protein selected from the group consisting of NLRP1 (NALP-1), ASC, caspase-1, or combinations thereof. In one embodiment, the p20 subunit of active caspase-1 is measured.

[0021] The terms "patient" or "subject" are used interchangeably herein, and is meant a mammalian subject to be treated, with human patients being preferred. In certain embodiments, the patient is a pediatric patient. Pediatric patients include newborns (birth to 1 month of age), infants (1 month to 2 years of age), children (2 to 12 years of age), and adolescents (12-21 years of age). In some cases, the methods of the invention find use in experimental animals, in veterinary application, and in the development of animal models for disease, including, but not limited to, rodents including mice, rats, and hamsters, and primates.

[0022] In certain embodiments, the present invention provides a method of evaluating a patient suspected of having a central nervous system (CNS) injury. In one embodiment, the method comprises providing a biological sample from a patient presenting with clinical symptoms consistent with a CNS injury; measuring the level of at least one inflammasome protein in the biological sample; determining the presence or absence of a protein signature associated with a CNS injury or a more severe CNS injury, wherein the protein signature comprises an elevated level of said at least one inflammasome protein; and selecting patients exhibiting the presence of the protein signature as having a CNS injury or a more severe CNS injury.

[0023] A patient may be suspected of having a CNS injury on the basis of neurologic symptoms (motor, sensory, cognitive) and/or radiological evaluation (MRI, CT scan, X-ray) consistent with a CNS injury, e.g., after a physician's exam. In some embodiments, a patient suspected of having a CNS injury, particularly a spinal cord injury, may having a rating of A or B on the American Spinal Cord Injury Association (ASIA) Impairment Scale. The ASIA Impairment Scale is a standard diagnostic tool that assess a patient's motor and sensory function. The classification ratings and accompanying descriptions of the ASIA Impairment Scale are as follows:

Classification/Rating	Description
A	Complete: no motor or sensory function is preserved below the level of injury, including the sacral segments S4-S5
B	Incomplete: sensory, but not motor, function is preserved below the neurologic level and some sensation in the sacral segments S4-S5
C	Incomplete: motor function is preserved below the neurologic level, however, more than half of key muscles below the neurologic level have a muscle grade less than 3 (i.e., not strong enough to move against gravity)
D	Incomplete: motor function is preserved below the neurologic level, and at least half of key muscles below the neurologic level have a muscle grade of 3 or more (i.e., joints can be moved against gravity)
E	Normal: motor and sensory functions are normal

Thus, a patient presenting with a classification rating of A or B on the ASIA Impairment Scale has no motor function below the level of the injury.

[0024] In other embodiments, a patient suspected of having a CNS injury may have a score of $\leq$ 12 (e.g. 3 to 12) on the Glasgow Coma Scale (GCS). In still other embodiments, the patient may have a GCS score of ≤ 8 (e.g. 3 to 8). The GCS is a neurological scale commonly used to assess the level of consciousness of patients after injury or trauma. The scale is composed of three tests (eye, verbal and motor responses), each of which is assigned a value on a scale up to 6. The three values separately as well as their sum are considered. The lowest possible GCS score (the sum) is 3 (deep coma or death), while the highest is 15 (fully awake person). A GCS score < 9 is indicative of severe brain injury whereas a GCS score $\geq$ 13 is indicative of minor brain injury. A GCS score between 9-12 is generally indicative of a moderate brain injury.

[0025] A patient suspected of having a CNS injury may have one or more signs and symptoms of CNS injury, such as temporary loss of consciousness, confusion, disorientation, memory or concentration problems, headache, dizziness, loss of balance, nausea or vomiting, sensory disruptions (e.g. blurred vision, ringing in the ears, bad taste in the mouth, loss of sensation in limbs), loss of motor function, sensitivity to light or sound, mood changes or mood swings, depression or anxiety, fatigue, drowsiness, and sleep disturbances.

[0026] In some embodiments, the level, concentration, or abundance of one or more inflammasome proteins is measured in a biological sample obtained from a patient (e.g. a patient suspected of having or suffering from a CNS injury). In particular embodiments, the levels, concentrations, or abundance of one or more inflammasome proteins is indicative of the severity

of CNS injury in the patient. A CNS injury includes, but is not limited to, a traumatic brain injury, a stroke- related injury, a cerebral aneurism-related injury, a spinal cord injury (*e.g.* contusions, compressions, lacerations), concussion-related injury (including post-concussion syndrome), cerebral ischemia, injury resulting from neurodegenerative diseases (including Parkinson's disease, Dementia Pugilistica, Huntington's disease, Alzheimer's disease, Creutzfeldt-Jakob disease), seizure-related injuries, multiple sclerosis, amyotrophic lateral sclerosis, and other CNS traumas. In certain embodiments, the levels, concentrations, or abundance of one or more inflammasome proteins is indicative of the severity of traumatic brain injury or spinal cord injury in the patient.

[0027] As used herein, "biological sample" refers to any bodily fluid or tissue obtained from a patient or subject. A biological sample can include, but is not limited to, whole blood, red blood cells, plasma, serum, peripheral blood mononuclear cells (PBMCs), urine, saliva, tears, buccal swabs, CSF, CNS microdialysate, and nerve tissue. In one embodiment, the biological sample is CSF, saliva, serum, plasma, or urine. In certain embodiments, the biological sample is CSF.

[0028] In some embodiments, the measured level, concentration, or abundance of one or more inflammasome proteins in the biological sample is used to prepare an inflammasome protein profile, wherein the profile is indicative of the severity of a CNS injury in the patient or the patient's prognosis or recovery potential from a CNS injury. The inflammasome protein profile may comprise the level, abundance, or concentration of one or more inflammasome proteins measured in the patient's sample optionally in relation to a pre-determined value or range of reference values as described herein. In certain embodiments, the inflammasome proteins in the profile include NLRP1 (NALP-1), ASC, and/or caspase-1 (*e.g.* p20 subunit of caspase-1). In one particular embodiment, the inflammasome protein profile comprises the level, abundance, or concentration of each of NLRP1 (NALP-1), ASC, and caspase-1 (*e.g.* p20 subunit of caspase-1).

[0029] In one aspect of the invention, the method of evaluating a patient suspected of having a CNS injury comprises determining the presence or absence of a protein signature associated with a CNS injury or a more severe CNS injury based on the measured level, abundance, or concentration of one or more inflammasome proteins in the patient sample or on the inflammasome protein profile prepared from the patient's sample. In certain embodiments, the protein signature comprises an elevated level of at least one inflammasome protein. The level of said at least one inflammasome protein in the protein signature may be enhanced relative to the

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level of the protein in a control sample or relative to a pre-determined reference value or range of reference values as further described herein. The protein signature may, in certain embodiments, comprise an elevated level for each of caspase-1 (*e.g.* p20 subunit of caspase-1), NLRP1, and ASC. Patients who exhibit the protein signature may be selected or identified as having a CNS injury or a more severe CNS injury.

[0030] The level or concentration of at least one inflammasome protein can be assessed at a single time point (*e.g.* after a potential CNS injury) and compared to a pre-determined reference value or range of reference values or can be assessed at multiple time points (*e.g.* two, three, four, five or more) after a potential CNS injury and compared to a pre-determined reference value or to previously assessed values. For instance, a biological sample for measuring levels or concentrations of inflammasome proteins can be obtained from a patient within one hour of a potential CNS injury to two weeks following a potential CNS injury. In some embodiments, the biological sample is obtained within one day, two days, three days, four days, five days, six days, seven days, ten days, or twelve days of a CNS injury or potential injury.

[0031] As used herein, "pre-determined reference value" refers to a pre-determined value of the level or concentration of an inflammasome protein ascertained from a known sample. For instance, the pre-determined reference value can reflect the level or concentration of an inflammasome protein in a sample obtained from a control subject (*i.e.*, an uninjured, healthy subject). The control subject may, in some embodiments, be age-matched to the patients being evaluated. Thus, in particular embodiments, the measured level or concentration of at least one inflammasome protein is compared or determined relative to the level or concentration of said at least one inflammasome protein in a control sample (*i.e.* obtained from an uninjured subject).

[0032] In other embodiments, the pre-determined reference value or range of reference values can reflect the level or concentration of an inflammasome protein in a sample obtained from a patient with a known severity of CNS injury as assessed by clinical measures or post mortem analysis. A pre-determined reference value can also be a known amount or concentration of an inflammasome protein. Such a known amount or concentration of an inflammasome protein may correlate with an average level or concentration of the inflammasome protein from a population of control subjects or a population of patients with known levels of injury. In another embodiment, the pre-determined reference value can be a range of values, which, for instance, can represent a mean plus or minus a standard deviation or confidence interval. A range of

reference values can also refer to individual reference values for a particular inflammasome protein across various levels of CNS injury severity. In certain embodiments, an increase in the level of one or more inflammasome proteins (*e.g.*, NLRP1 (NALP-1), ASC, or caspase-1) relative to a pre-determined reference value or range of reference values is indicative of a more severe central nervous system injury.

[0033] In some embodiments, the method of assessing the severity of a CNS injury further comprises measuring the level or concentration of one or more proteins described in U.S. Patent Publication No. 2011/0177974, which is hereby incorporated by reference in its entirety, in addition to measuring the level or concentration of one or more inflammasome proteins. For instance, in certain embodiments, the method further comprises measuring the level or concentration of one or more proteins selected from ubiquitin C-terminal hydrolase L1; vesicular membrane protein p-24; synuclein; microtubule-associated protein; synaptophysin; Vimentin; Synaptotagmin; Synaptojanin-2; Synapsin2; CRMP1, 2; Amphiphysin-1; PSD95; PSD-93; Calmodulin dependent protein kinase II (CAMPK)-alpha, beta, gamma; Myelin basic protein (MBP); Myelin proteolipid protein (PLP); Myelin Oligodendrocyte specific protein (MOSP); Myelin Oligodendrocyte glycoprotein (MOG); myelin associated protein (MAG); NF-H; NF-L; NF-M; BIII-tubulin-1 or combinations thereof in the biological sample obtained from the patient in addition to measuring the level or concentration of one or more inflammasome proteins. Thus, the protein signature may comprise an elevated level of one or more of these proteins in addition to the elevated level of one or more inflammasome proteins. In other embodiments, the method further comprises measuring the level or concentration of one or more proteins selected from S-100β, neuron-specific enolase, neurofilament light chain, glial fibrillary acidic protein (GFAP) or combinations thereof in the biological sample obtained from the patient in addition to measuring the level or concentration of one or more inflammasome proteins. In one embodiment, the protein signature associated with a CNS injury or a more severe CNS injury comprises an elevated level of one or more proteins selected from S-100β, neuron-specific enolase, neurofilament light chain, glial fibrillary acidic protein (GFAP) in addition to an elevated level of one or more inflammasome proteins (*e.g.* NLRP1 (NALP-1), ASC, or caspase-1).

[0034] In other embodiments of the invention, the methods of assessing the severity of a CNS injury in a patient or evaluating a patient suspected of having a CNS injury further comprise

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administering a neuroprotective treatment to the patient based on the measured level of said at least one inflammasome protein or when a protein signature associated with a CNS injury or a more severe CNS injury is identified. Such neuroprotective treatments include drugs that reduce excitotoxicity, oxidative stress, and inflammation. Thus, suitable neuroprotective treatments include, but are not limited to, methylprednisolone, 17α-estradiol, 17β-estradiol, ginsenoside, progesterone, simvastatin, deprenyl, minocycline, resveratrol, and other glutamate receptor antagonists (e.g. NMDA receptor antagonists) and antioxidants. In some embodiments, neuroprotective treatments are neutralizing antibodies against an inflammasome protein or binding fragments thereof, such as those described in U.S. Patent Publication No. 2009/0104200, which is hereby incorporated by reference in its entirety. For instance, in one embodiment, the neuroprotective treatment is an anti-ASC antibody or fragment thereof. Anti-ASC antibodies include antibodies that specifically bind to amino acid residues 178-193 of rat ASC (accession number BAC43754), e.g., amino acid sequence ALRQTQPYLVTDLEQS (SEQ ID NO:1), or antibodies that specifically bind to the amino acid sequence RESQSYLVEDLERS (SEQ ID NO:2) of human ASC. In another embodiment, the neuroprotective treatment is an anti-NLRP1 antibody or fragment thereof. Suitable neutralizing anti-NLRP1 antibodies or fragments thereof include antibodies that specifically bind to the amino acid sequence CEYYTEIREREREKSEKGR (SEQ ID NO:3) of human NLRP1 or the amino acid sequence MEESQSKEESNTEG (SEQ ID NO: 4) of rat NLRP1. The neutralizing antibodies or antibody fragments may be polyclonal antibodies, monoclonal antibodies, chimeric antibodies, humanized antibodies, single-chain variable fragments (scFvs) and the like. Aptamers that specifically bind to an inflammasome protein or epitope thereof (e.g., SEQ ID NOs: 1-4) may also be suitable neuroprotective treatments. Neuroprotective treatments also encompass therapeutic regimens or rehabilitative procedures, such as hypothermia treatment.

[0035] The success of, or response to, treatment can also be monitored by measuring the levels of at least one inflammasome protein. Accordingly, in some embodiments, the methods of evaluating a patient further comprise measuring the level of at least one inflammasome protein in a biological sample obtained from the patient following neuroprotective treatment, preparing a treatment protein signature associated with a positive response to the neuroprotective treatment, wherein the treatment protein signature comprises a reduced level of at least one inflammasome protein, and identifying patients exhibiting the presence of the treatment protein signature as

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responding positively to the neuroprotective treatment. A reduction in the level, abundance, or concentration of one or more inflammasome proteins (*e.g.* NLRP1, ASC, and caspase-1) is indicative of the efficacy of the neuroprotective treatment in the patient. The one or more inflammasome proteins measured in the sample obtained following treatment may be the same as or different than the inflammasome proteins measured in the sample obtained prior to treatment. The inflammasome protein levels may also be used to adjust dosage or frequency of a neuroprotective treatment.

[0036] The present invention also provides a method of determining a prognosis for a patient with a central nervous system injury. In one embodiment, the method comprises providing a biological sample obtained from the patient within a week of injury, and measuring the level of at least one inflammasome protein in the biological sample to prepare an inflammasome protein profile as described above, wherein the inflammasome protein profile is indicative of the prognosis of the patient. In certain preferred embodiments, the biological sample is obtained from the patient within one week, within five days, or within three days of injury. In some embodiments, an increase in the level of one or more inflammasome proteins (*e.g.*, NLRP1, ASC, caspase-1, or combinations thereof) relative to a pre-determined reference value or range of reference values is indicative of a poorer prognosis. For instance, an increase of about 20% to about 300% in the level of one or more inflammasome proteins relative to a pre-determined reference value or range of reference values is indicative of a poorer prognosis. In one embodiment, increased levels of caspase-1, particularly the p20 subunit of active caspase-1, relative to a pre-determined reference value or range of reference values acutely after injury (*i.e.* within a week of injury) is indicative of a poorer prognosis.

[0037] In particular embodiments, an elevated level of at least one inflammasome protein relative to a pre-determined reference value or range of reference values is predictive of the patient's recovery potential or long-term outcome as assessed by the Glasgow Outcome Scale (GOS). The GOS is a scale that allows for the objective assessment of a patient's recovery following brain injury. The scale is comprised of scores ranging from 1 to 5 with the following descriptions:

Score/Category	Description
1-Death	Severe injury or death without recovery of consciousness
2-Persistent Vegetative State	Severe damage with prolonged state of unresponsiveness and a lack of higher mental functions
3-Severe Disability	Severe injury with permanent need for help with daily living
4-Moderate Disability	No need for assistance in everyday life, employment is possible but may require special equipment
5-Low Disability	Light damage with minor neurological and psychological deficits.

In one embodiment, an elevated level of at least one inflammasome protein relative to a pre-determined reference value or range of reference values is predictive of the patient having a GOS score of 1 to 3 upon follow-up assessment (*i.e.* the patient having an unfavorable outcome, such as death or severe disability). In another embodiment, a reduced level of at least one inflammasome protein relative to a pre-determined reference value or range of reference values is predictive of the patient having a GOS score of 4 or 5 upon follow-up assessment (*i.e.* the patient having a favorable outcome, such as moderate to low disability). The inventors have found that the CSF levels of one or more inflammasome proteins within three days following a CNS injury are useful for predicting the long-term outcome or recovery potential of the patient. Elevated inflammasome proteins levels correlate with unfavorable outcomes for the patient, whereas reduced or low inflammasome protein levels correlate with favorable outcomes for the patient (Example 3).

[0038] The inflammasome proteins of the invention and other marker proteins can be measured in a biological sample by various methods known to those skilled in the art. For instance, proteins can be measured by methods including, but not limited to, liquid chromatography, gas chromatography, mass spectrometry, radioimmunoassays, immunofluorescent assays, FRET-based assays, immunoblot, ELISAs, or liquid chromatography followed by mass spectrometry (*e.g.*, MALDI MS). One of skill in the art can ascertain other suitable methods for measuring and quantitating any particular biomarker protein of the invention.

[0039] The present invention also includes kits for preparing an inflammasome protein profile associated with CNS injury, such as spinal cord injury or traumatic brain injury. The kits may include a reagent for measuring at least one inflammasome protein and instructions for

measuring said at least one inflammasome protein for assessing the severity of a central nervous system injury in a patient. As used herein, a "reagent" refers to the components necessary for detecting or quantitating one or more proteins by any one of the methods described herein. For instance, in some embodiments, kits for measuring one or more inflammasome proteins can include reagents for performing liquid or gas chromatography, mass spectrometry, immunoassays, immunoblots, or electrophoresis to detect one or more inflammasome proteins as described herein. In some embodiments, the kit includes reagents for measuring one or more inflammasome proteins selected from NLRP1, ASC, caspase-1, or combinations thereof.

[0040] In one embodiment, the kit comprises a labeled-binding partner that specifically binds to one or more inflammasome proteins, wherein said one or more inflammasome proteins are selected from the group consisting of NLRP1, ASC, caspase-1, and combinations thereof. Suitable binding partners for specifically binding to inflammasome proteins include, but are not limited to, antibodies and fragments thereof, aptamers, peptides, and the like. In certain embodiments, the binding partners for detecting NLPR1 are antibodies or fragments thereof, aptamers, or peptides that specifically bind to the amino acid sequence of SEQ ID NO: 3 or SEQ ID NO: 4 of human NLRP1 and rat NLRP1, respectively. In other embodiments, the binding partners for detecting ASC are antibodies or fragments thereof, aptamers, or peptides that specifically bind to the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2 of rat ASC and human ASC, respectively. Labels that can be conjugated to the binding partner include metal nanoparticles (e.g., gold, silver, copper, platinum, cadmium, and composite nanoparticles), fluorescent labels (e.g., fluorescein, Texas-Red, green fluorescent protein, yellow fluorescent protein, cyan fluorescent protein, Alexa dye molecules, etc.), and enzyme labels (e.g., alkaline phosphatase, horseradish peroxidase, beta-galactosidase, beta-lactamase, galactose oxidase, lactoperoxidase, luciferase, myeloperoxidase, and amylase).

[0041] In some embodiments, the kit can include reagents for measuring one or more inflammasome proteins in CSF samples. In other embodiments, the kits can include reagents for measuring one or more inflammasome proteins in other patient samples including nerve tissue, CNS microdialysate, blood, saliva, serum, plasma, or urine. In still other embodiments, the kits further comprise a set of reference values to which the measured level of one or more inflammasome proteins can be compared.

[0042] This invention is further illustrated by the following additional examples that should not be construed as limiting. Those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made to the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLES

Example 1. Inflammasome Proteins are Secreted into Cerebrospinal Fluid after Spinal Cord Injury

[0043] To determine whether NLRP1 inflammasome proteins were present in cerebrospinal fluid (CSF) following spinal cord injury (SCI), CSF samples from seven patients with SCI or control patients were analyzed for levels of nucleotide-binding leucine-rich repeat pyrin domain containing protein 1 (NLRP1; also known as NAcht leucine-rich-repeat protein 1 (NALP-1)), apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), and caspase-1. The American Spinal Cord Injury Association (ASIA) scale of the SCI patients at admission to the emergency department ranged from AIS A to B. Information regarding the diagnosis, procedures and outcomes of the patients is shown in Table 1. None of the patients had any complications. CSF from uninjured individuals was obtained as a control from three males and two females ranging from 67 to 91 years old.

[0044] For detection of inflammasome proteins, CSF samples were prepared with Laemali buffer. Immunoblot analysis was carried with the Criterion system (Bio-Rad) as described previously (de Rivero Vaccari *et al.*, 2008) using antibodies (1:1000 dilution) to NLRP1 (Bethyl Laboratories), Caspase-1 (Imgenex) and ASC (Santa Cruz). Proteins were resolved in 14-20% TGX Criterion precasted gels (Bio-Rad), transferred to polyvinylidene difluoride (PVDF) transfer membranes (Tropifluor – Applied Biosystems) and placed in blocking buffer (PBS, 0.1% Tween-20, 0.4% I-Block (Applied Biosystems) and then incubated for one hour with primary antibodies. Membranes were then incubated for one hour with anti-mouse, anti-rat or anti-rabbit horseradish peroxidase (HRP)-linked antibodies. Signal visualization was performed by enhanced chemiluminescence.

[0045] Immunoblot analysis of control CSF samples (n=5) revealed very low levels of NLRP1 inflammasome proteins (Figure 1). In contrast, immunoblot analysis of samples from 6 different SCI patients showed an increase in the levels of NLRP1, caspase-1 and ASC in the CSF when

compared to CSF from control subjects. It should be noted that patients 2, 3 and 4 (Figure 1) did not show increased levels of caspase-1 acutely (day 0 through day 2) after SCI. Interestingly, these patients demonstrated stark motor improvement at 2 days after SCI. Patients 1, 5, and 6 showed increased levels of caspase-1, ASC and NLRP1 inflammasome proteins acutely after SCI and these individuals had a poor prognosis and did not show motor improvements. Thus, it appears that individuals that present with low levels of caspase-1 in CSF acutely after SCI may have a better prognosis than those individuals who show increased levels of this biomarker.

[0046] The results from these experiments show that protein levels of NLRP1, ASC, and caspase-1 in CSF are increased following injury to the central nervous system and suggest that levels of these inflammasome proteins can serve as biomarkers of the severity of neuronal damage following injury thereby directing treatment and rehabilitation efforts, monitoring response to treatment, and aiding in the determination of prognosis of recovery in injured patients.

Example 2. Immunohistochemical Expression of NLRP1 Inflammasome Proteins in Spinal Cords After Injury

[0047] Spinal cord sections were obtained from nine decedents (8 males and 1 female with ages ranging from 20 to 77 years) who had injury to the spinal cord due to vertebral fractures. The spinal cord injury was assessed microscopically, using bright field optics, by examining one H&E or H&E/DAB-stained section from the lesion center of each case or from cervical, thoracic and lumbar sections from control cases. The spinal cord injuries were classified on the basis of their histological appearance as "contusion/cyst," massive compression, or laceration (Fleming *et al.*, 2006). Contusional injuries were characterized by an intact pia and relative preservation of the anatomical relations of various elements of the spinal cord, and variable degrees of injury ranging from involvement of the entire cross-sectional area to large usually asymmetric areas of tissue damage. Massive compression injuries were characterized by disruption of the pia and severe distortion and disruption of spinal cord parenchyma. Laceration injuries, which by definition were perforating or penetrating injuries caused by weapons or projectiles, were associated with breaching of the pia and linear tearing of the cord tissue.

[0048] All tissue samples had been removed within 24 h of death and fixed in neutral buffered formalin. Blocks from the spinal cords were dehydrated, embedded in paraffin wax, cut into 6

μm thick sections and placed on positively charged glass slides. One set of sections was stained with hematoxylin-eosin (H&E) and the remaining sets were used for immunohistochemistry. Paraffin-embedded sections were stained with anti-NLRP1 (Bethyl Laboratories as described in de Rivero Vaccari et al. 2008), anti-caspase-1 (Upstate), and anti-ASC (Chemicon) using diaminobenzidine (DAB) as the chromophore and hematoxylin. Negative controls included sections in which the primary antibody was omitted and sections incubated with isotype-matched antibodies (1:100-1:10,000 IgG). These positive and negative controls were processed with every batch of immunohistochemical slides.

[0049] In all cases, tissue samples from the center of injury and at various distances above and below the injury were obtained. The data from tissue from the center of the lesion were used to compare the inflammatory responses between cases whereas those from the remote, uninjured segments of the spinal cord served as within-case controls. Between-case comparison of the remote samples was not possible because, for different cases, the distance of these samples from the lesion center was variable.

[0050] Immunohistochemical analysis combined with light microscopy indicated that NLRP1 is expressed in neurons of the ventral horn (black arrows), myelinated axons (arrow heads) and oligodendrocytes (yellow arrows) in injured spinal cords (Figure 2). Moreover, NLRP1 immunoreactivity in areas of the penumbra (C7) was higher than in areas distant to the epicenter (L2).

[0051] DAB immunoreactivity for caspase-1 was detected in swollen axons (spheroids, blue arrows) (Figure 2), and arterioles (not shown). At areas of the penumbra, caspase-1 staining is present in motor neurons (black arrows) of the ventral horn, and in the white matter in oligodendrocytes (yellow arrows). Caspase-1 immunoreactivity in oligodendrocytes (yellow arrows) was the same at all levels of the spinal cords examined, regardless of proximity to the epicenter. At areas distant to the epicenter (T12), caspase-1 was also present in motor neurons (black arrows) but with decreased immunoreactivity than the penumbra (C7). This finding indicates that caspase-1 immunoreactivity in neurons decreases as the distance to the epicenter increases, similarly to NLRP1.

[0052] At areas of the penumbra (C7) and distant to the epicenter (L2), neurons in the ventral horn (black arrows) and white matter oligodendrocytes (yellow arrow) showed ASC immunoreactivity. In addition, ASC was also present in macrophages/microglia at the epicenter

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(blue arrow heads). Moreover, ASC immunoreactivity was also detected in the substantia gelatinosa (dorsal horn) at C7 and L2 (not shown).

[0053] Neuroinflammation has been considered to play a critical role in the pathogenesis of SCI and TBI, but the role of the innate immune response has not been examined directly. The innate immune system senses microbial and viral pathogen-associated molecular patterns and danger signals released from damaged or stressed cells to trigger conserved intracellular signaling pathways that drive proinflammatory responses that are critical for productive innate and adaptive immunity. Excessive inflammatory responses become deleterious leading to tissue destruction. The results of this experiment provide evidence demonstrating that the NLRP1 inflammasome signaling system is activated in the innate immune response in damaged human spinal cord and brain tissue after trauma. These findings support the idea that activation of the NLRP1 inflammasome signaling system is an early event in spinal cord and brain pathology and that these proteins may serve as biomarkers for SCI and TBI in humans.

Example 3. Inflammasome Proteins in Cerebrospinal Fluid of Brain-Injured Patients are Biomarkers of Functional Outcome

[0054] To determine whether inflammasome proteins may serve as biomarkers for other types of central nervous system injury, a total of 45 CSF samples were collected from 23 traumatic brain injury (TBI) patients on the day of injury and up to three days after the injury and analyzed by immunoblot for levels of NALP-1 (also known as NLRP1), ASC, and caspase-1. Each of the patients presented with the following inclusion criteria: severe or moderate head trauma (Glasgow Coma Scale (GCS) score $\leq$ 12), age 1 month to 65 years, and ventriculostomy. Twenty-two of the patients suffered severe brain trauma (GCS score $\leq$ 8) and 1 suffered moderate brain trauma (moderate TBI GCS score range 9–12). Nine patients (5 men and 4 women) with a mean age of 66.3 years (range 29–91 years) served as controls. Control patients required a ventriculostomy for nontraumatic pathology. Patients with acute meningitis, cerebral vasculitis, or other recent CNS infection were excluded. Information regarding patient demographics, intracranial pathology, GCS score at presentation, and Glasgow Outcome Scale (GOS) score at 5 months post-injury is shown in Table 2.

[0055] Cerebrospinal fluid samples were collected within 12 hours of injury and up to 72 hours after injury. Samples were centrifuged at 2000g for 10 minutes at 4°C to pellet cellular bodies

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and debris. Supernatants were resolved by gel electrophoresis and immunoblotted as previously described (de Rivero Vaccari *et al.*, 2008). Quantification of band density was performed with UNSCAN-IT gel digitizing software (Silk Scientific). Due to the low volume of sample available, NALP-1 was analyzed in 6 of the 9 controls, caspase-1 was analyzed in 43 of the 45 TBI samples, and NALP-1 was analyzed in 42 of the 45 TBI samples. Immunoblot analysis shows that the inflammasome proteins ASC, caspase-1 (p20), and NALP-1 are present in the CSF of patients with TBI and nontrauma controls. Quantitative data from a densitometric analysis are shown in Figure 3. Expression of the 22-kD isoform of ASC (Fig. 3A), the p20 subunit of cleaved caspase-1 (Fig. 3B), and NALP-1 (Fig. 3C) is significantly elevated in the CSF of TBI patients compared with nontrauma controls ($p < 0.0001$, $p = 0.0029$, and $p = 0.0202$, respectively).

[0056] To determine if the levels of inflammasome components correlate with outcome, we grouped study participants by outcome category (GOS Scores 1 and 3, unfavorable outcome; GOS Scores 4 and 5, favorable outcome). At 5 months postinjury, 3 patients had a GOS score of 1 (death), 11 patients had a GOS score of 3 (severe disability), 6 patients had a GOS score of 4 (moderate disability), and 3 patients had a GOS score of 5 (good recovery). Within the sample of patients with TBI, no patient remained with a GOS score of 2 (persistent vegetative state).We detected significantly higher levels of ASC (Fig. 4A), caspase-1 (p20) (Fig. 4B), and NALP-1 (Fig. 4C) in the CSF of TBI patients with unfavorable outcomes, including death and severe disability with complete dependence on others for activities of daily living ($p < 0.0001$).

[0057] To further understand the relationship between outcome and inflammasome proteins, we constructed modified scatter plots of expression levels of ASC, caspase-1 (p20), and NALP-1 and GOS (Figure 5). A calculated linear regression line is shown for each plot. Linear regression analysis shows that expression of ASC (Fig. 5A; $p < 0.05$), caspase-1 (p20) (Fig. 5B; $p < 0.01$), and NALP-1 (Fig. 5C; $p < 0.05$) correlate significantly with outcome at 5 months. Post hoc, the Dunn multiple comparison tests following a Kruskal-Wallis test showed that the levels of ASC are significantly higher in patients with severe disability (GOS Score 3) compared with patients with moderate disability (GOS Score 4) ($p < 0.001$) and patients with mild to no disability (GOS Score 5) ($p < 0.05$). Similarly, expression levels of caspase-1 (p20) and NALP-1 are significantly higher in patients with severe disability (GOS Score 3) than in those with moderate disability (GOS Score 4) ($p < 0.001$).

[0058] The results of this study show that inflammasome proteins (*e.g.* ASC, NALP-1, and caspase-1) are acutely elevated (*e.g.* within 72 hours) in the CSF of patients with TBI as compared with nontrauma controls. Elevation of these proteins likely reflects the extent of neuroinflammation, suggesting that inflammasome proteins can serve as acute biomarkers of CNS injury. These findings are clinically relevant, as CSF biomarkers are more specific indicators of neuropathology than serum biomarkers. Cerebrospinal fluid directly bathes the brain, closely reflecting the extracellular milieu and biochemical changes that are specific to the CNS. Sampling the CSF eliminates influences of multiorgan trauma or other systemic pathology represented in the serum, which is significant as patients with TBI often present with trauma to other organ systems.

[0059] The results also demonstrate that levels of inflammasome proteins are significantly higher in the CSF of patients who have died and those with severe disability than in patients with moderate to no disability, suggesting that inflammasome activation produces chronic neuroinflammation, contributing to secondary injury and poor outcome 5 months after TBI. The extent of acute elevation of these proteins can predict an unfavorable versus favorable outcome. Such markers could also direct treatment and rehabilitation efforts. The clinician would target therapies to patients identified as having a greater risk of inflammation-mediated secondary injury.

[0060] Response to treatment could be monitored by following the levels of ASC, active caspase-1, and NALP-1 in the CSF. One such treatment, therapeutic hypothermia, attenuates the endogenous inflammatory response of the CNS to TBI by decreasing cytokine production and reducing activation of astrocytes and microglia (Aibiki *et al.*, 1999; Goss *et al.*, 1995; Kumar *et al.*, 1997; Truettner *et al.*, 2005), and cortical neurons exposed to moderate hypothermia in culture show a decrease in activation of the inflammasome (Tomura *et al.*, in press). Thus, ASC, active caspase-1, and NALP-1 can serve as objective, biochemical indicators of treatment efficacy for patients with CNS injury.

Example 4. Hypothermia Decreases Caspase-1 Activation after Traumatic Brain Injury in Pediatric Patients

[0061] To evaluate whether inflammasome proteins, such as caspase-1, can also be used to monitor treatment efficacy in TBI patients, CSF caspase-1 levels obtained from pediatric patients

who received hypothermia treatment following TBI were compared to those obtained from pediatric patients who did not receive treatment following TBI. Cerebrospinal fluid of pediatric patients (ages 0.1 to 16 years) was obtained at different times after traumatic brain injury (day 1, 2 and 3). Patients were divided into those that received hypothermia treatment and those who did not (normothermia). As shown in Figure 6, the data indicate that within 24 hours after injury the levels of caspase-1 were lower in the hypothermia group when compared to the normothermia group. Thus post-traumatic therapeutic hypothermia lowers the levels of caspase-1 activation after brain injury, consistent with findings that those patients that receive hypothermia treatment present better outcomes when compared to those patients who do not get the hypothermia treatment.

[0062] It is understood that the disclosed invention is not limited to the particular methodology, protocols and materials described as these can vary. It is also understood that the terminology used herein is for the purposes of describing particular embodiments only and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

[0063] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

Table 1. Spinal Cord Injury Subjects

Patient	Age	Gender	Race	Mechanism of Injury	Spinal Injury	AIS Grade	Level	Surgery	Hypothermia	Other injuries	Exam at Rehab D/C
1	43	M	Black	Auto vs. Pedestrian	C7/T1 bilateral jumped facets	A	C7	C4-T2 Laminectomy w/instrumented fusion	Yes	None	C7 ASIA A
2	38	M	Hispanic	Fall from height	T3 & T4 Burst fractures	B	T3	T1-6 Laminectomy w/instrumented fusion	No	None	T3 ASIA D
3	21	M	White	Driving accident	C5/6 Fracture dislocation	A	C5	C5/6 Anterior disectomy & fusion	Yes	None	C5 ASIA C
4	19	M	Black	Motor vehicle accident	C4/5 Bilateral jumped facets	B	C4	C4/5 Anterior disectomy & fusion	No	None	C4 ASIA D
5	22	M	White	Rugby accident	C5/6 Bilateral jumped facets	A	C5	C5/6 Anterior disectomy & fusion	Yes	None	C6 ASIA A
6	48	M	Hispanic	Motorcycle vs. Pedestrian	T1/2 Fracture dislocation	A	T3	C5-T2 Laminectomy w/instrumented fusion	No	Degloving injury and fractures of face	T3 ASIA B

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Table 2. Summary of demographic data in patients with TBI*

Case No.	Age (yrs), Sex	Race	Mechanism of Injury	GCS Score†	GOS Score‡	Intracranial Pathology
1	26, M	White	MVA	3	4	bilat temporal cortical contusions, SAH, SDH
2	22, M	Hispanic	motorcycle accident	3	4	SDH, diffuse SAH
3	19, F	Hispanic	MVA	7	3	fronto- & temporoparietal & basal ganglia hemorrhagic contusions, frontoparietal SAH
4	30, F	Hispanic	MVA	8	4	scattered SAH, diffuse cerebral edema
5	21, M	Hispanic	motorcycle accident	5	3	frontal SDH & SAH
6	17, F	Hispanic	MVA	4	3	diffuse extraaxial & intraparenchymal hemorrhage
7	26, M	Hispanic	MVA	8	3	diffuse SAH, IVH, & parenchymal hemorrhagic contusions
8	36, M	White	ATV accident	3	5	SDH, SAH, & diffuse cerebral edema
9	16, M	Hispanic	MVA	7	5	frontotemporal SDH, frontal lobe contusion, cerebral edema
10	22, M	Hispanic	MVA	3	3	SDH
11	36, M	Black	Gunshot wound	3	1	SDH, bullet fragments in frontal lobe
12	54, M	Hispanic	Fall	3	3	frontal hemorrhagic contusions, parietooccipital

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Case No.	Age (yrs), Sex	Race	Mechanism of Injury	GCS Score†	GOS Score‡	Intracranial Pathology
						hemorrhagic contusion, SDH, brain edema
13	62, M	Black	MVA	8	3	diffuse frontoparietal hemorrhage, frontal & parietal lobe hemorrhagic contusions
14	49, F	White	Assault	8	3	temporal & frontal lobe hemorrhagic contusions, frontotemporal SAH, frontal dural hematoma
15	20, M	Hispanic	Motorcycle accident	6	4	bone fragments in frontoparietal brain parenchyma, scattered frontoparietal SAH, hemorrhagic contusions
16	28, M	Hispanic	Motorcycle accident	4	3	SDH
17	21, M	Black	MVA	5	4	mild cerebral edema
18	45, M	Hispanic	Gunshot wound	8	3	bullet fragments in occipital lobe, occipital SDH, minimal parietal & occipital pneumocephalus
19	21, M	White	MVA	3	3	diffuse axonal injury, scattered SAH, parietal SDH, mild hydrocephalus
20	17, M	Hispanic	Sports injury	4	4	SDH, SAH, diffuse cerebral edema
21	19, F	Hispanic	MVA	11	5	bone fragments in frontal lobe parenchyma, frontal lobe contusion, edema, pneumocephalus
22	65, M	White	MVA	7	1	parietal SDH, SAH, parietal hemorrhagic contusion, uncal herniation

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Case No.	Age (yrs), Sex	Race	Mechanism of Injury	GCS Score†	GOS Score‡	Intracranial Pathology
23	18, M	White	MVA	3	1	diffuse axonal injury, scattered hemorrhagic contusions in frontal, temporal, parietal lobes & corpus callosum, IVH

* IVH = intraventricular hemorrhage; SAH = subarachnoid hemorrhage; SDH = subdural hematoma. MVA = motor vehicle accident; ATV = all terrain vehicle

† Obtained on admission.

‡ Assessed at 5 months postinjury.

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CLAIMS:

1.   A method of evaluating a patient suspected of having a central nervous system (CNS) injury comprising:

measuring the level of at least one inflammasome protein in a biological sample obtained from a patient presenting with clinical symptoms consistent with a CNS injury, wherein the biological sample is cerebrospinal fluid (CSF), blood, serum or plasma;

determining the presence or absence of a protein signature associated with the CNS injury or a more severe CNS injury, wherein the protein signature comprises an elevated level of the at least one inflammasome protein; and

selecting patients exhibiting the presence of the protein signature as having the CNS injury or the more severe CNS injury.

2.   The method of claim 1, wherein the level of the at least one inflammasome protein in the protein signature is enhanced relative to the level of the at least one inflammasome protein in a control sample.

3.   The method of claim 1, wherein the level of the at least one inflammasome protein in the protein signature is enhanced relative to a pre-determined reference value or range of reference values.

4.   The method of claim 1, wherein the CNS injury is a stroke-related injury, a cerebral aneurism-related injury, concussion-related injury, cerebral ischemia, injury resulting from neurodegenerative diseases, seizure-related injuries, multiple sclerosis, amyotrophic lateral sclerosis, a spinal cord injury or a traumatic brain injury.

5.   The method of claim 1, wherein the at least one inflammasome protein is nucleotide-binding leucine-rich repeat pyrin domain containing protein 1 (NLRP1), apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), caspase-1, or combinations thereof.

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6. The method of claim 1, wherein the protein signature comprises an elevated level for each of caspase-1, NLRP1, and ASC.

7. The method of claim 6, wherein the protein signature comprises an elevated level for the p20 subunit of caspase-1.

8. The method of claim 1, wherein the biological sample is obtained within one week of the suspected injury.

9. The method of claim 1, wherein the biological sample is obtained within five days of the suspected injury.

10. The method of claim 1, wherein the biological sample is obtained within three days of the suspected injury.

11. The method of claim 1, wherein the patient has an A or B rating on the American Spinal Cord Injury Association (ASIA) Impairment Scale.

12. The method of claim 1, wherein the patient has a Glasgow Coma Scale (GCS) score of 3 to 12.

13. The method of claim 12, wherein the patient has a GCS score of 3 to 8.

14. The method of claim 1, wherein the patient is a pediatric patient.

15. The method of claim 1, wherein the level of the at least one inflammasome protein is measured by immunoblot or ELISA.

16. The method of claim 1, wherein the biological sample is CSF.
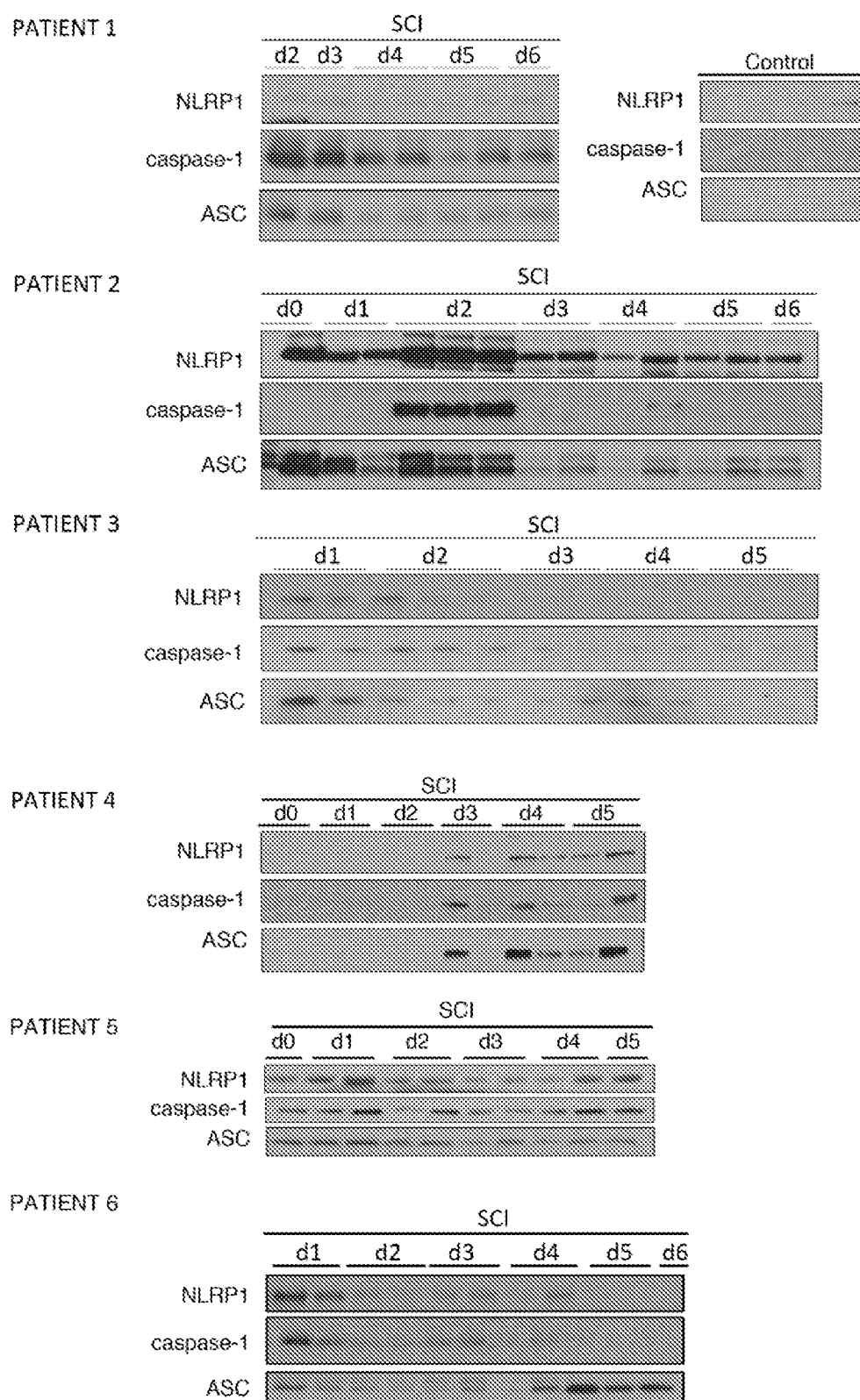
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17.   Use of a neuroprotective agent to treat a CNS injury in a patient, wherein the patient is selected according to the method of claim 1.

18.   The use of claim 17, where in the neuroprotective agent is a neutralizing antibody or binding fragment thereof against an inflammasome protein, an aptamer that specifically binds to an inflammasome protein or epitope thereof, methylprednisolone, 17α-estradiol, 17β-estradiol, ginsenoside, progesterone, simvastatin, deprenyl, minocycline, and resveratrol.

19.   The use of claim 17, wherein the method of claim 1 further comprises:
        measuring the level of the at least one inflammasome protein in a biological sample obtained from the patient following neuroprotective treatment, wherein the biological sample is cerebrospinal fluid (CSF), blood, serum or plasma;
        preparing a treatment protein signature associated with a positive response to the neuroprotective treatment, wherein the treatment protein signature comprises a reduced level of at least one inflammasome protein; and
        identifying patients exhibiting the presence of the treatment protein signature as responding positively to the neuroprotective treatment.

20.   The use of claim 19, wherein the biological sample is CSF.

21.   A method of determining a prognosis for a patient with a CNS injury comprising:
        providing a biological sample obtained from the patient within a week of injury; and
        measuring the level of at least one inflammasome protein in a biological sample obtained from a patient presenting with clinical symptoms consistent with a CNS injury to prepare an inflammasome protein profile, wherein the inflammasome protein profile is indicative of the prognosis of the patient, and wherein the biological sample is cerebrospinal fluid (CSF), blood, serum or plasma.

22.   The method of claim 21, wherein an elevated level of at least one inflammasome protein relative to a pre-determined reference value or range of reference values is predictive of the patient having a Glasgow Outcome Scale (GOS) score of 1 to 3 upon follow-up assessment.

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23.   The method of claim 21, wherein the at least one inflammasome protein is NLRP1, ASC, caspase-1, or combinations thereof.

24.   The method of claim 23, wherein the at least one inflammasome protein is the p20 subunit of caspase-1.

25.   The method of claim 21, wherein the CNS injury is a stroke-related injury, a cerebral aneurism-related injury, concussion-related injury, cerebral ischemia, injury resulting from neurodegenerative diseases, seizure-related injuries, multiple sclerosis, amyotrophic lateral sclerosis, a spinal cord injury or a traumatic brain injury.

26.   The method of claim 21, wherein a reduced level of at least one inflammasome protein relative to a pre-determined reference value or range of reference values is predictive of the patient having a GOS score of 4 or 5 upon follow-up assessment.

27.   The method of claim 21, wherein the level of the at least one inflammasome protein is measured by immunoblot or ELISA.

28.   The method of claim 21, wherein the patient is a pediatric patient.

29.   The method of claim 21, wherein the sample is CSF.

30.   A neuroprotective agent for use in treating a CNS injury in a patient, wherein the patient is selected according to the method of claim 1.

31.   The neuroprotective agent of claim 30, where in the neuroprotective agent is a neutralizing antibody or binding fragment thereof against an inflammasome protein, an aptamer that specifically binds to an inflammasome protein or epitope thereof, methylprednisolone, 17α-estradiol, 17β-estradiol, ginsenoside, progesterone, simvastatin, deprenyl, minocycline, and resveratrol.

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32. The neuroprotective agent of claim 30, wherein the method of claim 1 further comprises:

measuring the level of the at least one inflammasome protein in a biological sample obtained from the patient following neuroprotective treatment, wherein the biological sample is cerebrospinal fluid (CSF), blood, serum or plasma;

preparing a treatment protein signature associated with a positive response to the neuroprotective treatment, wherein the treatment protein signature comprises a reduced level of at least one inflammasome protein; and

identifying patients exhibiting the presence of the treatment protein signature as responding positively to the neuroprotective treatment.

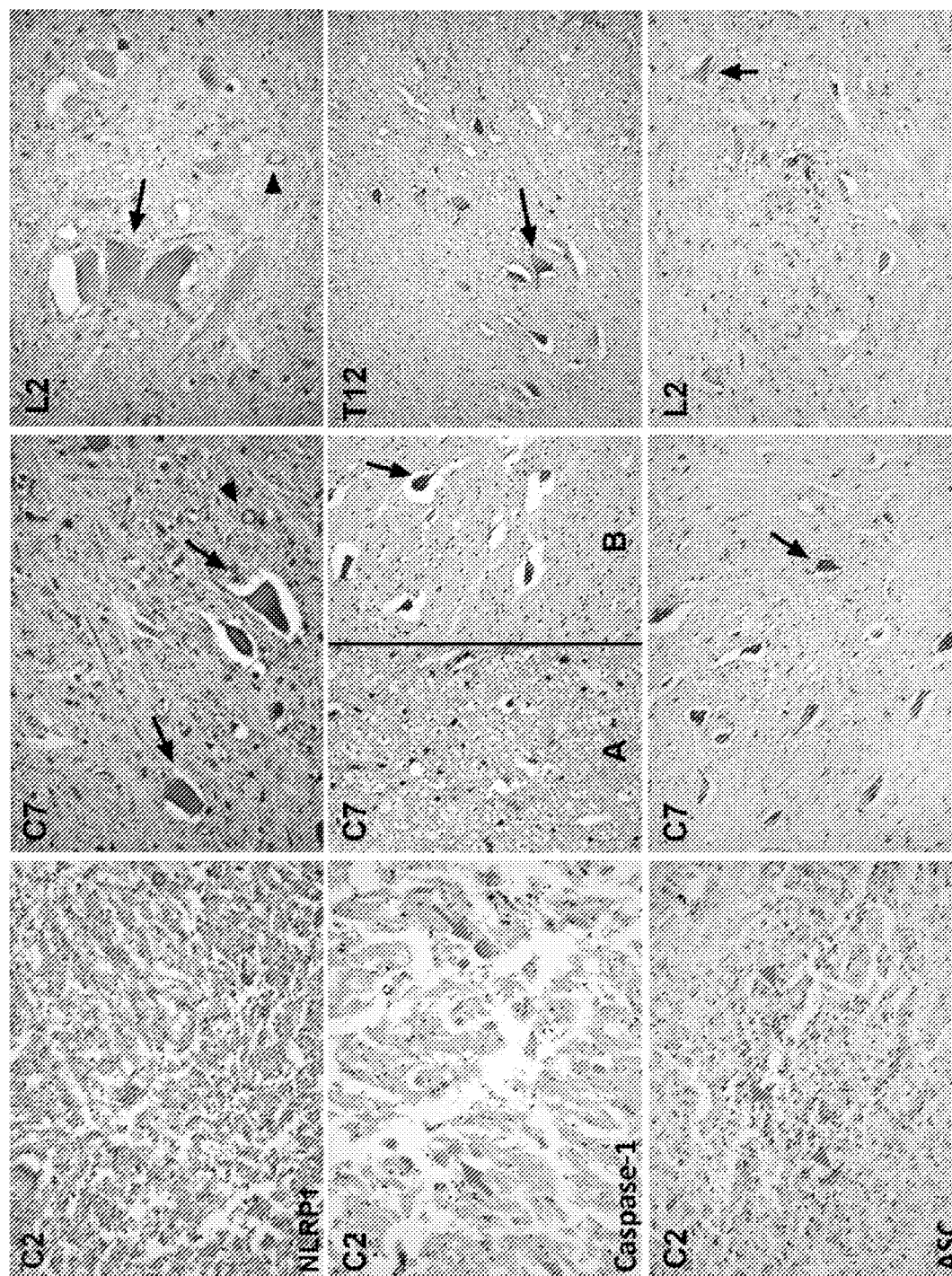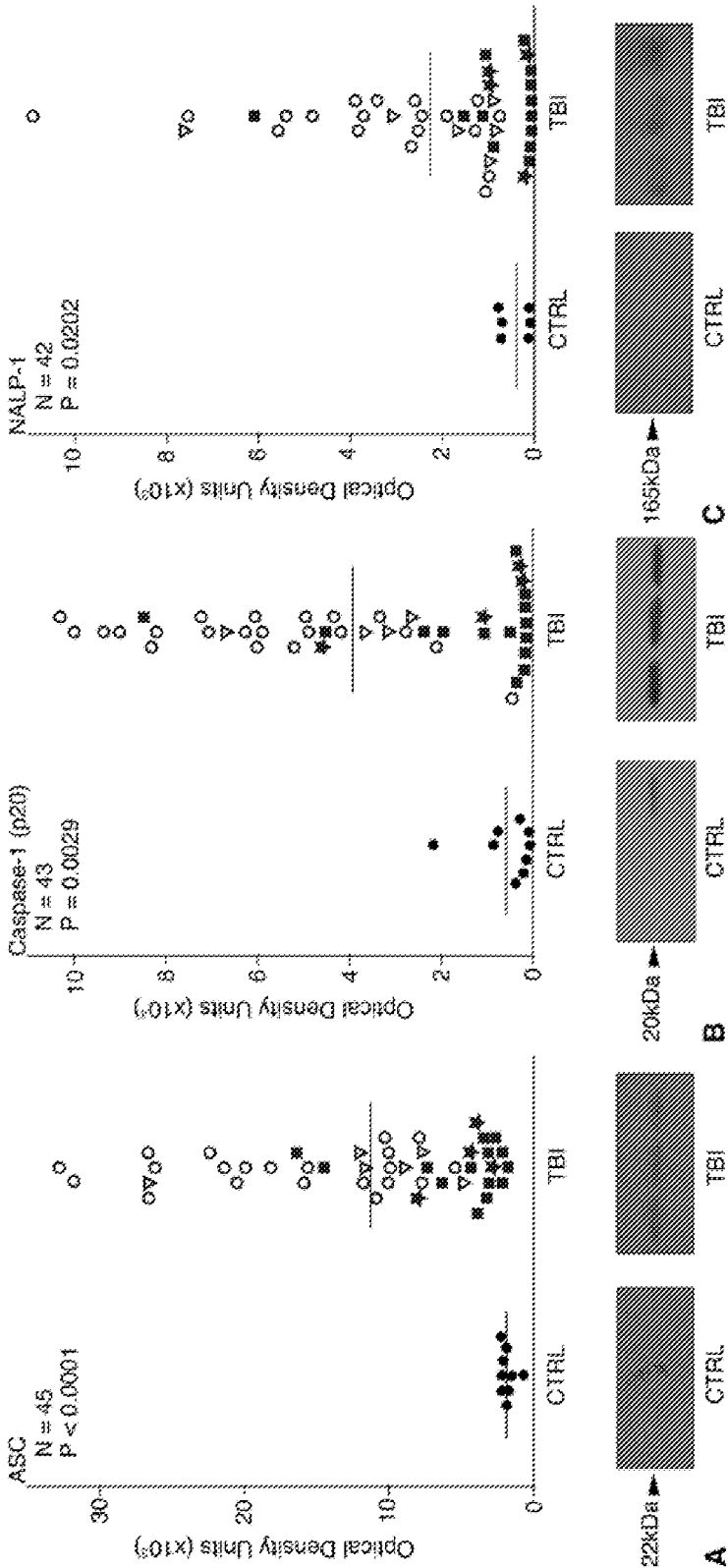33. The neuroprotective agent of claim 32, wherein the biological sample is CSF.

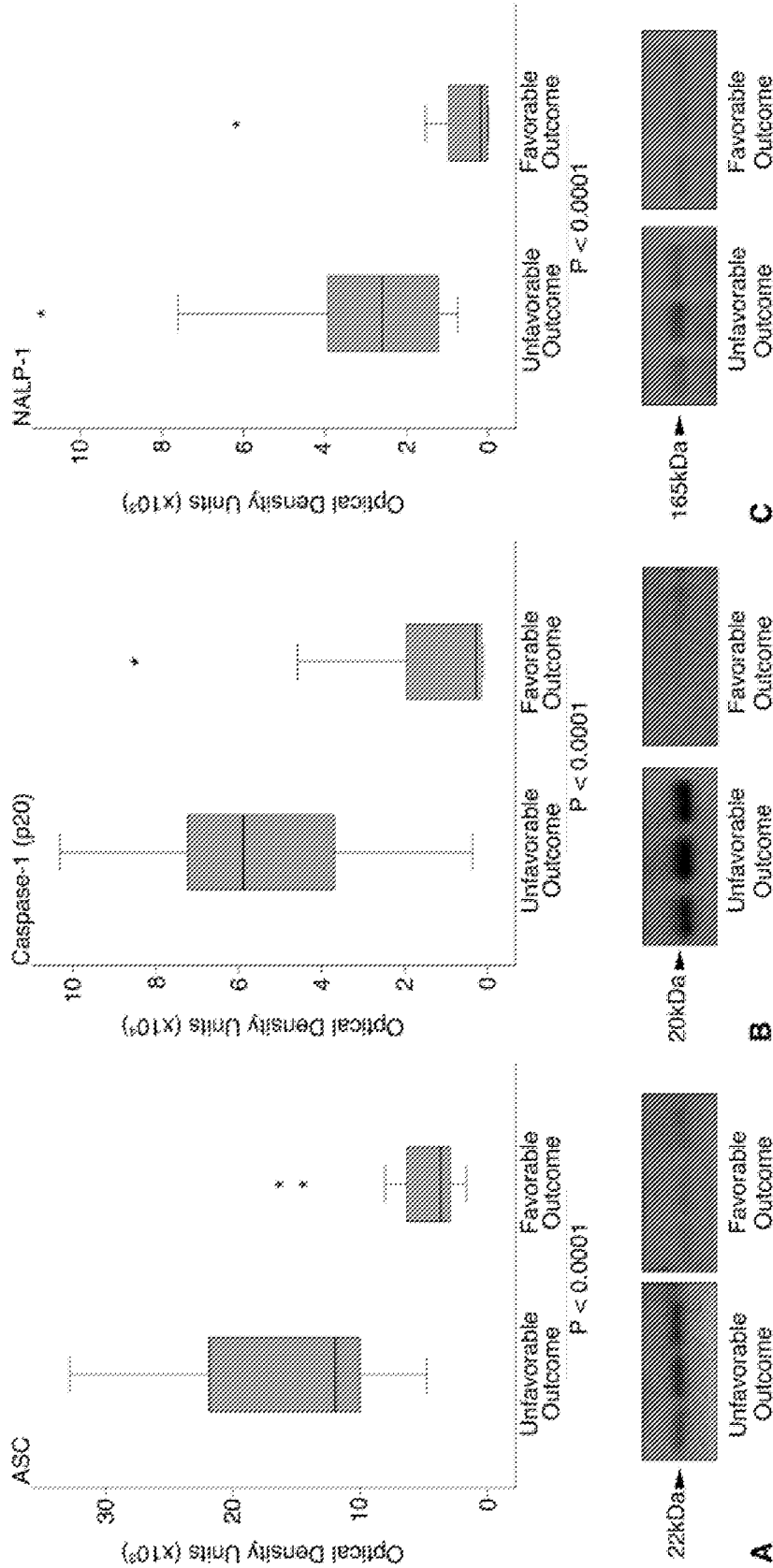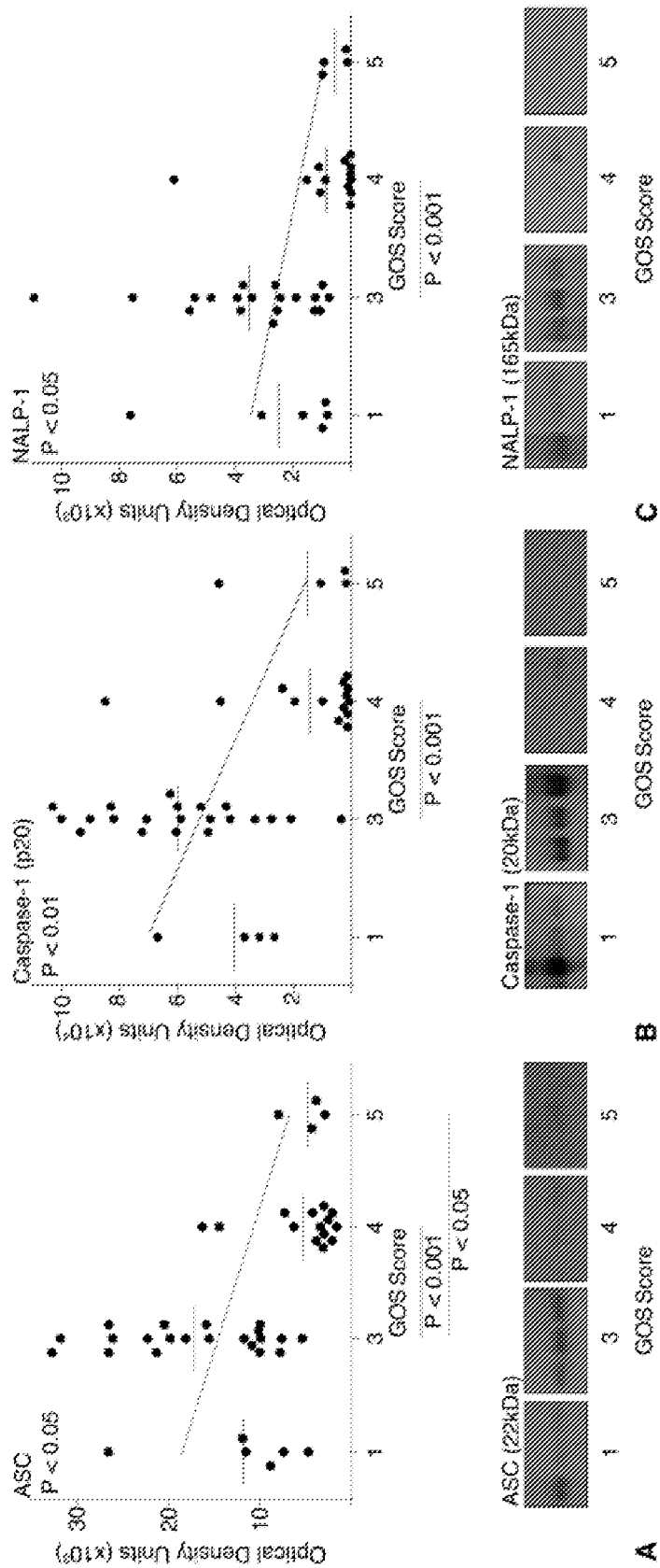FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

FIGURE 6

Caspase-1

A ASC — Optical Density Units (×10⁵). Unfavorable Outcome vs Favorable Outcome. P < 0.0001. 22kDa

B Caspase-1 (p20) — Optical Density Units (×10⁴). Unfavorable Outcome vs Favorable Outcome. P < 0.0001. 20kDa

C NALP-1 — Optical Density Units (×10³). Unfavorable Outcome vs Favorable Outcome. P < 0.0001. 165kDa